ผลของการเสริมฟรุคโตโอลิโกแซคคาไรด์ ต่อจุลินทรีย์ในทางเดินอาหาร การย่อยได้ของสารอาหาร พลาสมาโคเลสเตอรอล และการทำงานของฟาโกซัยท์ในสุนัขโต

นางสาว สิรีลักษณ์ ไมตรีปวิธ

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรมหาบัณฑิต
สาขาวิชาอาหารสัตว์ ภาควิชาสัตวบาล
คณะสัตวแพทยศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย
ปีการศึกษา 2551

THE EFFECTS OF FRUCTOOLIGOSACCHARIDE SUPPLEMENTATION ON GUT MICROFLORA, NUTRIENT DIGESTIBILITY, PLASMA CHOLESTEROL AND PHAGOCYTE ACTIVITY IN ADULT DOGS

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A Thesis Submitted in Partial Fulfillment of the Requirements
for the Degree of Master of Science Program in Animal Nutrition
Department of Animal Husbandry
Faculty of Veterinary Science
Chulalongkorn University
Academic Year 2008

Thesis Title	THE EFFECTS OF FRUCTOOLIGOSACCHARIDE SUPPLEMENTATION ON GUT MICROFLORA, NUTRIENT DIGESTIBILITY, PLASMA CHOLESTEROL AND PHAGOCYTE ACTIVITY IN ADULT DOGS
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สิรีลักษณ์ ไมตรีปวิธ : ผลของการเสริมฟรุคโตโอลิโกแซคคาไรด์ ต่อจุลินทรีย์ในทางเดินอาหาร การย่อยได้ของสารอาหาร พลาสมาโคเลสเตอรอล และการทำงานของฟาโกซัยท์ในสุนัขโต (THE EFFECTS OF FRUCTOOLIGOSACCHARIDE SUPPLEMENTATION ON GUT MICROFLORA, NUTRIENT DIGESTIBILITY, PLASMA CHOLESTEROL AND PHAGOCYTE ACTIVITY IN ADULT DOGS.) อ. ที่ปรึกษาวิทยานิพนธ์หลัก: ผศ. สพ.ญ. ดร. อุตรา จามีกร, อ. ที่ปรึกษาวิทยานิพนธ์ร่วม: รศ. น.สพ. เกรียงศักดิ์ พูนสุข, 53 หน้า.

การวิจัยครั้งนี้มีวัตถุประสงค์เพื่อศึกษาผลของการเสริมฟรุคโตโอลิโกแซคคาไรด์ (FOS) ต่อจุลินทรีย์ในทางเดินอาหาร การย่อยได้ของสารอาหาร พลาสมาโคเลสเตอรอล และการทำงานของฟาโกซัยท์ในสุนัขโต โดยใช้สุนัขพันธุ์ผสม เพศเมีย อายุระหว่าง 3.5-7 ปี น้ำหนักเฉลี่ย 14.3 ± 0.4 กิโลกรัม จำนวน 8 ตัว วางแผนการทดลองแบบ 4 × 4 จัตุรัสลาติน โดยแบ่งกลุ่มทดสอบออกเป็น 4 กลุ่มคือ กลุ่มที่ 1 สุนัขที่ได้รับอาหารพื้นฐานอย่างเดียว กลุ่มที่ 2 สุนัขที่ได้รับอาหารพื้นฐานร่วมกับ FOS ที่ระดับร้อยละ 0.5 กลุ่มที่ 3 สุนัขที่ได้รับอาหารพื้นฐานร่วมกับ FOS ที่ระดับร้อยละ 1.0 และกลุ่มที่ 4 สุนัขที่ได้รับอาหารพื้นฐานร่วมกับ FOS ที่ระดับร้อยละ 2.0 สุนัขทั้งหมดได้รับเพียงอาหารพื้นฐานในช่วงปรับตัวนาน 10 วัน จากนั้นจึงให้ FOS ติดต่อกันนาน 15 วัน และทำการหยุด FOS เป็นเวลา 10 วัน และมีน้ำสะอาดจัดให้กินอย่างเต็มที่ (ad libitum) ตลอดแต่ละช่วงการทดลองซึ่งนาน 35 วัน

ผลการทดลองพบว่า การเสริม FOS ที่ระดับร้อยละ 0.5, 1 และ 2 ไม่มีผลต่อน้ำหนักตัว การกินได้ และ ค่าการย่อยได้ของโปรตีน ไขมัน และ อินทรีย์สาร ของสุนัข ($P > 0.05$) สุนัขที่ได้รับอาหารพื้นฐานอาหารเพียงอย่างเดียวจะมีค่าวัตถุแห้งของมูลต่ำกว่ากลุ่มที่เสริม FOS ที่ระดับร้อยละ 0.5, 1 และ 2 แต่จะมีค่าการย่อยได้ของวัตถุแห้งสูงกว่าอย่างมีนัยสำคัญทางสถิติ ($P < 0.05$) สำหรับจุลินทรีย์ในทางเดินอาหารพบว่า แบคทีเรียกลุ่มแลคติคแอซิคแบคทีเรียมีจำนวนเพิ่มสูงขึ้น ในขณะที่จำนวนแบคทีเรียกลุ่มอี.โคลัยลดลงอย่างมีนัยสำคัญทางสถิติ ($P < 0.05$) ในกลุ่มที่เสริม FOS ที่ระดับร้อยละ 0.5, 1 และ 2 เมื่อเปรียบเทียบกับกลุ่มควบคุม ไม่พบความแตกต่างของค่าพลาสม่าโคเลสเตอรอลในสุนัขทุกกลุ่ม การทำงานของฟาโกซัยท์พบว่า สุนัขกลุ่มที่ได้รับการเสริม FOS ที่ระดับร้อยละ 2 จะมีค่าร้อยละการทำงานของฟาโกซัยท์ (%PA) สูงที่สุด ($P < 0.05$) ตามด้วยกลุ่มที่ได้รับ FOS ที่ระดับร้อยละ 1 และ 0.5 และกลุ่มควบคุมตามลำดับ

จากผลการทดลองสรุปได้ว่า การเสริมฟรุคโตโอลิแซคคาไรด์ที่ระดับร้อยละ 2 ซึ่งเป็นระดับที่มีการใช้ในอาหารสุนัขสำเร็จรูปทั่วไปในปัจจุบัน สามารถเพิ่มจำนวนจุลินทรีย์ที่เป็นประโยชน์ในทางเดินอาหาร เช่น แลคติคแอซิคแบคทีเรีย ในทางตรงกันข้ามจะลดจำนวนจุลินทรีย์ที่เป็นโทษเช่น อี.โคลัย รวมทั้งยังมีผลเสริมการทำงานของระบบภูมิคุ้มกัน คือ การทำงานของฟาโกซัยท์ในสุนัขที่โตเต็มที่แล้ว

ภาควิชา	สัตวบาล	ลายมือชื่อนิสิต.....……
สาขาวิชา	อาหารสัตว์	ลายมือชื่ออ.ที่ปรึกษาวิทยานิพนธ์หลัก…….....
ปีการศึกษา	2551	ลายมือชื่ออ.ที่ปรึกษาวิทยานิพนธ์ร่วม…….....

#4975574231: MAJOR ANIMAL NUTRITION

KEY WORDS: FOS/ GUT MICROFLORA/ NUTREINT DIGESTIBILTY/ PHAGOCYTE ACTIVITY/ ADULT DOGS

SIREELUK MAITREEPAWIT: THE EFFECTS OF FRUCTOOLIGOSACCHARIDE SUPPLEMENTATION ON GUT MICROFLORA, NUTRIENT DIGESTIBILITY, PLASMA CHOLESTEROL AND PHAGOCYTE ACTIVITY IN ADULT DOGS. THESIS PRINCIPAL ADVISOR: ASST. PROF. UTTRA JAMIKORN, D.V.M., M.S., Ph.D., THESIS CO-ADVISOR: ASSOC. PROF. KREINGSAK POONSUK, D.V.M., FRVAC. (DENMARK)., 53 pp.

The objectives of this experiment were to investigate the effects of FOS supplementation on gut microflora, nutrient digestibility, plasma cholesterol, and phagocyte activity in adult dogs and to determine the appropriate level of FOS supplementation in commercial dog foods. Eight healthy mixed breed female dogs, age between 3.5-7 years and average body weight between 14.3 ± 0.4 kg were used in this study. An experimental design was a replicated 4×4 Latin-square (LSD). Dogs had free access to water and were fed twice daily. Treatments composed of none (the control group), 0.5, 1.0, and 2.0% FOS (Beghim Meiji, France) supplementation. There were four experimental periods, each period last 35-d and composed of a 10-d adaptation period (no supplemental FOS) followed by a 15-d test period (supplemental FOS) and a 10-d post test period (no supplemental FOS).

Results of this experiment showed no effects of FOS supplementation on body weight and feed intake. The control dogs had lower ($P < 0.05$) fecal DM, but greater fecal DM digestibility than the dogs supplemented with FOS at 0.5, 1.0 and 2.0%. No differences ($P > 0.05$) in fecal digestibility of CP, crude fat and OM were observed. The dogs supplemented with FOS at 0.5, 1.0 and 2.0% had greater ($P < 0.05$) populations of fecal lactic acid bacteria than those of the control group. The fecal *E.coli* population decreased ($P < 0.05$) in all FOS supplement groups when compared to the control group. No differences ($P>0.05$) in plasma cholesterol concentration was observed between the treatments. The dog supplemented with 2.0% FOS had the greatest ($P < 0.05$) percentages of phagocyte activity, followed by 1.0 and 0.5% FOS supplement, and the control groups, respectively.

In conclusion, supplementation of FOS at 2% which is the level used in commercial pet food could demonstrate some effects on gut microflora by increase the population of beneficial lactic acid bacteria and decrease the population of fecal *E.coli*. These supplement also showed positive effect on immune system regarding increase the phagocyte activity.

Department:	Animal Husbandry	Student's Signature: ……
Field of Study:	Animal Nutrition	Principal Advisor's Signature: ……
Academic Year:	2008	Co-advisor's Signature: …….....

ACKNOWLEDGEMENTS

First of all, I would like to express my deep gratitude to my advisor, Assistant Professor Dr. Uttra Jamikorn for her valuable time, advice, guidance, helpful consultation and constant encouragement.

I would like to thank and to express my deep gratitude to my co-advisor, Associate Professor Kreingsak Poonsuk for his valuable time, advice, guidance, helpful consultation and constant encouragement and Associate Professor Dr. Sumolya Kanchanapangka for her helpful consultation.

I would like to thank Nutrix Co., ltd. for supplying the dry dog foods as the basal diet used in this study, and Beghim Meiji, Co., ltd. for supplying the fructooligosaccharide (FOS).

My thanks also expressed to the thesis committee, Associate Professor Somchai Chanpongsang and Associate Professor Chancharat Reodecha for their valuable suggestions.

My sincere and warm appreciation is expressed to Miss Pensuda Hongpu, Miss Punyaphat Ittitanawong, and Mrs. Supab Meungkaew for their kindness and laboratory technical suggestion.

I am also deeply grateful to my family and my friends for their kind encouragement throughout my study period.

Lastly, my thanks go to the financial support from Chulalongkron University and the Faculty of Veterinary Science, Chulalongkron University in Partial Fulfillment of the Requirements for the Master's Degree.

CONTENTS

Page

CHAPTER Page

LIST OF TABLES

Table Page

LIST OF FIGURES

Figure Page

LIST OF ABBREVIATIONS

APC	all plate counted
BGLB	brilliant green lactose bile broth
BW	body weight
CBC	complete blood count
CD	cluster of differentiation
CFU	colony forming units
CO_2	carbon dioxide
DLTB	double lauryl tryptose broth
DP	degree of polymerization
E.coli	*Escherichia coli*
EMB	eosin methylene blue
FI	feed intake
FOS	fructooligosaccharide
g	gram
g/d	gram per day
GIT	gastrointestinal tract
H_2	hydrogen
HMGCoA	3-hydroxy-3-methyl-glutaryl-CoA
Ig A	immunoglobulin A
kcal	kilocalorie
kg	kilogram
LAB	lactic acid bacteria
LDL	low-density lipoprotein
LTB	lauryl tryptose broth
ME	metabolizable energy
mg	milligram
min	minute
mL	milliliter

mm	millimeter
NADPHs	nicotinamide adenine dinucleotide phosphates
NH_3	ammonia
NH_4^+	ammonium ion
No	number
NSP	non starch polysaccharide
OF	oligofructose
PDS	peptone diluting saline
PMNs	polymorphonuclear neutrophils
PP	Peyer's patches
RBC	red blood cell
SCFA	short-chain fatty acid
SLTB	single lauryl tryptose broth
TG	triglyceride
VFA	volatile fatty acid
VLDL	very low density lipoprotein
µL	micro litter
ug/dL	microgram per dillitter
µg/day	microgram per day
WBC	white blood cell

CHAPTER I

INTRODUCTION AND AIMS

Fructooligosaccharide (FOS) also sometimes called oligofructose (OF) or oligofructan are classes of oligosaccharides. FOS have been identified as prebiotic that are not digested by small intestinal enzymes but beneficially affects the host by selectively stimulating the growth of some normal flora and improves host health (Gibson and Roberfroid, 1995). In general, term of "FOS" may include all nondigestible oligosaccharides composed of fructose and glucose units. Specifically, FOS refer to short chains of fructose units bound by ß – (2, 1) linkages attached to a terminal glucose units. Some beneficial bacteria in the intestinal tract can hydrolyze ß – (2, 1) bond and are thereby able to use FOS as a source of energy for promoting their multiplication with resulting to depress the population of specific enteropathogenic bacteria.

