ผลของไทโลซิน โปรไบโอติก และแลคทูโลส ต่อระดับเอนไซม์ที่ย่อยน้ำตาลโมเลกุลคู่ และระดับกรดไขมันสายสั้น ภายในลำไส้ของหนูแรท และหนูแรทที่ได้รับเชื้อ อี. โคไล

นางสาว ทิพย์มนต์ ใยเกษ

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรมหาบัณฑิต
สาขาวิชาสรีรวิทยาการสัตว์ ภาควิชาสรีรวิทยา
คณะสัตวแพทยศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย
ปีการศึกษา 2542

ISBN 974-334-756-9

EFFECTS OF TYLOSIN, PROBIOTIC AND LACTULOSE ON DISACCHARIDASE ACTIVITIES AND INTESTINAL SHORT-CHAIN FATTY ACIDS PRODUCTION OF RATS AND RATS ADMINISTERED WITH *E. coli* SUSPENSION

Miss Tipmon Yaigate

A Thesis Submitted in Partial Fulfillment of the Requirements
for the Degree of Master of Science in Animal Physiology
Department of Physiology
Faculty of Veterinary Science
Chulalongkorn University
Academic Year 1999

ISBN 974-334-756-9

Thesis title	Effects of tylosin, probiotic and lactulose on disaccharidase activities and intestinal short-chain fatty acids production of rats and rats administered with *E. coli* suspension
By	Miss Tipmon Yaigate
Department	Physiology
Thesis Advisor	Assistant Professor Kris Angkanaporn, Ph.D.

Accepted by the Faculty of Veterinary Science, Chulalongkon University in Partial Fulfillment of the Requirements for the Master's Degree.

.....Dean of Faculty of Veterinary Science
(Professor Narongsak Chaiyabutr, Ph.D.)

Thesis Committee

.....Chairman
(Associate Professor Dungnarumon Prachankhadee, Ph.D.)

.....Thesis Advisor
(Assistant Professor Kris Angkanaporn, Ph.D.)

.....Member
(Professor Narongsak Chaiyabutr, Ph.D.)

.....Member
(Associate Professor Chollada Buranakal, Ph.D.)

.....Member
(Associate Professor Jiroj Sasipreeyajan, Ph.D.)

ทิพย์มนต์ ใยเกษ : ผลของไทโลซิน โปรไบโอติก และแลคทูโลส ต่อระดับเอนไซม์ที่ย่อยน้ำตาลโมเลกุลคู่ และระดับกรดไขมันสายสั้น ภายในลำไส้ของหนูแรท และหนูแรทที่ได้รับเชื้อ อี.โคไล. (EFFECTS OF TYLOSIN, PROBIOTIC AND LACTULOSE ON DISACCHARIDASE ACTIVITIES AND INTESTINAL SHORT-CHAIN FATTY ACIDS PRODUCTION OF RATS AND RATS ADMINISTERED WITH *E.coli* SUSPENSION) อ. ที่ปรึกษา : ผศ.น.สพ.ดร. กฤษ อังคนาพร, 63 หน้า. ISBN 974-334-756-9.