Nowadays, there are 2 types of FOS products available in the market; powder and liquid types. Supplementation of FOS has been shown to enhance gut health in several ways. One principle is the dramatic change in the composition of gut microflora, generally increasing the beneficial bacteria especially *Bifidobacteria spp.* and decreasing potential pathogens including *Escherichia coli*, *Clostridium spp.*, *Bacteroides spp*. etc. (Chadwick et al., 1992). Fructans also effectively prevent and treat constipation, especially inulin, a long-chain FOS, has been reported to increase stool frequency and moisture content in constipated human (Hond et al., 2000). Short – chain fructooligosaccharide have been shown to improve the symptoms of constipation problem as well (Hidaka et al., 1986). In addition, the benefits of prebiotics on gut health include the exertion of an antibacterial effect on potentially enteropathogenic bacteria through production of acids. These acid products were affected by a reduction in intestinal pH, reduction of ammonia levels through protonation of $NH4^{+}$, production of B group vitamins, and

immunomodulation in the gut mucosa (Gibson and Roberfroid, 1995). Short chain fatty acid (SCFA) produced from colonic fermentation has been shown to stimulate intestinal peristalsis (Kamath et al., 1988). Supplementation of FOS also decrease mean fasting blood glucose, mean serum cholesterol and LDL in diabetic patients (Yamashita et al., 1984). However, the mechanisms of these metabolic effects are not clear. Previous studies had demonstrated some effects of FOS on immune function in rats. Manhart et al (2003) reported that FOS showed an immunostimulating effect on Peyer's patches (PP) lymphocytes under healthy and endotoxemic conditions. However, the publication of research study of FOS supplement on immune system in dog is quite limited.

At the present, FOS are generally recommended in corporate into the premium dog foods at the amount of 0.5-2% which are considered quite low when compared the research dose at 5-10% (Fiordaliso et al., 1995; Kok et al., 1996a). The hypothesis of this study is that adult dogs supplemented with FOS should be improving of gut health when compared to the non-supplemented dogs since FOS would be increase the number of beneficial bacteria, nutrients digestibility and phagocyte activity of leukocytes, in contrast to decrease the number of pathogenic bacteria and plasma cholesterol.

The objectives of this experiment were:

1. To investigate the effects of fructooligosaccharide supplementation on gut microflora, nutrient digestibility, plasma cholesterol and phagocyte activity of leukocytes in adult dogs.

2. To determine the minimum requirement of FOS for supplement in dog food to achieve the target for enhancing of lactic acid bacteria (LAB) in gut, nutrients digestibility and immunostimulant, as well plasma cholesterol in adult dogs.

CHAPTER II

BACKGROUND AND INFORMATIONS

Fructooligosaccharide as Prebiotics

Definition of Prebiotics

One application for enhancing the beneficial microflora in the gut is so called prebiotics. 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, and thus improves host health”. All of these non-digestible carbohydrates are expressed as non-digestible polysaccharides. They are not hydrolyzed by animal enzymes in the small intestine, but are fermented by colonic bacteria in the large intestine. Gibson et al. (1999) had identified some characteristics of them: active at nutritionally feasible dose, lack of side effects, fine control of microflora modulation, persistence throughout the colon and inhibit adhesion of pathogens. Today, FOS is one of the most popular prebiotic used in commercial pet foods.

Origin and compositional characteristics of FOS

FOS can be divided into two groups, short-chain FOS (sc-FOS) and Long-chain FOS, as follows:

Short-chain fructooligosaccharide (sc-FOS) compose of ß-D-fructofuranoses attached by ß -D-glucopyranosyl or ß -D-fructopyranosyl residues. They constitute a group of oligosaccharides derived from sucrose that are isolated from natural vegetable sources. FOS is define as degree of polymerizations (DP) <10 (Robertfroid, 1998). Lewis (1993) reported that a commercial FOS mixture containing three FOS species; 1-ketose (1-kestotriose; GF_2), nystose (1, 1-kestotetraose; GF_3), and 1F- ß -fructofuranosyl-nystose (1, 1, 1-kestopentaose; GF_4). The Molecular structure of the FOS is shown in Figure 1.

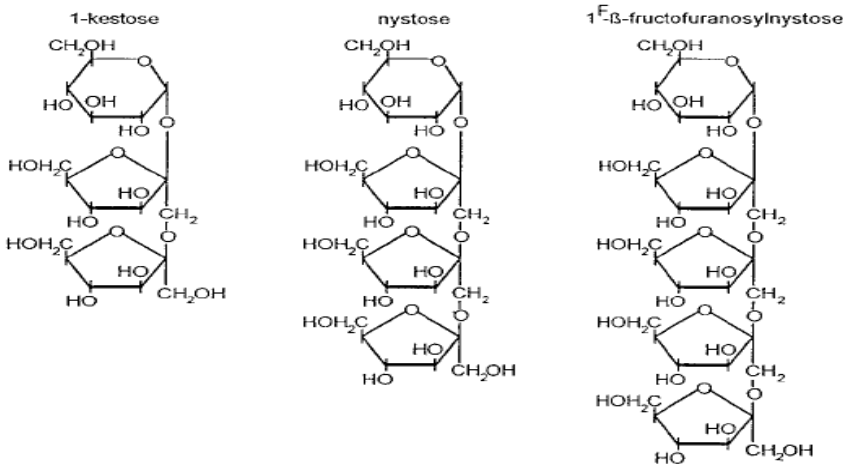

Figure 1. Molecular structure of the fructooligosaccharide (Hussein et al. 1998).

sc-FOS are naturally occur as constituents in plants and vegetable. The most common sources of sc-FOS are onions, Jerusalem artichokes, asparagus, wheat, rye, and garlic. Bornet et al. (2002) found that onion had the highest content of sc-FOS, ranging from 25-40% (dry matter, DM basis) of which 97% are SC-FOS. Garlic and chicory root had the least FOS content. sc-FOS are produced on a commercial scale by two different processes. One is from sucrose using a food grade fungal fructosyltransferase ,for example; *Aspergillus niger*, *Aureobasidium spp.*, *Arthrobacter spp.*, and *Fusarium spp.* etc. Another is from inulin by partial hydrolysis using endo-inulinase (Flickinger, 2003; Yun, 1996). sc-FOS have been described as 0.4-0.6 times as sweet as sucrose (Robertfroid, 1993).

Long-chain FOS which compose of fructans ranging between 2 and 60 fructose units are named inulin, and those with 2 to 20 fructose units are named oligofructose. Chemically, they are sucrose molecules to which one or more fructose units have been added by ß – (1, 2) – glycosidic linkage to the fructose units of sucrose (Gibson et al., 1995). Long-chain FOS present in edible parts of variety of plants, such as onion, Jerusalem artichokes, chicory, leek, rye, barley and garlic. For commercial production, these FOS are obtained either by extraction from plant

sources with subsequent enzymatic hydrolysis or produced by enzymatic transglycosylation reactions using fructosyltransferase (Van et al., 1995).

Jenkins et al. (1999) indicated the potential effects of oligofructose and inulin on physiologic effects as shown in table 1.

Table 1 The effects of oligofructose and inulin on physiologic effects.

Local	Systemic
↑ Fecal bulk	↓ (↑) Cholesterol
↑ Bacteria	↓ TG (↓ insulin; ↓ glucose)
Selective ↑ bacteria	↓ NH_3
↑ SCFA production	↓ Urea
Selective ↑ in SCFA	↑ B vitamins
↑ Mineral absorption	↑ Immune function
↑ B vitamin synthesis	(↑ Glutamine?)

Source: modified from Jenkins et al. (1999)

All FOS can escape digestion in upper part of the intestine. The ß - (2, 1) linkages present in the fructans have been shown to be resistant to mammalian enzymes. Hidaka et al. (1986) reported that <0.5% of sc-FOS was hydrolyzed by human salivary enzymes and rat pancreatic and small intestinal enzymes. Similar result was reported by Oku et al. (1984) that <0.5% of scFOS was hydrolyzed by rat intestinal mucosal enzyme of rat. Furthermore, *in vitro* work performed by Nilsson et al. (1988) reported that at pH>1.8, <1% oligofructose (OF) was hydrolyzed by human gastric juice. The recovery of OF from the small intestine of rats was similar to that of polyethylene glycol, a nondigestible marker, proving that it was not digested by mammalian enzymes. Tokunaga et al. (1989) proved that sc-FOS was degraded by microbial populations by incubating ^{14}C-labelled scFOS in cecal contents of rats.

After incubation, approximately 90% of the ^{14}C was detected in fermentative end-products, SCFAs (66%), CO_2 (12%), and fecal biomass (6 to 10%). Therefore, most fructans could reach the colon and were highly digestible substrates for bacteria.

The reports about effects of sc-FOS and long-chain FOS supplementation on nutrient digestibility in dog are quite limited. Some previous studies demonstrated that supplementation of sc-FOS at 0.5 to 1.5% of dogs did not affect nutrient digestibility (Swanson et al., 2002a; Flickinger et al., 2003). On the other hand, Propst et al. (2003) and Twomey et al. (2003) found that the dogs supplemented with oligofructose and inulin at 0.3 to 6% of diet had lower ($P < 0.05$) fecal CP, CF, and crude fat digestibility than the control dogs but no difference ($P > 0.05$) in fecal digestibility of DM and ash were observed. In addition, FOS can also be used as an alternative growth promoter, as with antibiotics, in animal feed. Furthermore, they resist stomach and intestinal digestion, and heat of the food processing (Saroj, 2004).

Shim et al. (2005) described mode of actions of FOS in the gastrointestinal tract of weaned pigs as follows (Table 2);

Table 2 Mode of actions of FOS in the gastrointestinal tract of weaning pigs.

Fructooligosaccharide				
Small Intestine		**Large Intestine (caecum + colon)**		
Villous height (↑)		Fermentation metabolized		
Endogenous enzyme activity (↑)		by *Bifidobacteria spp*. & *Lactobacilli spp*.		
Digestibility (↑)		Absorption (VFA ↑)		
		Acidic pH (↓)		
		E. coli (↓)		
		Putrefactive agents (↓)		
		Immunity (↑)		
Benefits:	Diarrhoea (↓)	Gut Health (↑)	Feed Intake (↑)	Growth (↑)

Source: adapted from Shim et al. (2005)

The gut microflora

Rastall (2004) reviewed microbial ecology of companion animals as follow. Microbiologically, the gut could be thought of in terms of three principle regions: the stomach, small intestine, and colon. For microbial population, the stomach had very low bacterial numbers; facultative anaerobes such as lactobacilli, streptococci, and yeast were present at about 100 colony forming units (CFU) per milliliter due to the low environment at pH. The small intestine had a larger bacterial load that consisted of facultative anaerobes such as lactobacilli, streptococci, and enterobacteria as well as anaerobes such as *Bifidobacterium spp.*, *Bacteroides spp.*, and clostridia as levels of approximately 10^4-10^8 CFU/ml. The most heavily colonized region, however, was the colon, with a total population of 10^{11}-10^{12} CFU/ml of contents. The colonic microflora was the predominant target for dietary intervention in the gut ecology.

In terms of health, the most significant active organisms are believed to be the bifidobacteria (Gibson and Roberfroid, 1995). Bifidobacteria are the major component of the microbial barrier to the intestinal infection. Bifidobacteria produce a range of antimicrobial agents that are active against gram-positive and – negative organisms (Gibson and Wang, 1994). Lactobacilli are also health positive and produce a range of antimicrobial agents. In addition to the production of antimicrobial agents, a large population of beneficial bacteria competitively excludes pathogens by occupying receptor sites and competing for space, nutrients competitors etc.

Effect of FOS on gut microflora

FOS are dietary components that are not digested by the host, but they benefit the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the gastrointestinal tract (GIT). They have great potential to modulate colonic microflora and discourage the colonization of enteric pathogens.

Supplementation of FOS has been shown to enhance gut health in several ways. Their consumption may result in dramatic changes in the composition of gut

microflora. FOS selectively feed the health-promoting bacteria *Bifidobacterium*, and lactic acid bacteria, such as *Lactobacillus acidophilus*, and a few other *Lactobacilli* species. The *Lactobacillus acidophilus*, *Bifidobacterium* and *Enterococcus faecium* bacteria can be correctively referred to as health-promoting or beneficial bacteria for many reasons suggested by some research studies. Each of these species is benefits in both small and large intestines. However, the *L. acidophilus* and *E. faecium* tend to be more active in the small intestine, while the *Bifidobacterium* are more active in the large intestine. These health-promoting bacteria are also considered lactic acid-producing bacteria and assist in maintaining and regulating digestive tract pH to restrict *E. coli* and *Salmonella* growth and the attachment or colonization by the beneficial bacteria prevents harmful bacteria from attaching and increasing in number.

Gebbink et al. (1999) found that the pigs supplemented with 0.5% FOS in the diet had decreased ($P < 0.05$) the *E. coli* population and increased *Bifidobacteria* population in the proximal and distal colon. Indeed, Gibson and Wang (1994) reported that some species of *Bifidobacteria* are able to exert antimicrobial effects on various gram-positive and gram-negative entero pathogens including *Salmonella spp.*, *Campylobacter spp.* and *E. coli*. In addition to the production of bacteriocins, acetate and lactate produced by *Bifidobacteria* decrease luminal pH, creating an unfavorable environment for many pathogens.

FOS were utilized as rapidly fermentable source of carbohydrates. They are fermented by colonic bacteria to produce short-chain fatty acids (SCFAs; acetate, propionate and butyrate). All these three predominant SCFAs stimulate the growth and differentiation of epithelial cell in the colon and small intestine. SCFAs in the large bowel account for approximately 80% of total SCFAs produced by humans. Han et al. (1984) reported that as the most important oxidative fuel, more than 70% of the metabolic energy supplied for the colonocyte was these SCFAs, especially butyrate. Roediger (1982) supported that SCFAs were the main energy source for colonocytes, in particular, butyrate, which is the preferred energy substrate of colonic epithelium. Gibson and Wang (1994) found that the dogs supplemented with

FOS had greater concentrations of fecal butyric acid than those of the control group. Both of them have been shown to increase mucosal villus height, crypt depth and number of goblet cells, thus reinforcing and stabilizing the gut mucosal barrier (Kleessen et al., 2003). Similar to lactate, these organic acids decrease luminal pH and assist in pathogen resistance. These were the mechanisms regarded as being responsible for increasing the ability of the normal intestine microbes to inhibit pathogen colonization and increasing digestibility and availability of vitamins and minerals (Crittenden and Playne, 1996). Hesta et al. (2002) found that the cats fed 6% long-chain FOS had greater amount of (P <0.05) total fecal SCFA and valeric acid than the control cats.

Effect of FOS on Cholesterol

Cholesterol is an important metabolic compound occurring in cell membranes and lipoproteins. It is also a precursor to bile acids and steroid hormones. The steroid hormones have only small structural differences which cause major differences in their functions. The body has the ability to synthesize and redistribute cholesterol. The main organ that synthesizes cholesterol is the liver. The amount of cholesterol synthesized by the body can be two to three times or more than the amount ingested. Cholesterol is not an essential nutrient and can be made in the body from simple compound via acetyl CoA (Spady et al., 1993).

Effectively, three acetyl CoAs are combined to from hydroxmethylglutaryl CoA (HMGCoA), which is reduced by NADPH and catalyzed by HMGCoA reductase (Spady et al., 1993). This enzyme, the rate-limiting enzyme of cholesterol synthesis, is located on the endoplasmic reticulum. The subcellular localization of cholesterol synthesis, cytosol, and endoplasmic reticulum, compared to ketone-body formation in the mitochondria, assures that the fate of HMGCoA is determine by subcellular localization of HMGCoA formation. The reduction of HMGCoA by HMGCoA reductase, using 2 NADPHs as the source of reducing power, forms mevalonate . Mevalonate, with the input of two ATPs, forms 5-pyrophosphomevalonate. Decarboxylation and intramolecular rearrangements of 5-pyrophosphomevalonate

produced 3, 3-dimethyallyl pyrophosphate and Δ3-isopentenyl pyrophosphate, both isopentenyl units (C5). Three C5 units combine to make a C15 pyrophosphate unit, farnesyl pyrophosphate. Two C15 pyrophosphate units plus NADPH produce squalene, a C30 unit. After enzymatic rearrangements, oxidation to form the 3-OH and further reductions utilizing NADPH, the squalene forms cholesterol as shown in figure 2.

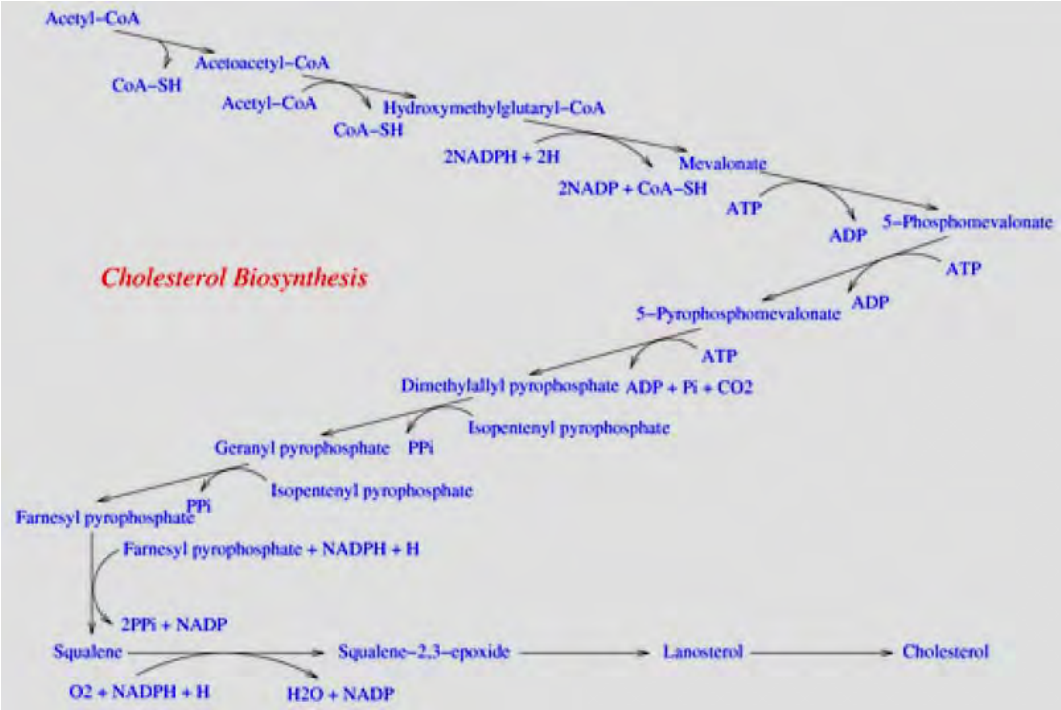

Figure 2. Biosynthesis of Cholesterol (www.cellml.org/.../metabolic_models_doc.html [online, 2008])

Cholesterol storage and delivery are controlled by a number of LDL receptors. Low-density lipoprotein is a cholesterol-rich lipoprotein which can deliver cholesterol to many tissues via LDL receptors. As tissue cholesterol levels rise, the importance of cholesterol delivery is less important. When cellular cholesterol levels are high, there is a down-regulation of LDL receptors (a decrease in the number of receptors), thus decreasing cholesterol delivery to that tissue. The tissues rich in

LDL receptors are liver, adrenal gland, ovaries, testes, and others that convert cholesterol to important metabolic products.

Quantitatively, the greatest loss of cholesterol is via bile acids and bile salts formed by the liver. These bile acids and bile salts are important in digestion for emulsification of lipids, including fat-soluble vitamins. The bile acids and bile salts are secreted from the liver to the intestines via bile duct. A considerable portion of bile acids returned to the liver by the enterohepatic circulation. This decreases the need to convert as much cholesterol to bile acids. Therefore, another attempt to decrease cholesterol levels is by increasing conversion of cholesterol to bile acids, by slowing recirculation of the bile acids and bile salts, has been used. This has been suggested as one of the benefits of dietary fiber, which binds bile acids and causes greater excretion in the feces instead of recirculation back to the liver (Voet and Voet, 1995). The normal ranges of canine cholesterol between 110 – 314 mg/dl.

FOS, besides their effect on the GIT, are also able to exert systemic effect, namely by modifying the hepatic metabolism of lipids in several animal models. But a research study in dog is quite limit and the mechanisms of these metabolic effects are not clear. Like dietary fiber, FOS escapes digestion in the small intestine and enters the cecum without significant changes in their structure. They are fermented by the resident microflora into SCFA (acetate, propionate, and butyrate), lactic acid acid, CO_2, and H_2, which influences the lipid metabolism in human beings (Luo et al., 1996). Andersson et al. (2001) described that the mechanism by which lactic acid bacteria and *Bifodobacterium* might be able to reduce total and LDL cholesterol. *Lactobacilli* produce deconjugate the bile salts enzymes (Bile salt hydrolase, BHS) in the intestine to form bile acids and thereby inhibit micelle formation. This leads to decreased absorption of cholesterol. Another factor thought to be elaborated by *lactobacilli* is HMGCoA which inhibits HMGCoA reductase, the rate limiting enzyme in endogenous cholesterol synthesis. Studies in rats have demonstrated that feeding inulin or oligofructose as 10% of the diet reduced hepatic triglyceride synthesis and serum VLDL (Fiordaliso et al., 1995; Kok et al., 1996a; Kok et al., 1996b). Furthermore, FOS decreased pre-prandial urea, cholesterol and

triglyceride concentrations, and lowered post-prandial glucose, urea, cholesterol and triglyceride concentrations in healthy dogs (Diez et al., 1997). However, evidence has recently been presented that the metabolic effects of dietary fibers are associated with, and possibly mediated through, changes of gastrointestinal hormones such as glucagons-like peptide- I (Reimer and McBurney, 1996; Reimer et al., 1997; Kok et al., 1998). Thus, the metabolic effects of FOS, which has many of the properties of dietary fiber, might be mediated by this or other hormones.

Effect of FOS on Phagocyte activity

Although specific nutrients are known to be important in the development and function of the immune system (Alexander, 1995), less is known about the potential of dietary fibers to impact on immune function. The properties of FOS are similar to physiological effects of a dietary fiber (Roberfroid, 1998). It can be speculated that the fermentative property of dietary FOS may have positively influenced immunity. Schley and Field (2002) reported the immune-enhancing effects of dietary fiber that changes in the intestinal microflora that occur with the consumption of prebiotic fiber may potentially mediate immune changes via: the direct contact of lactic acid bacteria or bacterial products (cell wall or cytoplasmic components) with immune cells in the intestine and the production of short-chain fatty acids from fiber fermentation or by changes in mucin production.

The immune system is defined as the host's defense against destructive forces from both outside (e.g. bacteria, viruses, parasites) and within (e.g. malignant and auto reactive cells in the body. Immune responses are generally classified as either innate (inborn components of the immune system) or acquired (adaptive). The components and cells that comprise these two arms of the immune system are presented in Table 3.

The innate immunity or nonspecific immunity comprises the cells and mechanisms that defend the host from infection by other organisms, in a non-specific manner. This means that the cells of the innate system recognize, and

respond to, pathogens in a generic way, but unlike the adaptive immune system, it does not confer long-lasting or protective immunity to the host.

The innate immunity provides immunity to invading organisms without the need for prior exposure to these antigens and includes physical barriers such as the skin and mucous membranes, cell-mediated barriers, including phagocytic cells, inflammatory cells, dendritic cells, and natural killer cells, and soluble mediators such as cytokines, complement and acute-phase proteins (Delves and Roitt, 2000a).

The acquired, or adaptive, immune system develops over an individual's lifetime. Immune responses by this system generally occur after those of the innate immune system; they are antigen-specific, and are more efficient upon secondary exposure to the pathogen (Goust and Bierer, 1993). Lymphocytes are an important cellular component of this arm of the immune system that modulate the function of other immune cells or directly destroy cells infected with intracellular pathogens (Table 3). Each developing T- or B-cell generates a unique receptor, or recognition molecule, by rearranging its receptor genes, such that a set of cells expressing a vast array of diverse receptors is produced, allowing immune cells to selectively eliminate virtually any foreign antigen that enters the body (Delves and Roitt, 2000a). B-cells, abundant in lymph nodes, recognize foreign antigen through membrane-bound antibodies, or immunoglobulins, and upon activation become antibody secreting plasma cells to effectively remove soluble bacteria/ antigens (Delves and Roitt, 2000a). Antibodies are secreted in soluble form and bind foreign particles to facilitate clearance by phagocytes (Delves and Roitt, 2000b). B-cells can also serve as antigen presenting cells and in this respect influence T-cell function (Delves and Roitt, 2000a).

Table 3 The immune system.

Arm of immune system	Defenses	Components	Functions
Innate immune system	Physical barriers	Skin and mucous membranes	Prevent the entry of antigens into systemic circulation
	Cell-mediated barriers	Phagocytic cells, e.g. neutrophils, macrophages	Engulf foreign antigens
		Inflammatory cells, e.g. basophils, mast cells	Release inflammatory mediators, e.g. Histamine, Prostaglandins
		Natural killer cells	Destroy infected or malignant cells
		Dendritic cells	Present antigens to lymphocytes
	Soluble factors	Cytokines	Activate/recruit other cells
		Complement	Enhance phagocytosis
		Acute-phase proteins	Promote repair of damaged tissue
Acquired immune system	B-lymphocytes	Plasma cells	Secrete antibody
	T-lymphocytes	CD4+ T- cells	Induce activation of lymphocytes
		Th1 cells	Promote cell-mediated responses

	Th2 cells	Promote humoral (antibody) responses
	CD8+ T-cells	
	Cytotoxic T-cells	Destroy infected or malignant cells
	Suppressor T-cells	Destroy infected or malignant cells

Source: Schley and Field (2002)

Phagocytosis is an important clearance mechanism for the removal and disposition of foreign agents and particles or damaged cell. Macrophages, monocytes, and polymorphonuclear cells are phagocytic cells.

Phagocytosis of microorganisms involves several steps: attachment, internalization, and digestion. After attachment, the particle is engulfed within a membrane fragment and a phagocytic vacuole is formed. The vacuole fuses with the primary lysosome to form the phagolysosome, in which the lysosomal enzymes are discharged and the enclosed material is digested. Remmants of indigestible material can be recognized subsequently as residual bodies. Polymorphonuclear neutrophils (PMNs), eosinophils and macrophages play an important role in defending the host against microbial infection. PMNs and occasional eosinophils appear first in response to acute inflammation, followed later by macrophages. Chemotactic factors are released by actively multiplying microbes. These chemotactic factors are power attractants for phagocytic cells which have specific membrane receptors for the factors. Certain pyogenic bacteria may be destroyed soon after phagocytosis as a result of oxidative reactions. However, certain intracellular microorganisms such as *Mycobacteria* or *Listeria* are not killed merely by ingestion and many remain viable unless there is adequate cell-mediated immunity induced by γ interferon activation of macrophages as shown in Figure 3.

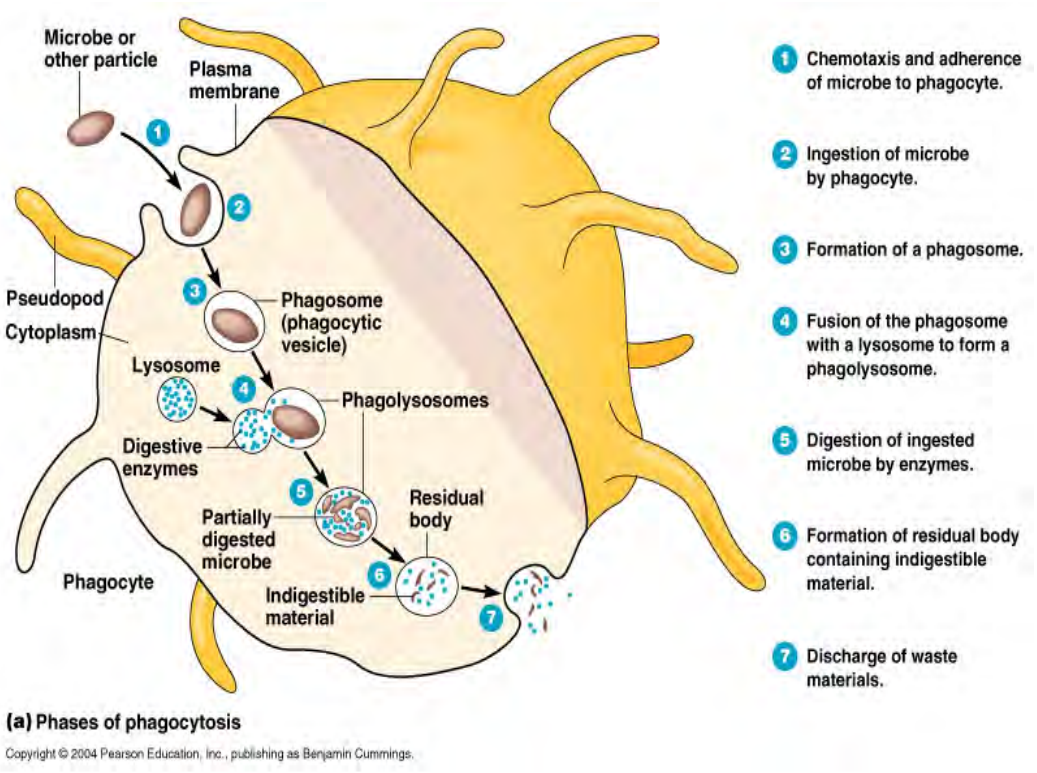

Figure 3. Phogocytosis activity (http://diverge.hunter.cuny.edu/~weigang/Lecture-syllabus.html [online, 2008])

Nevertheless, a research study of FOS supplementation on the phagocyte activity in dog is quite limited. Herich et al. (2002) who found that the weaned pigs supplemented with FOS had greater numbers of lymphocytes, leukocyte, neutrophils, $CD4^+$ T cells, and the phagocyte activity than the single administration of *Lactobacillus* and the control groups. Pierre et al. (1997) demonstrated that oligofructose enhanced the T-lymphocyte function in mice. It has been suggested that prebiotic such as FOS or inulin may be beneficial for the immune system and health of weaned pig.