คำสำคัญ: โปรไบโอติก เอนไซม์ย่อยน้ำตาลโมเลกุลคู่ กรดไขมันสายสั้น

การวิจัยครั้งนี้มีวัตถุประสงค์เพื่อศึกษาผลของไทโลซิน โปรไบโอติก และ แลคทูโลส ต่อระดับเอนไซม์ที่ย่อยน้ำตาลโมเลกุลคู่ และระดับกรดไขมันสายสั้น ภายในลำไส้ของหนูแรท และหนูแรทที่ได้รับเชื้อ อี.โคไล โดยหนูทดลองพันธุ์วิสตาร์ เพศผู้ อายุ 3 สัปดาห์ จำนวน 150 ตัว ถูกแบ่งออกเป็น 2 การทดลอง การทดลองละ 75 ตัว การทดลองที่ 1 เลี้ยงหนูในห้องทดลอง การทดลองที่ 2 ป้อนเชื้อ อี.โคไล (10^8 โคโลนี ต่อ มล.) 1 มล. เป็นเวลา 5 วันให้หนูทดลองแต่ละตัว และเลี้ยงในห้องทดลอง แต่ละการทดลองแบ่งเป็น 5 กลุ่ม กลุ่มละ 15 ตัว กลุ่มที่ 1 หนูได้รับน้ำ 1 มล. เป็นกลุ่มควบคุม กลุ่มที่ 2 หนูได้รับสารละลายไทโลซิน ทราเทรท (0.1 มก. ต่อ 1 มล.) กลุ่มที่ 3 หนูได้รับสารละสายโปรไบโอติก (50 มก.ต่อ 1 มล.) กลุ่มที่ 4 หนูได้รับสารละลายแลคทูโลส (667 มก. ต่อ 1 มล.) และกลุ่มที่ 5 หนูได้รับโปรไบโอติกละลายในสารละลายแลคทูโลส (50 มก. ต่อ 1 มล.) หนูทดลองทุกตัวได้รับสารทดลองทางเข็มป้อนอาหารวันละ 1 ครั้งเป็นเวลา 14 วัน ชั่งน้ำหนักอาหารที่กินทุกวัน ในวันที่ 0 วันที่ 7 และ วันที่14 ของการทดลองชั่งน้ำหนักหนูทดลองทุกตัว และสุ่มหนูทดลองมากลุ่มละ 5 ตัว ทำการเก็บตัวอย่างเลือดดำนำมาตรวจวัดค่าเม็ดเลือดแดงอัดแน่น ฮีโมโกลบิน และนับจำนวนเม็ดเลือดขาว เก็บตัวอย่างอาหารที่ย่อยในลำไส้นำมาวัดระดับของกรดไขมันสายสั้นโดยวิธีแก๊สโครมาโตรกราฟฟี่ เก็บตัวอย่างเยื่อบุผนังลำไส้เล็กส่วนกลางตอนบนและตอนล่าง และลำไส้เล็กส่วนปลายนำมาวัดระดับเอ็นไซม์ที่ย่อยน้ำตาลโมเลกุลคู่โดยการตรวจวัดปริมาณกลูโคสที่เกิดจากการย่อยน้ำตาลโมเลกุลคู่และตัวอย่างเยื่อบุผนังทางเดินลำไส้เล็กส่วนกลางตอนบนและตอนล่าง ลำไส้เล็กส่วนปลาย ไส้ตัน และลำไส้ใหญ่นำมาตรวจวัดปริมาณดีเอ็นเอ และอาร์เอ็นเอ

จากการทดลองพบว่า การเจริญเติบโตและค่าโลหิตวิทยาของหนูทดลองที่ได้รับสารทดลองไม่แตกต่างจากกลุ่มควบคุม ในทั้ง 2 การทดลอง ในการทดลองที่ 1 พบว่าโปรไบโอติกเพิ่มระดับความเข้มข้นของกรดอะซิติก ในลำไส้เล็กของหนูทดลอง ($P<0.05$) ส่วนในลำไส้ใหญ่สารละลายไทโลซิน ทาเทรท มีผลลดระดับความเข้มข้นของกรดวาเรอริก ($P<0.05$) ระดับเอนไซม์ย่อยน้ำตาลมอลโตสในลำไส้เล็กส่วนปลายของหนูที่ได้รับแลคทูโลส และหนูที่ได้รับสารละลายโปรไบโอติกในแลคทูโลส เพิ่มขึ้นอย่างมีนัยสำคัญ ($P<0.05$) ในขณะที่เอนไซม์ย่อยน้ำตาลแลคโตสของหนูทดลองที่ได้รับสารละลายไทโลซิน ทาเทรท โปรไบโอติก แลคทูโลสและสารละลายโปรไบโอติกในแลคทูโลส เพิ่มขึ้นอย่างมีนัยสำคัญ ($P<0.05$) สารละลายไทโลซิน ทาเทรท และโปรไบโอติก มีผลเพิ่มระดับเอนไซม์ย่อยน้ำตาลแลคโตสในบริเวณลำไส้เล็กส่วนปลายอย่างมีนัยสำคัญ ($P<0.05$) ในการทดลองที่ 2 พบว่าโปรไบโอติกมีผลเพิ่มระดับความเข้มข้นของกรดอะซิติก กรดโปรปิโอนิก และกรดบิวทิริกในลำไส้ใหญ่อย่างมีนัยสำคัญ ($P<0.05$) และยังพบว่าแลคทูโลสมีผลเพิ่มระดับความเข้มข้นของกรดโปรปิโอนิก และกรดบิวทิริกอย่างมีนัยสำคัญ ($P<0.05$) แต่สารทดลองเหล่านี้ไม่มีผลทำให้ระดับเอนไซม์ที่ย่อยน้ำตาลโมเลกุลคู่ดีขึ้น