CHAPTER III

MATERIALS AND METHODS

Experimental design and animals

1. Animals

Eight healthy female mixed breed dogs, age between 3.5-7 years and average body weight between 14.3 ± 0.4 kg were subject to used in this study. The experimental designed was the replicated 4 ×4 Latin-square. All dogs were housed individually in the metal cages (1.5 x 2.5 x 1.5 meters) with a plastic slat floor and the temperatures were ranging between 25.7 and 30.3 $^{\circ}$C. Each individual cage was cleaned twice daily. In the pre-test period, the dogs were fed a basal diet for 10 days after that they were fed the basal diets with various levels of FOS supplement in the diet for 15 days (test period) and all dogs were fed a basal diet for the last 10 days (post-test period). Fresh water was available ad libitum throughout the experiment. Animal care procedures were approved by the Animal Care Committee guidelines of Faculty of Veterinary Science, Chulalongkorn University.

2. Feed and Feeding

The commercial extruded dog diet formulated with no FOS supplementation in accordance with the AAFCO (2003) nutrient guide for adult dog was used as the basal diet. Major ingredients of the basal diet composed of broken rice, corn gluten meal, chicken meal, soybean meal, salt, limestone, monocalciumphosphate, and potassium chloride. Chemical analysis of nutrient composition in the basal diet is presented in Table 4.

Treatments composed of none (the control group), 0.5, 1.0, and 2.0% FOS (Beghim Meiji, France) supplementation by coated FOS in the diet (Table 5).

Table 4 Chemical analysis of nutrient composition in the basal diet (DM basis).

Item	Amount
Metabolizable energy (kcal/g)[a]	3.4
Crude protein (%)[b]	22.6
Ether extract (%)[b]	7.8
Crude fiber (%)[b]	3.1
Ash (%)[b]	11.7
Calcium (%)[b]	1.1
Phosphorus (%)[b]	0.9

[a]Calculated by use of equation from NRC (2006): ME, kcal/g = [(3.5×CP)+(8.5×EE)+(3.5×NFE)]/100

[b]Analyzed according to the AOAC (1990) procedures.

Table 5 Experimental groups.

Group	Description
1	Basal diet (no supplemental FOS)
2	Basal diet + 0.5% FOS in diet
3	Basal diet + 1.0% FOS in diet
4	Basal diet + 2.0% FOS in diet

The amount of food was calculated using standard equations to determine energy requirements of active adults dogs (ME requirement, kcal = 132 $BW_{kg}^{0.75}$; NRC, 2006) and adjusted every week. Each day, food was weighed and divided into two equal portions and fed to dogs at 0800 and 1600 hours in stainless steel bowls. Body weight of all animals was determined every week and the amount of feed intake was recorded daily. On d 15 through d 24 of each period, dogs were dosed with 0.5 g Cr_2O_3 at each feeding via gelatin capsule for a total of 1.0 g marker/d. This chromic oxide was used as a digestible marker.

Sample Collection and Determination

1. Feed

Throughout the experiment, the food sample was collected daily and pooled into plastic bags and stored at -20°C until nutrient content analysis. It was analyzed in duplicate for DM, CP, EE, CF, ash, Ca and P using AOAC (1990) procedure.

2. Blood

At Each period, blood collection was performed at d 10, 25 and 35 in the morning. Five ml of blood from cephalic vein was divided into three tubes as the following;

2.1 Blood samples 1 ml was collected into a micro-centrifuge tube with containing EDTA for complete blood count [CBC; RBC, hemoglobin, hematocrit, total white blood cell (WBC), neutrophil, eosinophil, basophil, lymphocyte and monocyte] determination.

2.2 Blood samples 1 ml was collected into heparinzed polypropylene (PP) tubes for determination of plasma cholesterol.

Both of Plasma cholesterol and CBC were determined by the laboratory of small animal hospital, Faculty of Veterinary Science, Chulalongkorn University.

2.3 Another 3 ml of blood samples were collected into heparinzed polypropylene (PP) tubes and placed on ice then centrifuged at 1500 × g for 10 minutes at room temperature for determination of the percentage of phagocyte activity modified from Weir (1978) as follows: 30 µl of *E.coli* (stain ATCC 25922) (1-2 × 10^7 micro-organisms/ml) and 30 µl of serum are combined and incubated at 37 °C under continuous rotation (4 rev./min) for 0, 15, 30, and 60 min, respectively. Next, cells are fixed with methanol and strained with Geimsa stain. The percentage of cells that have ingested bacteria is determined from counts of at least 100 phagocytic cells as according to the following formula:

$$\% \text{ of phagocyte activity} = \frac{\text{No. of phagocyte cells have ingested bacteria}}{\text{Total of phagocyte cells}} \times 100$$

$$\text{Index of Phagocyte activity} = \frac{\text{No. of ingested bacteria by phagocyte cells 100 cells}}{100}$$

3. Feces

3.1 Fecal sample was collected on d 10, 25 and 35 in the morning. Total stools of each dog were removed from the floor of the pen and kept at 4 ºC for bacterial enumeration. Microbiological analyses were as follows;

3.1.1 Enumeration of mesophilic lactic acid bacteria (ISO 15214, 1998)

The plating was performed into MRS medium (de Man, Rogosa and Sharpe, Difco®) from the prepared dilutions (10^{-1} to 10^{-3}) by a duplicated pour plate method. The colonies were counted after incubation at 37 °C for 48 hours under anaerobic conditions by double – layer MRS medium (ISO 15214, 1998). The dishes containing 15 to 300 colonies were examined. The calculations of mesophilic lactic acid bacteria were done as follows;

3.1.1.1. General case; Calculation of APC was done according to the following formula:

$$N = \frac{\sum C}{\left[\left(n_1 \times 1\right) + \left(n_2 \times 0.1\right)\right] \times \left(d\right)}$$

Where

N = Number of colonies per gram of product

$\sum C$ = Sum of all colonies on all plate counted

n_1 = Number of plates in first dilution counted

n_2 = Number of plates in second dilution counted

d = Dilution from which the first count were obtained

3.1.1.2. Estimation of low numbers

3.1.1.2.1 If the two dishes contained less than 15 colonies, the formula was simplified and only the arithmetical mean was used for calculation.

$$N = y/d$$

Where

y = arithmetical mean of the colonies counted on two dished

d = the dilution factor of the initial suspension

3.1.1.2.2 If the two dished did not contain any colonies, the results are to be expressed as follows;

- Less than 1/d aerobic bacteria per gram where d is the dilution factor of the initial suspension

3.1.2 Enumeration of *Escherichia coli* (ISO-4831, 1991)

The total numbers of *Escherichia coli* were determined by the three tubes most probable number (MPN) method as shown in Figure 4. Lauryl Sulphate Tryptose broth (LTB) was used as selective enrichment medium. Brilliant Green Lactose Bile Broth (BGLB) and EC- medium were used as confirmation medium. The number of tubes that showed gas formation in the BGLB and EC– confirmation-broth were counted. The probable numbers of *E.coli* were calculated according to the MPN tables (de Man, J.C. MPN tables. IS0 4831, 1991)

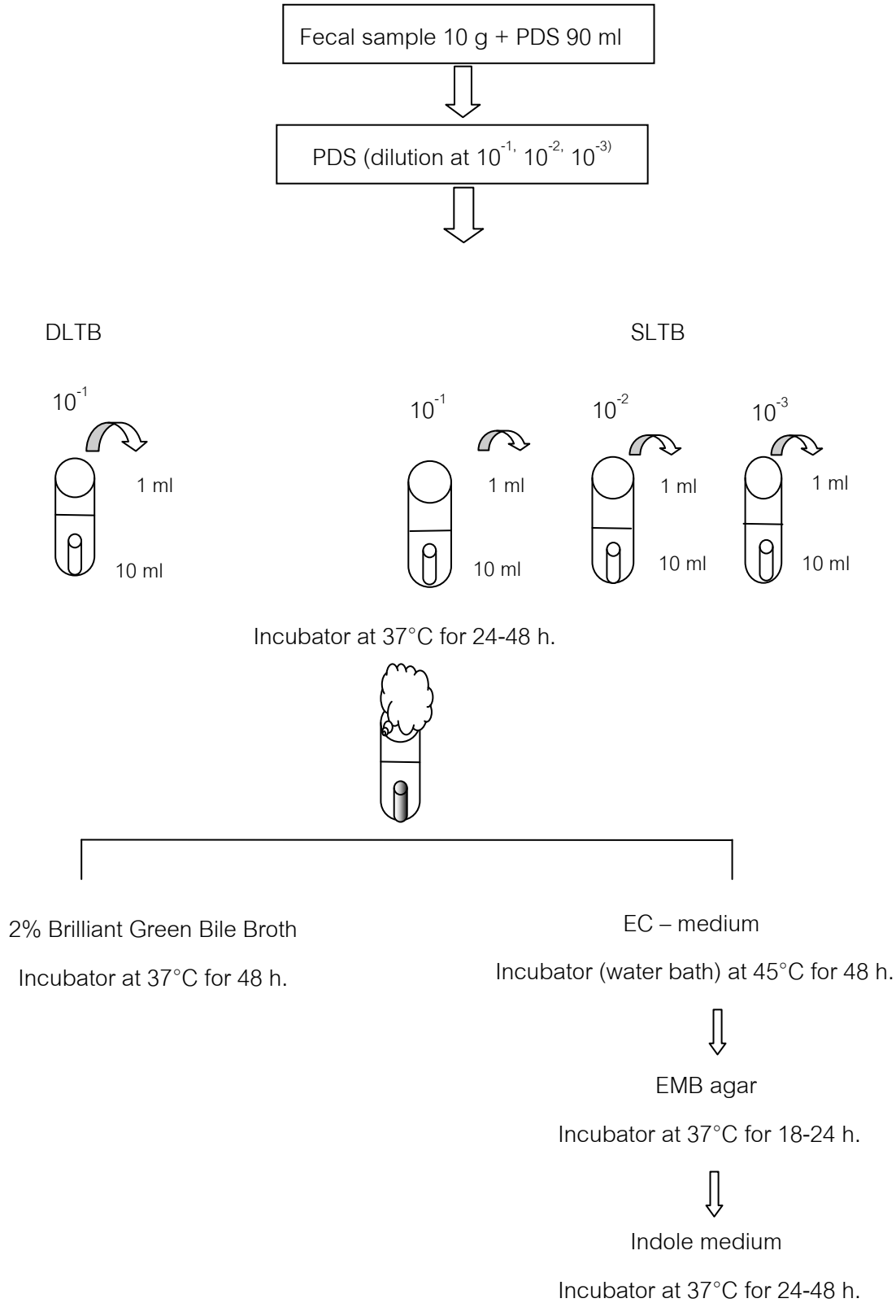

Figure 4. Most probable number (MPN) method adapted from ISO-4831 (1991).

3.2 From d 15 to 24 of the experiment before each feeding, the dogs were dosed orally twice a day with a gelatin capsule containing 500 mg of chromic oxide. This Cr_2O_3 would be use as a digestible marker for calculation of nutrient digestibility. On the first day of fecal collection (d22), all feces before 0700 hours were removed from the cages and discarded. Fecal output after that was collected until 2000 hours on d 24 from individual dog and placed into labeled plastic bags. Fecal samples of each dog were stored at -20°C and dried at 60°C in a forced-air oven. After drying, the samples were ground through a 1-mm screen mill (cyclotec 1093 sample mill) and collected in labeled plastic bottles at room temperature until further analysis. Fecal samples were examined for Cr using Williams et al. (1962) procedures.

3.3 Every week, all fecal samples were scored according to the following system: 1 = watery: liquid that can be poured; 2 = soft, unformed stool; assumes shape of container; 3 = soft, formed, a moist stool; 4 = hard, formed, dry stool; remain firm and soft; and 5 = hard, dry pellets; small, hard mass (Fig. 5)

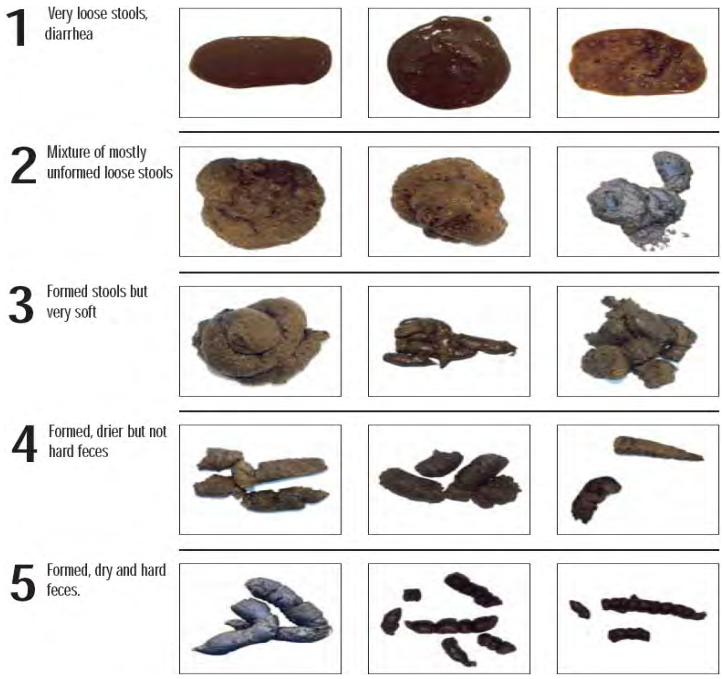

Figure 5. Fecal score characteristics (Royal Canin, 2006).

The diagram of sample collection is shown in Figure 6.

Statistical Analysis

All data were expressed as mean $\pm$ SD. These data were analyzed as a replicated 4 ×4 Latin-square using the general linear model. Each dog represented an experimental unit. The model included square, dog (square), period, rep, and treatment, and the error was residual error mean square. The mean differences between treatments were tested by Duncan's New Multiple Range test and the mean differences between groups were tested by t -test using the commercially computer program (SAS, 2002). Differences were considered significant when $P < 0.05$.

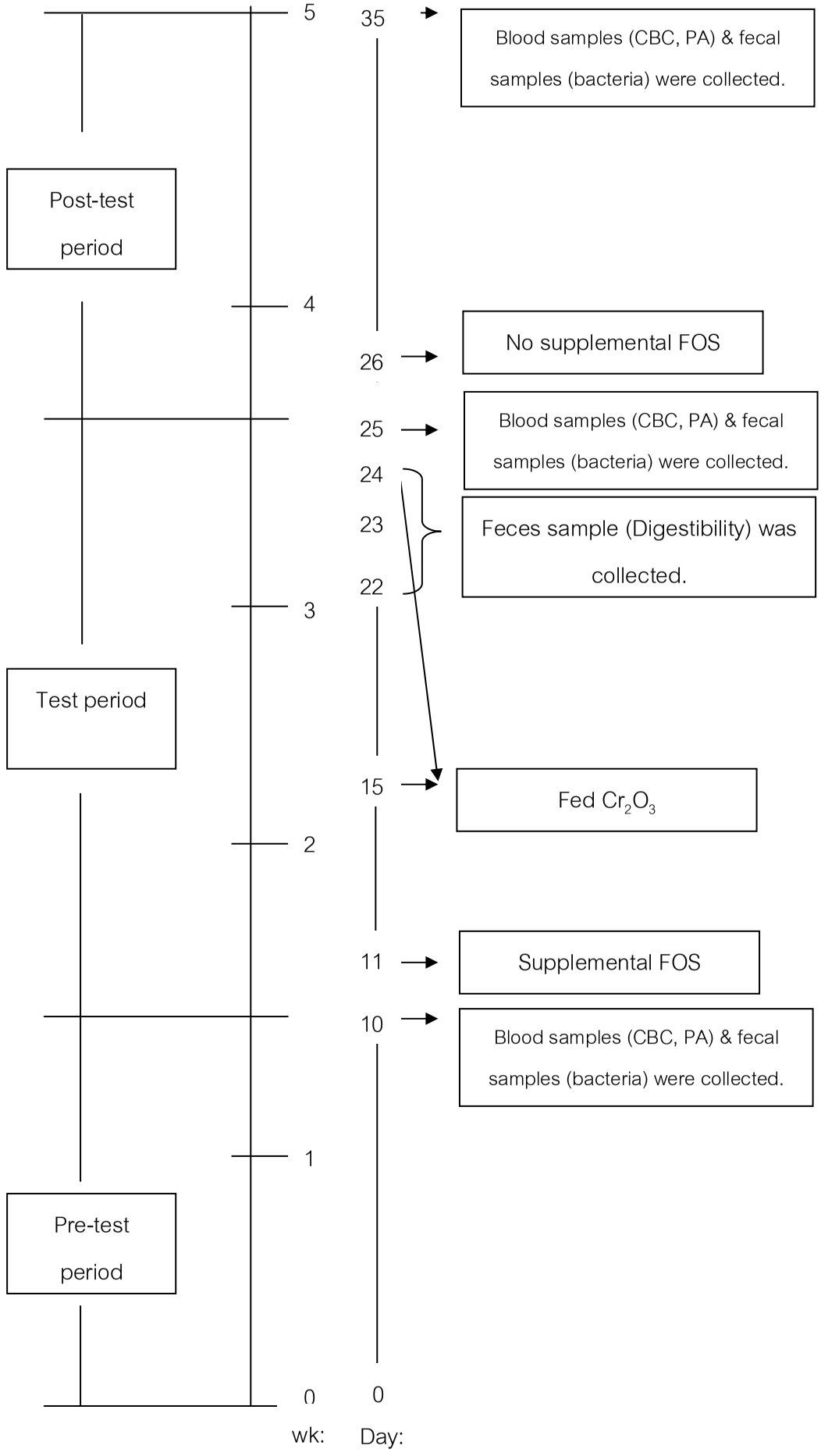

Figure 6. The diagram of sample collection in this experiment.

CHAPTER IV

RESULTS

Effect of FOS supplementation on body weight (BW) and feed intake (FI) of dogs

No effects of FOS supplementation on body weight and feed intake were observed in this experiment (Table 6).

Table 6 The average BW and Feed Intake of the dogs in all experimental groups.[1]

Item	FOS level			
	Control	0.5%	1.0%	2.0%
BW (kg)	14.4 ± 3.8	14.4 ± 3.8	14.1 ± 3.8	14.0 ± 3.9
Feed Intake (g/d)	278 ± 52.9	277 ± 57.2	276 ± 57.3	276 ± 53.5

[1]Mean ± SD

Effect of FOS supplementation on fecal characteristics of dogs

The control dogs had significant lower ($P < 0.05$) fecal DM than the dogs supplemented with FOS at 0.5, 1.0 and 2.0% (28.1, 29.4, 30.1, and 30.8%, respectively). The dog supplemented with 2.0% FOS had the greatest amount ($P < 0.05$) of wet and dry fecal output (g/d), follow by 1.0 and 0.5% FOS, and the control groups, respectively (175.7, 161.2, 144.8 and 133.7 g/d, respectively and 47.2, 50.6, 53.4, and 61.3 g/d, respectively). The control group had greater significantly ($P < 0.05$) fecal scores than all FOS supplement groups (Table 7).

Table 7 The effect of FOS supplementation on fecal characteristics of dogs.[1]

Item	FOS level			
	Control	0.5%	1.0%	2.0%
Fecal DM (%)	28.1 ± 0.1[d]	29.4 ± 0.3[c]	30.1 ± 0.3[b]	30.8 ± 0.5[a]
Wet fecal output (g/d)	133 ± 18.7[d]	144 ± 20.1[c]	161 ± 15.3[b]	175 ± 14.6[a]
Dry fecal output (g/d)	47.2 ± 6.4[d]	50.6 ± 4.8[c]	53.4 ± 5.0[b]	61.3 ± 6.2[a]
Fecal score[2,3]	4	3	3	3

[1]Mean ± SD

[2] Scores based on the following scale: 1 = watery: liquid that can be poured; 2 = soft, unformed stool; assumes shape of container; 3 = soft, formed, a moist stool; 4 = hard, formed, dry stool; remain firm and soft; 5 = hard, dry pellets; small, hard mass.

[3] Fecal score was analyzed by Sigma statistics, as individuals Mean ± SE followed; control = 0.11, 0.5% FOS = 0.09, 1.0% FOS = 0.08, and 2.0% FOS = 0.09 respectively.

[a,b,c,d] Mean ± SD within the same row with different superscripts differ significant ($P < 0.05$)

Effect of FOS supplementation on nutrient digestibility of dogs

Fecal DM digestibility of the control dogs were greater ($P< 0.05$) than the dogs supplemented with FOS at 0.5, 1.0 and 2.0% (70.2, 68.9, 68.7 and 68.4%, respectively). No significant differences ($P> 0.05$) in fecal digestibility of CP, crude fat and OM were observed when compared between the treatment groups (table 8). The control dogs and dogs supplemented 0.5% with FOS had greater ($P < 0.05$) fecal CF digestibility than the dogs supplemented with 1.0 and 2.0% FOS (58.9%, 61.2%, 56.5%, and 56.4%, respectively) as shown in table 8.

Table 8 The effect of FOS supplementation on nutrient digestibility of dogs.[1]

Fecal digestibility, %	FOS Level			
	Control	0.5%	1.0%	2.0%
Dry Matter	70.2 ± 0.1[a]	68.9 ± 0.2[b]	68.7 ± 0.2[b]	68.4 ± 0.3[b]
Crude Protein	80.2 ± 3.8	79.5 ± 5.7	79.3 ± 5.6	79.1± 4.8
Crude Fat	93.9 ± 2.6	93.9 ± 1.8	93.9 ± 2.0	94.6 ± 1.6
Crude Fiber	58.9 ± 3.7[a]	61.2± 7.5[a]	56.5± 1.8[b]	56.4± 5.4[b]
Organic Matter	64.9 ± 4.5	63.9 ± 3.9	63.9 ± 3.9	62.2 ± 4.0

[1]Mean ± SD

[a,b] Mean ± SD within the same row with different superscripts differ ($P < 0.05$)

Effect of FOS supplementation on complete blood count (CBC) and plasma cholesterol of dogs

There were no significant differences of CBC between all FOS supplement and the control groups as shown in Table 9.

Table 9 The effect of FOS supplementation on complete blood count (CBC) of dogs.[1]

Item	FOS level			
	Control	0.5%	1.0%	2.0%
WBC, % 10^3 per µl	6.4 ± 1.6	6.6 ± 1.7	6.6 ± 1.3	6.7 ± 1.7
Lymphocytes, %per µl	34.0 ± 7.2	34.6 ± 6.9	34.7 ± 7.4	34.9 ± 7.5
Neutrophils, %per µl	55.5 ± 6.7	55.5 ± 7.9	56.1 ± 5.6	57.9 ± 5.2
Monocytes, %per µl	2.4 ± 0.6	2.9 ± 0.4	2.9 ± 0.7	3.0 ± 1.1
Eosinophils, %per µl	4.4 ± 3.2	4.0 ± 3.4	3.9 ± 3.1	4.5 ± 3.2
RBC, 10^6 per µl	4.8 ± 1.1	5.3 ± 1.1	5.4 ± 1.2	5.4 ± 1.3
Hemoglobin, g/dl	12.9 ± 2.0	13.8 ± 2.1	13.9 ± 2.9	14.1 ± 2.7
Hematocrit, %per µl	43.8 ± 8.6	44.6 ± 9.2	44.8 ± 10.1	45.6 ± 11.4

[1]Mean ± SD

Table 10 showed the comparison of plasma cholesterol concentration between the pre-test, test, and post-test periods.

No differences ($P > 0.05$) in concentration of plasma cholesterol was observed when compared between the treatment groups during the pre-test (10-d adaptation period), test (15-d test period), and post-test (10-d post test period) periods.

Table 10 The effect of FOS supplementation on plasma cholesterol of dogs during the pre-test, test, and post-test periods.[1]

Plasma Cholesterol (mg/dl)	FOS Level			
	Control	0.5%	1.0%	2.0%
10 D Before FOS	141 ± 71.4	140 ± 66.4	142 ± 85.2	142 ± 85.7
15 D FOS	142 ± 81.3	138 ± 63.4	122 ± 37.7	121 ± 30.3
10 D After FOS	142 ± 84.5	134 ± 62.5	123 ± 44.6	122 ± 41.9

[1] Mean ± SD

Effect of FOS supplementation on fecal microbial populations of dogs

Fecal microbial populations are reported in Table 11. This result was also conducted to compare the numbers of lactic acid bacteria and *E. coli* between the pre-test (10-d adaptation period), test (15-d test period), and post-test (10-d post test period) periods.

There were no differences ($P > 0.05$) in fecal LAB of all dogs during the 10-d adaptation period (no supplemental FOS). At test period, the dogs received 2.0% FOS have greatest ($P < 0.05$) populations of fecal LAB, follow by 1.0 and 0.5% FOS supplement, and the control groups (1.6×10^9, 2.5×10^9, 3.2×10^9, and 4×10^9 cfu/g, respectively) and also greater than the pre - test and post – test periods . The control dogs had lower ($P < 0.05$) fecal LAB than the dogs supplemented with 0.5, 1.0, and 2.0% FOS during the 10-d post test (1.6×10^9, 2.5×10^9, 2.5×10^9, and 3.2×10^9 cfu/g, respectively). These amounts had also greater than the amounts at the pre – test, but lesser than the amounts at the test period. There were also positive correlation (r = 0.9429) between number of fecal LAB (cfu $\log_{10}$/g) and the levels of FOS during test period as shown in Figure 7.

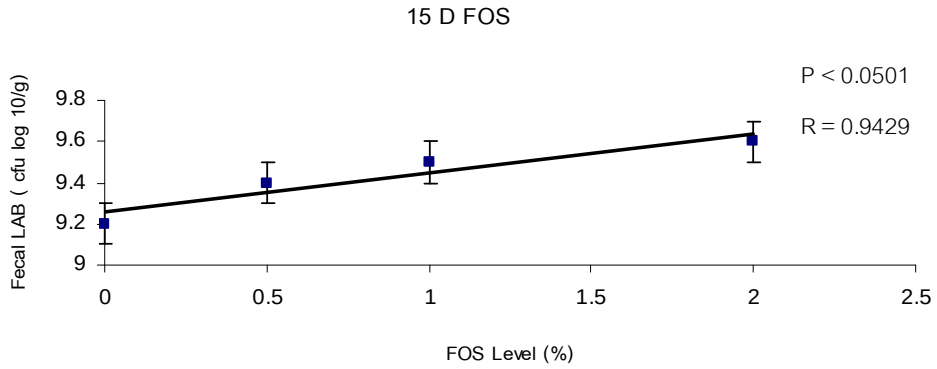

Figure 7. Linear correlations between numbers of fecal LAB and the levels of FOS.

No change was observed in fecal *E.coli* population of the control group at all periods. The *E.coli* population of all FOS supplement groups at test and post – test period were lower than the adaptation period. The dogs received 2.0% FOS had the lowest amount ($P < 0.01$) of fecal *E.coli* (cfu/g), follow by 1.0 and 0.5% FOS, and the control groups during test and post –test periods, respectively (2.0×10^5, 3.2×10^5, 4.0×10^5, and 1.0×10^6 cfu/g, respectively and 1.0×10^5, 1.6×10^5, 2.5×10^5, and 1.0×10^6 cfu/g, respectively). There were also an invert correlation (r = -0.9303) between number of fecal *E.coli* (cfu $\log_{10}$/g) and the levels of FOS during test period as shown in Figure 8.