จากการศึกษาครั้งนี้สรุปได้ว่า การให้สารละลายไทโลซิน โปรไบโอติก หรือแลคทูโลส มีผลเพิ่มระดับเอนไซม์ย่อยน้ำตาลมอลโตส และเอนไซม์ย่อยน้ำตาลแลคโตสของหนูที่เลี้ยงในสภาวะปกติ และพบว่ามีผลเด่นชัดต่อการเปลี่ยนแปลงระดับความเข้มข้นของกรดไขมันสายสั้นในลำไส้ใหญ่ โดยโปรไบโอติก แลคทูโลส และโปรไบโอติกละลายในสารละลายแลคทูโลส มีผลเพิ่มระดับความเข้มข้นของกรดอะซิติก กรดโปรปิโอนิก กรดบิวทิริก และกรดวาเรอริก ในลำไส้ใหญ่ของหนูที่ได้รับเชื้อ อี.โคไล ในขณะที่สารละลายไทโลซิน ทาเทรท มีผลลดระดับความเข้มข้นของกรดไขมันสายสั้นในลำไส้ใหญ่ของหนูที่เลี้ยงในสภาวะปกติ และหนูที่ได้รับเชื้อ อี.โคไล

ภาควิชา สรีรวิทยา ลายมือชื่อนิสิต.....

สาขาวิชา สรีรวิทยาการสัตว์ ลายมือชื่ออาจารย์ที่ปรึกษา.....

ปีการศึกษา 2542

3970627731 : MAJOR ANIMAL PHYSIOLOGY
KEY WORDS : PROBIOTIC, DISACCHARIDASE, SHORT-CHAIN FATTY ACID
TIPMON YAIGATE : EFFECTS OF TYLOSIN, PROBIOTIC AND LACTULOSE ON DISACCHARIDASE ACTIVITIES AND INTESTINAL SHORT-CHAIN FATTY ACIDS PRODUCTION OF RATS AND RATS ADMINISTERED WITH *E.coli* SUSPENSION. THESIS ADVISOR: ASSISTANT PROFESSOR KRIS ANGKANAPORN, D.V.M., M. Sc., Ph.D.; 63 pp. ISBN 974-334-756-9.

The objective of this investigation was to study the effect of oral administration of tylosin, probiotic and lactulose on disaccharidase activities and short-chain fatty acids (SCFAs) concentrations in the intestinal contents of rats and rats administered with *E.coli* suspension. One hundred and fifty, three-week-old, male Wistar rats were divided into two experiments with 75 rats each. In the experiment 1, the rats were reared in a conventional condition. The rats in the experiment 2 were administered with 1 ml of *E.coli* (10^8 CFU/ml) suspension for 5 days prior to the experiment and reared in a conventional condition. There were 5 treatments with 15 rats in each experiment. The treatments were C:control (1 ml of water), T:tylosin tartrate solution (0.1 mg/ml), P:probiotic (50 mg/ml), L:lactulose (667 mg/1 ml) and PL:combination of P and L (50 mg of P/ml of L). All rats were given each treatment using feeding tube once daily for 14 days. The feed intake was measured everyday. At days 0, 7 and 14 of the experiment, the rats were weighed and 5 rats in each treatment were sacrificed. Blood samples were collected for examining the blood picture values (hematocrit, hemoglobin, and white blood cell). The intestinal contents were collected for SCFAs determination using gas chromatography. Mucosal tissue samples from the proximal jejunum (PJ), the distal jejunum (DJ) and the ileum (I) were collected for the determination of disaccharidase activities by measuring the glucose production from disaccharides digestion. Those mucosal samples, caecum (CE) and colorectum (CR) were collected for the determination of total deoxyribonucleic acid (DNA) and ribonucleic acid (RNA) concentrations.

The growth performance and blood picture values were not affected by the treatments in both experiments. In the experiment 1, P increased acetate concentrations ($P<0.05$) in the small intestinal contents of the rats while T lowered valerate concentration in the large intestinal contents ($P<0.05$). L and PL increased maltase activities in the ileum ($P<0.05$). T, P, L and PL increased lactase activities in the DJ ($P<0.05$) while T and P increased lactase activities in the ileum ($P<0.05$). In the experiment 2, P increased acetate, propionate and butyrate ($P<0.05$) in the large intestinal contents while L increased propionate and butyrate ($P<0.05$). The treatments did not affect the disacchridase activities of the rats administered with *E.coli* suspension.

In conclusion, the supplementation of tylosin, probiotic or lactulose did not promote the growth and changes in blood pictures of the rats and rats administered with *E.coli* suspension. The administration of tylosin, probiotic or lactulose improved maltase and lactase activities of the rats in the experiment 1 but did not affect disaccharidase activities of the rats administered with *E.coli* suspension. The major effects of the supplementation were on the SCFAs concentrations in the lower gut of the experimental rats. Probiotic, lactulose and probiotic combined with lactulose affected the SCFAs concentration in the large bowel contents of the rats administered with *E.coli* suspension. They increased acetate, propionate, butyrate and valerate concentrations. Tylosin decreased SCFAs concentrations in both experiments.

ภาควิชา สรีรวิทยา ลายมือชื่อนิสิต .....

สาขาวิชา สรีรวิทยาการสัตว์ ลายมือชื่ออาจารย์ที่ปรึกษา .....

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ACKNOWLEDGEMENT

I would like to express my deep gratitude to my advisor, Assistant Professor Dr. Kris Angkanaporn for his kind advice, guidance, critical reading of manuscript and constant encouragement throughout this study.

My thanks also expressed to the thesis committee for their valuable suggestions.

My thanks would also express to Miss Siripen Komolvanich, Mr. Somchai Poldeenana, Sumphan Thamachareon D.V.M., Kittipong Thajumpa, D.V.M., Mr. Wanchai Yenpetch and Miss Petcharat Nampibul for their help and technical suggestion.

My warmest thanks must go to Mrs. Aphisada Srichreaudong of the Department of Medicine, Faculty of Veterinary Science, Chulalongkorn University for her technical help and thanks for the donation of probiotic by All-tech Co. Ltd. (Thailand).

Finally, I am deeply grateful to my family, my co-worker at Tubkwang Research Station and my friends for their kind encouragement throughout my study period.