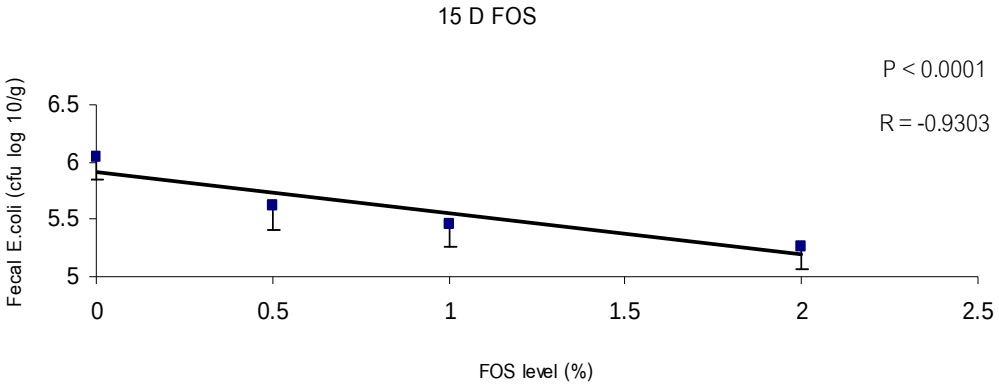

Figure 8. Linear correlations between numbers of fecal *E.coli* and the levels of FOS.

Table 11 The effect of FOS supplementation on fecal microbial populations of dogs during the pre-test, test, and post –test periods.[1]

Item	FOS Level			
	Control	0.5%	1.0%	2.0%
LAB (cfu[2]/g)				
10 D Before FOS	$1.6 \times 10^9 \pm 1.0$	$1.6 \times 10^9 \pm 1.0^{,B}$	$1.6 \times 10^9 \pm 1.0^{,B}$	$1.6 \times 10^9 \pm 1.0^{,B}$
15 D FOS	$1.6 \times 10^9 \pm 1.0^{c}$	$2.5 \times 10^9 \pm 0.5^{c, A}$	$3.2 \times 10^9 \pm 0.5^{b, A}$	$4.0 \times 10^9 \pm 0.6^{a, A}$
10 D After FOS	$1.3 \times 10^9 \pm 1.1^{b}$	$2.5 \times 10^9 \pm 0.5^{b, A}$	$2.5 \times 10^9 \pm 0.5^{b, A}$	$3.2 \times 10^9 \pm 0.5^{a, A}$
E. coli (cfu[2]/g)				
10 D Before FOS	$1.0 \times 10^6 \pm 4.8$	$1.0 \times 10^6 \pm 4.8^{,B}$	$1.0 \times 10^6 \pm 4.8^{,B}$	$1.0 \times 10^6 \pm 4.8^{,B}$
15 D FOS	$1.0 \times 10^6 \pm 4.8^{a}$	$4.0 \times 10^5 \pm 0.5^{b, A}$	$3.2 \times 10^5 \pm 0.7^{c, A}$	$2.0 \times 10^5 \pm 0.5^{d, A}$
10 D After FOS	$1.0 \times 10^6 \pm 4.8^{a}$	$2.5 \times 10^5 \pm 0.4^{b, A}$	$1.6 \times 10^5 \pm 0.4^{b, A}$	$1.0 \times 10^5 \pm 0.4^{c, A}$

[1] Mean ± SD

[2] cfu, colony-forming units

[a,b,c] Mean ± SD in the same row with different superscripts differ significant ($P < 0.05$)

[A,B] Mean ± SD in the same column with different superscripts differ significant ($P < 0.05$)

Effect of FOS supplementation on the percentages of phagocyte activity and index of phagocyte activity of dogs

There were no significant differences ($P > 0.05$) of the percentages of phagocyte activity (%PA) between all FOS supplement and the control groups at the pre-test period. During the test period, all dogs supplemented with FOS had the percentages of phagocyte activity (%PA) and index of phagocyte activity greater than both pre-test and post-test period as shown in Table12.

The dog supplemented with 2.0% FOS had the greatest ($P< 0.05$) of the percentages of phagocyte activity, followed by 1.0 and 0.5% FOS, and the control groups during the test and post – test periods, respectively (36.7, 34.1, 33.7, and 29.6%, and 33.2, 31.9, 31.4, and 29.3%, respectively).

There were no significant differences ($P > 0.05$) of index of phagocyte activity between all FOS supplement and the control groups at the pre-test period. During the test period, the dog supplemented with 2.0% FOS had the greatest ($P< 0.05$) index of phagocyte activity, followed by 1.0 and 0.5% FOS, and the control group, respectively (4.9, 4.5, 4.4, and 4.1, respectively). During the post-test period, dogs supplemented with 2.0% FOS had the greatest ($P < 0.05$) of IPA, followed by 1.0 and 0.5% FOS and the control group.

Table 12 The effect of FOS supplementation on the percentages of phagocyte activity (%PA) and index of phagocyte activity (IPA) of dogs during the pre-test, test, and post –test periods.[1]

Item	FOS Level			
	Control	0.5%	1.0%	2.0%
%PA				
10 D Before FOS	29.4 ± 2.1	29.5 ± 2.1^{,B}	29.5 ± 2.1^{,B}	29.7 ± 2.2^{,B}
15 D FOS	29.6 ± 2.0^{c}	33.7 ± 1.7^{b,A}	34.1± 1.8^{b,A}	36.7 ± 1.8^{a,A}
10 D After FOS	29.3 ± 2.1^{b}	31.4 ± 2.7^{b,B}	31.9 ± 1.6^{b,B}	33.2 ± 0.9^{a,B}
IPA				
10 D Before FOS	4.0 ± 0.4	4.1 ± 0.4^{,B}	4.1 ± 0.4^{,B}	4.3 ± 0.5^{,B}
15 D FOS	4.1 ± 0.4^{b}	4.4 ± 0.4^{a,A}	4.5 ± 0.6^{a,A}	4.9 ± 0.9^{a,A}
10 D After FOS	4.0 ± 0.4^{b}	4.2 ± 0.5^{ab,B}	4.2± 0.5^{ab,B}	4.4 ± 0.4^{a,B}

[1] Mean ± SD

^{a,b,c} Mean ± SD with the same row with different superscripts differ significant ($P < 0.05$)

^{A,B} Mean ± SD with the same column with different superscripts differ significant ($P < 0.05$)

As time passed, the number of engulfed *E.coli* (strain ATCC 25922) increased as shown in Figure 9.

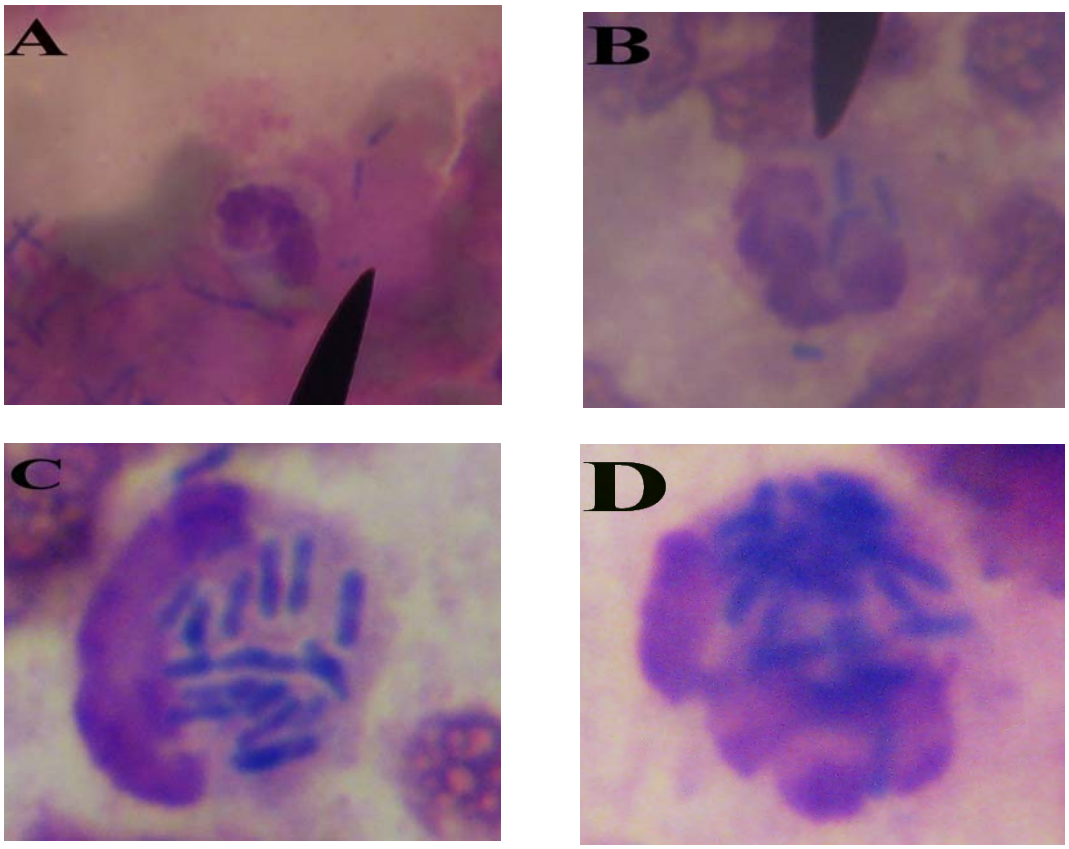

Figure 9. Microscopic photographs of phagocyte activity from the dogs supplemented with 2.0% FOS in this experiment (Magnification x 100); (A), (B), (C), (D); Canine granulocytes engulfing *E. coli* (strain ATCC 25922) after incubation for 0, 15, 30, and 60 min, respectively.

CHAPTER V

DISCUSSIONS

The Effect of FOS Supplementation on Body Weight and Feed Intake

The supplementation of FOS demonstrated no effect on body weight and feed intake. This result was similar to other studies (Houdijk et al., 1998; Olsen and Maribo, 1999; Swanson et al., 2002a, b). The difference might be associated with the different chemical structure of FOS (degree of polymerization), or length of oligosaccharides and presence of other fermentable sources especially non-starch polysaccharides in the diets.

The Effect of FOS Supplementation on Fecal Characteristics

The dogs supplemented with FOS at 0.5, 1.0, and 2.0% had greater ($P < 0.05$) fecal DM, wet fecal output and dry fecal output than the control dogs. These observations were surprising because FOS had been shown to increase wet fecal weight in the previous experiments (Deiz et al., 1997; Flickinger et al., 2003). But the results of the current experiment were in agreement with Hesta et al. (2000) who showed that the cats supplemented with FOS at 6 and 9% of diet resulted in the greater ($P < 0.05$) amount of wet fecal output and dry fecal output than the control cats. In addition, fecal scores of the current study decreased ($P < 0.05$) with higher levels of FOS supplementation. Similar result was reported by Schneeman (1999) who found increase dietary FOS caused deterioration in fecal quality with softer and water stools. The effect of FOS on stool consistency was caused by two reasons (Schneeman, 1999). First, the FOS molecules had a direct effect by increase fecal bulk regarding a portion that would remain undigested in the intestinal tract. Therefore, FOS provided volume to the feces via physical and osmotic effects. Second, FOS could make stool softness through the increased bacterial mass that occurred with more fermentation in the large intestine. With increased microbial fermentation, the bacteria in the gut multiply giving more bulk to the feces, causing a

laxative effect (Schneeman, 1999). Fermentation products can also act osmotically to increase fecal water content (Rowe et al., 1997). These two factors interplay to cause softer stools with higher levels of FOS in the diet. Although higher FOS levels had a significant effect on fecal quality, the level of 2.0% FOS in the diet is probably greater than would normally be used in a commercial dog food. As a result, the supplementation of FOS could prevent the constipation and provide fecal softness without diarrhea in dogs. However, these results contradicted to the reported by Swanson et al. (2002a) who found that supplementation of FOS did not influence neither fecal output nor fecal scores.

The Effect of FOS Supplementation on Fecal Microbial Population

FOS are not digested by small intestinal enzymes of mammals but beneficially affects the host by selectively stimulating the growth of some gut microflora and improves host health (Gibson and Roberfroid, 1995). Addition of these compounds into the diet of food animals was reported to be able to inhibit the growth of pathogenic microorganisms in the intestines of the animal (Gibson and Wang, 1994). One benefit of FOS supplement in the diet of adult dogs is to maintain a healthy microbial balance by selectively stimulate the growth of beneficial bacteria such as bifidobacteria and lactobacilli in the colon. These would maintain healthy gut ecology by increasing VFA production and decreasing pH of the intestinal content. Consequently, the growth of harmful bacteria such as *E. coli*, Salmonella, and many gram-negative microorganisms would be suppressed (Kruse et al., 1999). Roland (1995) reported that some bacterial species could utilize certain complex sugars for survival, whereas others could not.

The results of this experiment indicated that the supplement of FOS at all levels did have effect on the microbial populations by increase fecal LAB ($P < 0.05$) and decrease fecal *E.coli* ($P < 0.05$) numbers during the test period. Several studies in dogs found that diets supplemented with FOS increased the population of bifidobacteria and lactobacilli while decreased the number of *E. coli* (Russell, 1998; Howard et al., 2000; Swanson et al., 2002b; Flickinger et al., 2003; Twomey et al.,

2003). In chicken, Baily et al. (1991) observed that FOS had a positive effect on susceptibility of the animals to *Salmonella spp.* infections. In addition, lactobacillus strains have been reported to inhibit enteropathogenic *E. coli* from binding to intestinal cells (Bernet et al., 1994) and decrease enzyme (ß-glucuronidase, azoreductase, and nitroreductase) levels responsible for the production of carcinogenic compounds (Gorbach and Goldin, 1977). On the other hand, some studies in young pigs did not find the stimulating effects of FOS on lactobacilli and bifidobacteria in the gut (Orban et al., 1997; Bolduan et al., 1993).