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ABBREVIATION

GI	=	gastrointestinal
BW	=	body weight
SCFA	=	short chain fatty acid
CFU	=	colony forming unit
ml	=	milliliter
mg	=	milligram
kg	=	kilogram
PJ	=	proximal jejunum
DJ	=	distal jejunum
I	=	ileum
CE	=	caecum
CR	=	colorectum
w/v	=	weight per volume
µl	=	microliter
mg%	=	milligram percent
µmol	=	micromole
mmol	=	millimole
PCA	=	perchloric acid
KOH	=	potassium hydroxide
RNA	=	ribonucleic acid
DNA	=	deoxyribonucleic acid
Hct	=	hematocrit
Hb	=	hemoglobin
WBC	=	white blood cell

CHAPTER I
INTRODUCTION AND AIM

Animals are easily prone to stress at the age of weaning because of changing environmental factors such as housing, temperature, companion and nutrition (Bolduan et al., 1988). Post-weaning diarrhea are commonly found in the weaning piglets. High incidence of post-weaning diarrhea can cause the economic lost due to mortality and medical cost.

Antibiotics are usually employed to solve these problems. However, there are concerns on using antibiotics in animal production. Including the problems about bacterial resistance to antibiotics and antibiotic residues in animal products. The use of antibiotic presents some risk to human health. The risk is that a resistant strain of pathogenic or disease-causing bacteria may develop. The resistant strain of pathogen may cause human disease that does not response to the treatment with antibiotics or antibacterial agents. In addition, the antibiotic residues in animal products may increase the exposure to antibiotics and thus cause either the development of antibiotic-resistant pathogenic bacteria in human or the allergic reactions from the antibiotic residues.

Many countries realized of these problems and restricted the usage of antibiotics in animal feed. In 1977, the US Food and Drug Administrations (FDA) proposed the restriction on the subtherapeutic use of procaine penicillin and tetracycline in animal feeds. In Sweden, antibiotics and other chemotherapeutics were restricted in animal feed since January 1, 1986. At present, various scientific organizations and the association of veterinary practitioners in Germany have demanded the ban on all antibiotic substances as feed additive.

Due to those problems of antibiotic, the probiotic has been proposed as an alternative growth promoter in animal feed. Probiotics are live microorganism such as lactic acid bacteria that benefit to the host animal. It was found to improve the performance of the animal by decrease *E.coli* count and increase lactobacilli in the gastrointestinal tract (Spieler, 1995; Tortuero, et al., 1995; Kornegay and Risley, 1996). However, the effect of probiotic on the growth of animal has not yet clarified.

The weaning animals were usually susceptible to enterotoxigenic *Escherichia.coli*. After *E.coli* adhered to the gut wall, it produced enterotoxin that damaged intestinal mucosa. Transit rate of enterocyte from crypt of Liberkuhn to the tip of villi would increase to recover the damaged enterocyte, so there were less mature enterocyte at the tip of villi. Nousiainen (1991) reported that bacteria might form a protective barrier on the gut wall alleviating the luminal effect of toxins and resulted in decreased renewal rate of enterocytes. It is possible that there are more mature enterocytes at the tip of villi, and thus improving the intestinal functions such as increasing disaccharidase activity.

There are a number of mechanisms involved the growth promoting effect of probiotic. It is likely that probiotic may alter activities of enzymes in the small intestine. Witt and Savage (1987) proposed that the effects of probiotics on animal growth and food utilization were not related to the direct alteration effect of bacteria on host enzyme activities. In contrast, Collinton et al., (1990) found that there was a significant effect of probiotic on the development of sucrase, lactase, and tripeptidase activities in weaning pig. Therefore further investigations are needed to reveal the mechanism of probiotic on host enzyme activities.

Probiotic such as lactic acid bacteria, especially *Lactobacilli* spp, inhibit the colonization of pathogenic bacteria by the production of organic acid. The lactic acid as a product of the bacterial fermentation is further metabolized to volatile fatty acid which subsequently reduce the intestinal pH (Nemcova, 1997). In contrast, some

researchers found no effect of lactic acid bacteria on gastrointestinal pH of experimental animal (Apgar, et al., 1993; Tortero, et al., 1995). The main product of microorganism fermentation is short-chain fatty acid (SCFA). It affects colonic mucosal blood flow, ileal motility, caecal mucin secretion, colonocyte cellular differentiation and mucosal cell proliferation (Scheppach, 1994).

The lactic acid bacteria use carbohydrates as substrates for fermentation so the diet may affect the gut flora and their products. It was found that lactulose, the nondigestible carbohydrate stimulated the gut bifidobacteria (Gibson et al., 1995). The term prebiotic has been used to describe these dietary carbohydrates which are able to stimulate the growth of potentially beneficial bacteria at the expense of pathogenic microorganisms (Gibson and Roberfroid, 1995). The possibility that prebiotics may have additive effect when using with probiotics has attracted considerable attention.

The objective of the present study was therefore to examine the effects of oral administration of tylosin, probiotic, lactulose and combination of probiotic and lactulose on disaccharidase activities, intestinal SCFAs, intestinal RNA and DNA. The effects of those treatments were examined in two conditions of the experimental rats. First, the rats that reared in conventional condition and the rats that administered with *E.coli* suspension to create imbalance intestinal microflora.

CHAPTER II
BACKGROUND INFORMATION

The roles of gut microflora

The newborn mammals are sterile and acquire their characteristic gut microflora from their mothers and the surroundings. The gastrointestinal microflora of animals is a complex system. There are many interrelationships between different microorganisms and the host. The gastrointestinal tract microflora serves at least three purposes. First, it provides a source of energy from ingested materials. Second, it serves as a host-defense barrier in the prevention of disease by agents seeking entry via the intestinal tract; and third, the microbial inhabitants condition the immunology components of the gastrointestinal tract to respond to antigenic materials in a highly efficient manner. However, proteolytic bacteria can produce toxic compounds damaging the host. Other individual activities of gut bacteria with varying health consequences are summarized in Figure 1.

The microflora which, establishes itself in the gut, is very stable, but it can be influenced by some dietary and environmental factors. The three most important factors are excessive hygiene, antibiotic therapy and stress (Fuller, 1989).

Gastrointestinal tract development after weaning

The gastrointestinal (GI) tract of the animal is immature at birth. In rats, during the 3rd week of life, the GI tract displays dramatic changes of cellularity and enzymology. In suckling period, the capacity of the gastrointestinal tract is suitable for mother's milk and then adapted for solid diet after weaning.