This experiment was also conducted to compare the numbers of lactic acid bacteria and *E. coli* between the pre-test (10-d adaptation period), test (15-d test period), and post-test (10-d post test period) periods. No difference of fecal LAB numbers was observed during the pre-test period. For the test and post-test periods, the dogs supplemented with FOS at 0.5, 1.0, and 2.0% had greater ($P < 0.05$) fecal LAB numbers than the control dogs. At the post – test period, the dogs supplemented with 2.0% FOS had the greatest number ($P < 0.05$) of LAB when compared to other groups. The amounts of these LAB at the end of post-test period was greater than the amount at the amount at the end of pre-test, but lesser than the amount at the end of test period. In addition, fecal *E.coli* numbers decreased ($P < 0.05$) in all FOS supplement groups when compared to the control group during the test and post-test periods. The difference in microbial population between these periods could cause by FOS remaining in the GI tract and interfering the gut microbial ecology of the dogs.

The Effect of FOS Supplementation on Nutrient Digestibility

Little information is available regarding the effect of FOS on fecal nutrient digestibility in dogs. The results of this experiment showed that the dogs supplemented with FOS at 0.5, 1.0 and 2.0% had fecal DM digestibility significantly lower ($P < 0.05$) than the control dogs. No differences ($P >0.05$) in fecal digestibility of CP, crude fat and OM were observed. These results were similar to Swanson et al. (2002a), who reported no difference in total tract nutrient digestibility of CP and OM

in dogs supplemented with 2 g of scFOS/d. However, in this present study, the dogs supplemented with 1.0 and 2.0% FOS had lower ($P < 0.05$) fecal CF digestibility than dogs supplemented with 0.5% FOS and the control dogs. The inclusion of high levels of FOS could lead to loose stool, increased microbial fermentation (Niness, 1999), and reduced nutrient availability for the host (Diez et al., 1997; Strickling et al., 2000). Previous research done by Jørgensen et al. (1988) and Sutton (1992) reported that fermentable carbohydrates such as non-starch polysaccharide (NSP), resistant starch and oligosaccharides which would not be digested in the small intestine and would pass through to the hind gut to be the source of energy for microbial fermentation in the large intestine. The most important end products of microbial fermentation are volatile fatty acids and lactic acid that can make a contribution to the energy supply (Wenk, 1992). This can also happen even when the fecal digestibility of energy and nutrients are not improved. Houdijk et al. (1999) did not find any effect of either oligofructose or transgalactooligosaccharide (TOS) on fecal digestibility of CP and crude fat. Diez et al. (1997, 1998) found that the apparent protein digestibility in the dogs was reduced when 8% oligofructose and 2% sugar beet fiber were added to the diet. Whereas the addition of 7% inulin resulted in the reduced digestibility of the organic matter, crude protein and ether extract. The reduced apparent digestibility of protein could be the consequence of bacterial proliferation leading to a higher crude protein content in the feces.

The Effect of FOS supplementation on Plasma Cholesterol

The supplementation of FOS in this experiment did not significantly affect concentration of plasma cholesterol in adult dogs. However, such research done in human and some animal model suggested that FOS and inulin might lower serum total and LDL cholesterol (Yamashita et al., 1984). The research studies in dog are limit.

The reductions in serum cholesterol levels have been reported in rats consuming relatively high doses (10 g/d) and with long term feeding of oligofructose (Delzenne et al. 1995; Fiordaliso et al. 1995). Some previous studies have shown

that human consumed various levels of FOS (6 to 10 g/d) reduce serum cholesterol and LDL cholesterol. Yamashita et al (1984) found that consumption of FOS 8 g/d for 14 d significantly decreased fasting glycemia and total cholesterol concentrations in humans with non-insulin-dependent diabetes mellitus. FOS could enhance the production of propionate, a by product from microbial fermentation of FOS (Luo et al., 1996). Propionate was found to be an inhibitor of cholesterol synthesis by inhibiting both 3-hydroxy-3-methylglutaryl- CoA (HMG-CoA) synthase (Bush and Milligan, 1971) and HMG-CoA reductase (Rodwell et al., 1976). However, the mechanisms by which FOS can improve lipid metabolism are still not clear (Bornet et al., 1994) and fecal SCFA concentrations in this current study were not measured.

The Effect of FOS on Complete Blood Count, Phagocyte activity, and Index of Phagocyte activity

There were no significant different in the numbers of lymphocyte, neutrophil, monocyte, WBC, RBC, and hemoglobin concentrations. These results contradicted to the work done by Herich et al. (2002) who found that the weaned pigs supplemented with FOS had greater numbers of lymphocytes, leukocyte, neutrophils, and $CD4^+$ T cells as compared to the single administration of *Lactobacillus* and the control groups.

For the test period, the results of this experiment showed that the percentages of phagocyte activity (%PA) and index of phagocytic (IPA) activity of the dog supplemented with 2.0% FOS were the greatest (P <0.05), follow by 1.0 and 0.5% FOS, and the control groups, respectively. As time passed, the number of engulfed *E.coli* (strain ATCC 25922) increased in all treatment groups. These results were similar to Schiffrin et al. (1995) who reported the phagocyte activity of leukocytes increased from 40% to 85% in humans after consumption of fermented product containing FOS in humans. Herich et al. (2002) reported that the phagocyte activity of non-specific immune cells of piglets supplemented FOS at 1.5% revealed increase of %PA, IPA, phagocyte activity of neutrophils (%PANe), and Index of phgocytic activity of neutrophils (IPANe). Swanson. (2002) observed that prebiotics

altered the immune function of human and dogs by enhancing the number of lactic acid bacteria. The rise in intestinal lactic acid bacteria stimulated phagocyte activity (cellular immune response) and/or IgA secretion (humoral immune response) that would affect the colonization of pathogens, such as salmonella and rotavirus (Manning and Gibson, 2004). The beneficial effect of FOS on colonization of the gut by lactobacilli was presented not only by increased immune functions and counts of total lactobacilli in the feces but also in better utilization of the feed. However, LAB were not different among the treatments in this experiment. In addition, oligosaccharides exerted the same effect on the immune system as do probiotic bacteria using the signal system on the memory cell level in the lamina propria and the Peyer's patches (Manning and Gibson, 2004). Contrary to Roller et al. (2003) who found that prebiotics did not stimulate neutrophil and monocyte phagocytosis in rats.

This experiment was also designed to compare of %PA and IPA between the pre-test, test, and post-test periods. These results demonstrated that during the test period, the dog supplemented with 2.0% FOS had the greatest ($P < 0.05$) %PA, followed by 1.0 and 0.5% FOS, and the control groups, respectively. These percentages of the test period were also greater ($P < 0.05$) than the percentages at the pre-test and post-test periods. In the present study, the %PA increase as the LAB number increase. It is possible that there is a positive relationship between the number of LAB and %PA.

In conclusion, supplementation of FOS demonstrated some effects on the gut microflora as increase the population of beneficial LAB and decease the population of pathogenic *E.coli* in dogs. The supplementation of FOS at 2.0% into the commercial diet increased the population of beneficial gut microflora more ($P < 0.05$) than supplement at 0.5 and 1.0% FOS. The FOS supplement at 0.5, 1.0 and 2.0% did increase both wet and dry fecal mass and made the stool softener. However, supplementation of FOS at all levels did not affect fecal digestibility of crude protein, crude fat and organic matter and plasma cholesterol concentration.

The greatest amount of FOS supplement use in this study enhanced the immune function though the phagocyte activity regarding increase %PA and IPA. The results from this study suggested that the appropriate level of FOS supplementation in commercial dog foods could possibly be at 2.0%. Further studies could be continued either sc-FOS or long chain-FOS supplementation in commercial dog foods for long term feeding. It is also interesting to determine the remaining effect on microbial ecology, cholesterol levels, and immune systems. Since the FOS used in this study was kind of sc-FOS which could possibly have some effect difference from the long chain-FOS.

REFERENCES

AAFCO. 2000. Association of American Feed Control Officials: Official Publication. The Association, Atlanta, GA.

Alexander, J.W. 1995. Specific nutrients and the immune response. J. Nutr. 11: 229–232.

Andersson, H., Asp, N.G. and Bruce, A. 2001. Health effects of probiotics and prebiotics. J. Nutr. 45: 48-75.

AOAC. 1990. Method of analysis. Association of Official Analytical Chemists. Washington, DC.

Bailey, J. S., Blanken, L. C. and Cox, N. A. 1991. Effect of fructooligosaccharide on *Salmonella* colonization of the chicken intestine. J. Poult. Sci. 70: 2433-2438.

Bernet, M.F., Brassart, D., Neeser, J.R. and Servin, A. L. 1994. *Lactobacillus acidophilus* LA1 binds to cultured human intestinal cell lines and inhibits cell attachment and cell invasion by enterovirulent bacteria. Gut. 35: 4983-489.

Bornet, F.R.J. 1994. Undigestible sugars in food products. Am. J. Clin. Nutr. 59 (suppl): 763S-769S.

Bolduan, G., Beck. M. and Schubert, C. 1993. Effects of oligosaccharides on piglets. J. Anim. Nutr. 44: 21-27.

Bornet, F.R.J., Brouns, F., Tashiro, Y. and Duvillier, V. 2002. Nutritional aspects of short-chain fructooligosaccharide : natural occurrence, chemistry, physiology and health implications. Dig. Liver. Dis. 34 (Suppl.2): S111-20.

Bush, R.S. and Milligan, L.P. 1971. Study of the mechanism of inhibition of ketogenesis by propionate in bovine liver. Can. J. Anim. Sci. 51: 121-7.

Chadwick, R.W., George, S. and Claxton, L.D. 1992. Role of the gastrointestinal mucosa and microflora in the bioactivation of dietary and environmental mutagens and carcinogens. Drug. Metab. Rev. 24: 425-492.

Chatherine, J. 2008. Biosynthesis of cholesterol. Available from: http://www.cellml.org/examples/repository/qualitative/metabolic_models_doc.html.

Crittenden, R.G. and Playne, M.J. 1996. Production, properties and applications of food-grade oligosaccharides. Trends in Food. Sci. and Tech. 7: 353-360.

Delves, P.J. and Roitt, I.M. 2000a. The immune system: first of two parts. New. Eng. J. Med. 343: 37-49.

Delves, P.J. and Roitt, I.M. 2000b. The immune system: second of two parts. New. Eng. J. Med. 343: 108-117.

Delzenne, N., Aertssens, J., Verplaetse, H., Roccaro, M. and Roberfroid, M. 1995. Effect of fermentable fruto-oligosaccharides on mineral, nitrogen and energy digestive balance in rat. Life Sci. 57: 1579-1587.

Diez, M., Hornick, J., Baldwick, P., Istasse, L., 1997. Influence of a blend of fructo-oligosaccharides and sugar beet fiber on nutrient digestibility and plasma metabolite concentrations in healthy beagles. Am. J. Vet. Res. 58: 1238–1242.

Estrada, A., Drew, M.D. and Van Kessel,. A. 2001. Effects of the dietary supplementation of fructooligosaccharides and *bifidobacterium longum* to early weaned pigs on performance and fecal bacterial populations. Can. J. Anim. Sci. 81:141-148.

Fiordaliso, M.F., Kok, N., Desagar, J.P., Goethals, F., Deboyser, D., Roberfroid, M. and Deizenne, N. 1995. Dietary oligofructose lowers triacylglycerols, phospholipids and cholesterol in serum and very low density lipoproteins of rat. Lipids. 30: 163 - 167.

Flickinger, E.A. 2003. Oligosaccharides as functional foods : can we improve gut health? In Lyons, T.P. and K.A. Jacques,. eds. Biotechnology in the Feed Industry. Proc. Alltech's 19th Ann. Symp. Nottingham University Press. Loughborough, U.K.: 345-353.

Gebblink, G.A.R., Sutton, A.L., Richert, B.T., Patterson, J.A., Neilsen, J., Kelly, D.T., Verstegen, M.W.A., Williams, B.A., Bosch, M., Cobb, M., Kendall, D.C., Decamp, S. and Bowers, K. 1999. Effect of Addition of Fructooligosaccharide (FOS) and Sugar Beet Pulp to Weanling Pig Diets on Performance. Micro. Intes. Health: 53-59.

Gibson, G.R. and Wang, X. 1994. Inhibitory effects of bifidobacteria on other colonic bacteria. J. Appl. Bacteriol. 77: 412-420.

Gibson, G.R. and Roberfroid, M.B. 1995. Dietary modulation of the human colonic microbiota : Introducing the concept of prebiotics. J. Nutr. 125: 1401-1412.

Gibson, G.R., Beatty, E.R., Wang, X. and Cummings, J.H. 1995. Selective stimulation of *bifidobacteria* in the human colon by oligofructose and inulin. Gastroenterology. 108: 975-982.

Gibson, G. R., Rastall, R. A. and Robertfroid, M. B. 1999. Prebiotcs. In GR Gibson and MB Robertfroid (ed.), Colonic Microbiota, Nutrition and health, pp. 101. The Netherlands: Kluwer academic publishers.

Gorbach, S.L., Chang, T. and Goldin, B. 1987. Successful treatment of relapsing *Clostridium difficile* colitis with Lactobacillus GG. Lancet 2: 1519.

Goust, J.M. and Bierrer, B. 1993. Cell-mediated immunity. Immunol. Series 58: 187-212.

Han, I. K., Kim, J.D., Lee, J.H., Lee, S.C., Lee, K.K. and Lee, J.C. 1984. Studies on growth promoting effects of probiotics III. The effects of *Lactobacillus sporogenes* in the growing performance and the chage in microbial flora of the feces and intestinal contents of the broiler chicks. Kor. J. Anim. Sci. 26: 150 – 157.

He, G., Baidoo, S.K., Yang, Q., Golz, D., Tungland, B. 2002. Evaluation of chicory inulin extracts as feed additive for early-weaned pigs. J. Anim. Sci. 80 (S1):81.

Herich, R., Révajová, V., Levkut, M., Bomba, A.,Nemcová, R., Guba, P. and Gancarciková, S. 2002. The effect of *Lactobacillus paracasei* and Raftilose P95 upon the Non-specific Immune Response of Piglets. Food. Agric. Immunol. 14: 171-179.

Hesta, M., Janssens, G. P. J., Debraekeleer, J. and De Wilde, R. 2002. The effect of oligofructose and inulin on faecal characteristics and nutrient digestibility in healthy cats. J. Anim. Physiol. &. Anim. Nutr. 85: 135-141.

Hidaka, H., Eida, T., Takizawa, T., Tokunaga, T., and Tashiro, Y. 1986. Effects of fructooligosaccharides on intestinal flora and human health. Bifido. Microflora. 5: 37-50.

Hond, E.D., Geypens, B. and Ghoos, Y. 2002. Effect of high performance chicory inulin on constipation. J. Nutr . Res. 20: 731-736.