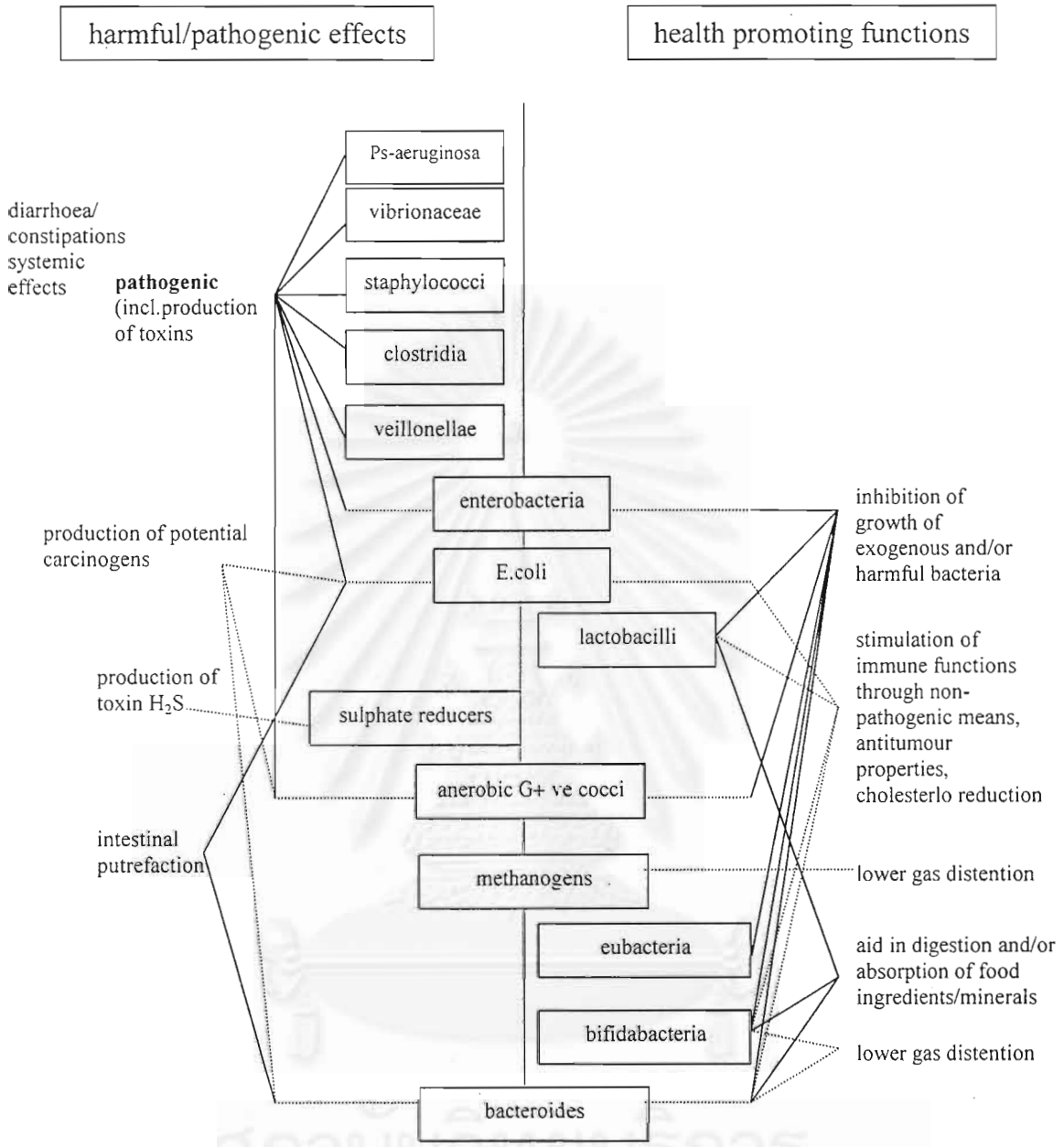


Figure 1. Generalized scheme of the human gut microbiota composition. The different bacterial groups are divided on the basis of whether they exert properties, which is potentially damaging or health promoting for the host (Fuller and Gibson, 1997).

There were some changes in intestinal morphology of the animals after weaning. It was reported that villi shortened and crypts deepened significantly after weaning (Hampson, 1986). However, two weeks after weaning villi length of weaned pigs were greater than those on the day of weaning and even greater in the weaned pigs than those in the unweaned littermates (Hampson, 1986; Nabuurs, 1995). Microorganisms in the gastrointestinal tract had an influence on the transit rate of the small intestinal epithelial cells from the crypts of Lieberkuhn to the tips of the villi. Savage et al. (1981) found that the germfree mice had 115 h epithelial cell transit rate but only 53 h was found in the mice with indigenous microorganisms. Such changes in the intestinal morphology reduce the small intestinal absorptive area and cause less mature enterocyte population. Because of these results, the weaned animals are obviously susceptible to diarrhea and growth retardation at the post-weaning periods.

The developmental changes of digestive function can be explained. The principal source of energy during suckling period comes from lactose in milk. Correspondingly, brush-border lactase activities are high from birth through the 2^{nd} postnatal week. On the other hand, brush-border maltase shows low activity during the first 16 days of life and then rises to adult levels a week after. In case of sucrase activities, they can be detected at the 17^{th} postnatal day and reach adult activities by the middle of the 4^{th} week of age (Henning, 1981).

The young animals were always susceptible to infect with *Escherichia coli* (*E.coli*). Nabuurs et al. (1993) studied the effect of *E. coli* enterotoxin on the net fluid absorption of the small intestine by the small intestine segment perfusion test method (SISP). They found that the weaned pig had significantly less absorption of fluid in the uninfected segments than those of the unweaned pig.