Hosono, A., Kitazawa, H. and Yamaguchi, T. 1997. Antimutagenic and antitumour activities of lactic acid bacteria. In Probiotics 2: Applications and Practical Aspects ed. Fuller, R. London: Chapman & Hall.:89–132.

Houdijk, J. G. M., Bosch, M.W., Tamminga, S., Verstegen, M.W.A., Berenpas, E.B. 1999. Apparent ileal and total-tract nutrient digestion by pigs as affected by dietary nondigestible oligosaccharides. J. Anim. Sci. 77:148-158.

Howard, M.D., Kerley, M.S., Sunvold, G.D. and Reinhart, G.A. 2000. Source of dietary fiber fed to dogs affects nitrogen and energy metabolism and energy metabolism and intestinal microflora populations. J. Nutr. Res. 20: 1473-1484.

Hussein, S.H., Campbell, J.M., Bauer, L.L, Fahey, G.C., JR., Hogarth, A.J.C.L., Wolf, B.W. and Hunter, D.E. 1998. Selected Fructooligosaccharide Composition of Pet-Food Ingredients. Am.J. Nutr. 128: 2803-2805.

ISO- 4831. 1991. Microbiology-General guidance for enumeration of presumptive *Escherichia coli* – Most probable Number technique.: 70-83.

ISO- 15214. 1998. Microbiology of Food Animal Feeding stuffs-Horizontal Method for the enumeration of Mesophilic Lactic Acid Bacteria-Colony-Count Technique at 30 $^{\circ}$C. Microbiology.: 23-41.

Jenkins, D.J.A., Kendall, C.W.C. and Vuksan, V. 1999. Inulin, Oligofructose and Intestinal Function. J. Nutr. 129: 1431-1433.

Jørgensen, H. and Just. A. 1988. Effect of different dietary components on site of absorption/ site of disappearance of nutrients. Proc. 4th Symp. on Digestive Physiology in the Pig, Jablonna.: 230-239.

Kamath, P.S., Phillips, S.F. and Zinsmeister, A.R. 1998. Short-chain fatty acids stimulate ileal motility in humans. Gastroenterology. 95: 1496-1502.

Kleessen, B., Sykura, B., Zunft, H.J. and Blaut, M. 1997. Effects of inulin and lactose on fecal microflora, microbial activity and bowel habit in elderly constipated persons. Am. J. Clin. Nutr. 65: 1397-1402.

Kok, N., Roberfroid, M. and Deizenne, N. 1996a. Involvement of lipogenesis in the lower VLDL secretion induced by oligofructose in rats. Brit. J. Nutr. 76: 881-890.

Kok, N., Roberfroid, M. and Deizenne, N. 1996b. Dietary oligofructose modifies the impact of fructose on hepatic triacylglycerol metabolism. Metabolism. 458: 1547-1550.

Kok, N. N., Roberfroid, M. B. and Delzenne, N. M. 1996. Involvement of lipogenesis in the lower VLDL secretion induced by oligofructose in rats. Br. J. Nutr. 76: 881–890.

Kruse, H.P., Kleessen, B. and Blaut, M. (1999) Effects of inulin on faecal bifidobacteria in human subjects. Br. J. Nutr. 82: 375–382.

Lewis, D. H. 1993. Nomenclature and diagrammatic representation of oligomeric fructans-a paper for discussion. New. Phytol. 124: 583-594.

Luo, J., Rizkalla, S.W., Alamowitch, C., Boussairi, A., Blayo, A., Barry, J.L., Laffifle, A., Ouyon, F., Bornet, F.R.J. and Slama, V.S. 1996. Chronic consumption of short-chain fructooligosaccharides by healthy subjects decreased basal hepatic glucose production but had no effect on insulin-stimulated glucose metabolism. Am. J. Clin. Nutr. 63: 939–945.

Manhart, N., Spitter, A., Bergmeister, H., Mittlböck, M. and Roth, E. 2003. Influence of Fructooligosaccharides on Peyer's Patch Lymphocyte Numbers in Healthy and Endotoxemic Mice. J. Nutr. 19: 657-660.

Manning, T.S., and G.R. Gibson. 2004. Prebiotics. Best Pract. Res. Clin. Gastroenterol. 18:287-298.

Nakamura, Y., Nosaka, M., Suzuki, S., Nagafuchi, T., Takahashi, T., Yajima, N., Takenouchi-Ohkubo, N., Iwase, T. 2004. Dietary fructooligosaccharides up-regulate immunoglobulin A response and polymeric immunoglobulin

receptor expression in intestines of infant mice. Clin. Exp. Immunol.137: 52–58.

National Research Council. 2006. Nutrient requirements of dogs and cats. The national academics press. Washington, D.C: 398.

Nilsson, U., Oste, R., Jagerstad, M. and Birkhead, D. 1998. Cereal fructans : *In vitro* and *In vivo* studies on availability in rats and humans. J. Nutr. 118: 1325-1330.

Niness, K.R., 1999. Inulin and oligofructose: what are they? J. Nutr. 129: 1402S–1406S.

Oku, T., Tokunaga, T. and Hosoya, N. 1984. Non digestibility of a new sweetener, “Neosugar”, in the rat. J. Nutr. 114: 1574-1581.

Olsen L.E. and Maribo, H. 1999. Company products for feed for piglets-Igalac, FUT and Bokashi F. In Danish Slaughterhouse Report #443. National Committee for Pig Breeding, Health and Production, Denmark.: 431-465.

Orban, J. I., Patterson, J. A., Sutton, A. L., and Richards, G. N. 1997. Effect of sucrose thermal oligosaccharide caramel (STOC) on growth performance and intestinal microbial populations in growing pigs. Proc. 6th symposium on Digestive Physiology in Pigs. Bad Doberan, Germany. EAAP Publ. 80: 280-282.

Patil, A.R., Carrion, P.A. and Holmes, A.K. 2001. Effect of chicory supplementation on fecal microflora of cat. F.A.S.E.B.J. 15: 288.

Pierre, F., Perrin, P., Champ, M., Bornet, F., Meflah, K. and Menanteau, J. 1997. Short-chain fructo-oligosaccharides reduce the occurrence of colon tumours and develop gutassociated lymphoid tissue in mice. Can. Res. 57: 225–228.

Propst, E.L., Flickinger, E.A., Bauer, L.L., Merchen, N.R. and Farhey, G.C., Jr. 2003. A dose-response experiment evaluating the effects of oligofructose and inulin on nutrient digestibility, stool quality, and fecal protein catabolites in healthy adult dogs. J. Anim. Sci. 81: 3057-3066.

Rastall, R.A. 2004. Bacterial in the gut: friends and foes and how to alter the balance. Waltham international science symposium. Am. J. Nutr. 134: 2022-2026.

Reimer, R. A., and McBurney, M. I. 1996. Dietary fiber modulates intestinal proglucagon messenger ribonucleic acid and postprandial secretion of glucagon-like peptide-I and insulin in rats. Endocrinology. 137: 3948–3956.

Reimer, R. A., Thomson, A. B. R. Rajotte, R. V. Basu, T. K. Ooraikul, B. and McBurney, M. I. 1997. A physiological level of rhubarb fiber increases proglucagon gene expression and modulates intestinal glucose uptake in rats. J. Nutr. 127: 1923–1928.

Robertfroid, M. B. 1993. Dietary fiber, inulin and oligofructose: a review comparing their physiological effects. Crit. Rev. food sci. nutr. 33,103-48.

Roberfriod, M.B. 1998. Prebiotics and synbiotics: concepts and nutritional properties. Br. J. Nutr. 80: 197-202.

Rodwell, V.W., Nordstrom, J.L. and Mitschelen, J.J. 1976. Regulation of HMG CoA reductase. Adv. Lipid. Res. 14: 1-74.

Roediger, W.E.W. 1982. Utilization of nutrients by isolated epithelial cells of the rat colon. Gastroenterology. 83: 424-429.

Roller, M., Rechkemmer, G and Watzl, B. 2003. Prebiotic inulin enriched with oligofructose in combination with the probiotics Lactobacillus rhamnosus and Bifidobacterium lactis modulates intestinal immune function in rats. J. Nutr. 134: 153–156.

Roland, N., Nugon-Baudon, L., Andrieux, C. and Szylit, O. 1995. Comparative study of the fermentative characteristics of inulin and different types of fiber in rats inoculated with a human whole fecal flora, Br. J.Nutr. 74(2): 239–249.

Rowe, J. B. 1997. Acidic gut syndrome: is it a problem for animals and humans? In: Corbett, J. L., Choct, M., Nolan, J. V., Rowe, J. B. (Eds.), Recent Advances in Animal Nutrition in Australia. University of New England, Armidale, NSW, pp. 47-54.

Royal canin. 2006. Fecal score. Available from: http://www.royalcanin.us/focus special edit.html.

Russell, T.J. 1998. The effect of natural sources of non-digestible oligosaccharides on the fecal microflora of the dog and effects on digestion. Friskies R&D center/ Missouri, copyright © Friskies-Europe: 157.

Saroj K. 2004. Feeds and feeding nonruminants. Khon Kaen university. Vol 2. Krang Naa Vithaya Publication. 699p.

SAS Institute Inc., 2002. SAS/STAT User's Guide: Statistics. SAS Institute, Cary, NC.

Schiffrin, E.J., Brassart, D., Servin, A., Rochat, F. and Donnet-Hughes, A. 1997. Immune modulation of blood leukocytes in humans by lactic acid bacteria: criteria for strain selection. Am. J. Clin. Nutr. 66: 515S-520S.

Schley, P.D. and Field, C.J. 2002. The immune-enhancing effects of dietary fibres and prebiotics. Br. J. Nutr. 87: 221-230.

Schneeman, B.O., 1999. Fiber, inulin and oligofructose: similarities and differences. J. Nutr. 129, 1424S–1427S.

Shim, S.B., Verstegen, M.W.A. and Verdonk, J.M.A.J. 2005. Prebiotics and probiotics or synbiotics in the diets of newly weans pigs – with regard to gut fermentation. The Asian-Aust. J.Anim. Sci: 42-66.

Spady, D.K., Woollett, L.A., and Dietschy, J.M. 1993. Regulation of plasma LDL-cholesterol levels by dietary cholesterol and fatty acids. Ann. Rev. Nutr. 13: 355-381.

Sparkes, A.H., Papasouliotis, K., Sunvold, G., Werrett, G., Gruffydd-Jones, E.A., Egan, K., Gruffydd-Jones, T.J. and Reinhart, G. 1998. Effect of dietary supplementation with fructooligosaccharides on fecal flora of healthy cats. Am.J.Vet. Res. 59: 436-440.

Strickling, J.A., Harmon, D.L., Dawson, K.A., Gross, K.L., 2000. Evaluation of oligosaccharide addition to dog diets: influences on nutrient digestion and microbial populations. Anim. Feed Sci. Technol. 86: 205–219.

Swanson, K.S. 2002. Prebiotics and probiotics: impact on gut microbial populations, nutrient digestibilities, fecal protein catabolite concentrations and immune functions of humans and dogs. Diss. Abstr. Int. 63:746.

Swanson, K.S., Grieshop, C.M., Flickinger, E.A., Bauer, L.L., Healy, H.P., Dawson, K.A., Merchen, N.R. and Fahey, G.C., Jr. 2002a. Supplemental fructooligosaccarides (FOS) and mannanoligosaccharides (MOS) influence immune function, ileal and total tract digestibilities, microbial populations and concentrations of fecal protein catabolites in the large bowel of dogs. J. Nutr. 132: 980-989.

Swanson, K.S., Grieshop, C.M., Flickinger, E.A., Bauer, L.L., Chow, J., Wolf, B.W., Garleb, K.A. and Fahey, G.C., Jr. 2002b. Fructooligosaccharides and *Lactobacillus acidophilus* modify gut microbial populations, total tract nutrient digestibilities and fecal protein catabolite concentrations in healthy adult dogs. J. Nutr. 132: 3721-3731.

Sutton, A. L., Mathew, A. G., Scheidt, A. B., Patterson, J. A., and Kelly, G. T. 1992. Effects of carbohydrate sources and organic acids on intestinal microflora and performance of the weaning pig. Proc. 5th symposium on Digestive Physiology in the Pig, Wegeningen.: 422-427.

Tokunaga, T., Oku, T. and Hosoya, N. 1989. Ultilization and excretion of a new sweetner fructooligosaccharide (Neosugar) in rats. J. Nutr. 119: 553-559.

Twomey, L.N., Pluske, J.R., Rowe, J.B., Choct, M., Brown, W. and Pethick, D.W. 2003. The effects of added fructooligosaccharide (Raftilose® P 95) and inulinase on faecal quality and digestibility in dogs. Anim. Feed. Sci.Technol. 108: 83-93.

Van, L. J, Coussement, P., De Leenheer, L., Hoebregs, H., and Smits, G. 1995. On the presence of inulin and oligofructose as natural ingredients in the western diet. Cri. Rev. Food. Sci. Nutr. 35: 525-552.

Voet, D. And Voet., J.G. 1995. Biochemistry. John Wiley & Sons, Inc. 2nd Edition: 1361.

Weigang. 2004. Phagocytosis activity. Available from: http://diverge.hunter.cuny.edu/~weigang/Lecture-syllabus.html.

Weir, D.M. 1978. Handbook of Experimental Immunology. Vol.2 Cellular Immunology. 3rd. Oxford; Blackwell Scientific Publication: 46.1-46.21.

Wenk, C. 1992. Enzymes in the nutrition of monogastric farm animals. Proc. 8th symposium on "Biotechnology in the Feed Industry", Kentucky.: 205-218.

Williams, C.H., David, D.J. and Lismaa, O. 1962. The determination of Chromic oxide in feces samples by atomic absorption spectrophotometry. J. Agr. Sci. 59:381.

Williams, C.M. and Jackson, K.G. 2002. Inulin and oligofructose: effects on lipid metabolism from human studies. Br. J. Nutr. 87: 261-264.

Yamashita, K., Kawai, K. and Itakura, M. 1984. Effect of fructo-oligosaccharides on blood glucose and serum lipids in diabetic subjects. J. Nutr. Res. 4: 961-966

Yun, J.W. 1996. Fructooligosaccharides-occurrence, preparation, and application. Enz. Microb. Technol. 19: 107-117.

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