Antibiotic

Antibiotics or antibacterial agents can be divided into two groups based on the method of production. Antibiotics such as bacitracins, tylosin and penicillin are produced by microorganism and the other group such as carbadox and sulfa drugs are produced by chemical synthesis.

Antibiotics are widely used in livestock production to treat sick animals at the therapeutic dosage, prevent diseases in exposed animals at the prophylactic dosage and to be supplemented as feed additive to improve feed efficiency and animal growth at the subtherpeutic dosage. Antibiotics were first appeared for the use as animal feed additives in 1950. The benificial effects of antibiotics are, increasing the growth rate, improving feed utilization, and reducing mortality and morbidity in clinically and subclinically infected animals. Antibiotics were used as the growth promoter in cattle, sheep, pigs and poultry because of their effectiveness on the inhibitory effect against wide spectrum of microbiota in the alimentary tract (Parker and Amstrong, 1987).

Effect of antibiotic on growth and intestinal function of the animals.

The antibiotics that commonly used in livestock production as dietary additives are bacitracins, lincomycin, penicillin, streptomycin, tetracyclin, tiamulin, tylosin and virginiamycin. They affect the intestinal microbial ecosystem and animal growth.

Beames (1969) found that tylosin phosphate (110 ppm) increased feed intake and body weight gain of pig but did not have any effect on feed conversion and the addition of tylosin phosphate to the diet of the rats decreased feed intake and growth rate.

Parker and Amstrong (1987) found that there were some alteration in the pig given diets supplemented subtherapeutic level of antibiotics. It was shown that there were some changes in intestinal morphology for instances the elongation of the villi and higher villi:crypt ratio. It was proposed that the effects were due to the lower level of toxic by-products produced from microbial activities, which reduced the damage of the enterocyte and therefore lower the cell renewal rates. They also reported that sucrase activity increased throughout the length of the small intestine of pig fed on diets containing antimicrobial feed additive.

Antibiotic was found to affect the concentration of SCFAs in gut contents. Cherbut et al. (1991) reported that the amount of SCFAs in the faeces of rat given high level antibiotic solution [bacitracin, 0.2 mg/g body weight (BW); gentamicin, 0.2 mg/g (BW); and nystatin 2×10^2 IU/g (BW)] using intragastric tube was dramatically lowered.

Probiotic

Probiotic is a live microbial feed supplement which beneficially affects the host animal by improving its intestinal microbial balance (Fuller, 1989). Probiotic may contain one or several strains of microorganisms and may be presented to the animal in the forms of powder (loose or in capsule), tablet, granule or paste. They may be either directly administered into the mouth or included in the food or water. The probiotics had numerous advantages on animal production for instances, improving growth rate of farm animals, improving the animal utilization of feed, improving milk production in dairy cows, increasing egg production and improved animal health (Fuller, 1992).

The microorganisms that are normally used as probiotic are including lactic acid bacteria (LAB) which produce lactic acid from fermentation of carbohydrates. There are many strains of LAB such as *Lactobacillus* spp. and *Streptococcus faecium*. Lactic acid bacteria are active against a wide spectrum of Gram positive (G+) bacteria, Gram negative (G-) bacteria, yeast and fungi. The inhibitory components of the LAB comprise the production of bacteriocins, toxic metabolites of oxygen and organic acids (Chateau, et al., 1993; Nemcova, 1997).

Effect of probiotic on growth and intestinal function of the animals.

According to researches concerning probiotic in animal feed, it was found that probiotics increased growth performance and feed utilization of the animals (Apgar et al., 1993; Abe et al.,1995). Witt and Savage (1987) suggested that the effects of lactobacilli on growth and food utilization of the animals may be due to the alteration of the enzymes in the small intestine. Germfree mice were inoculated with *Lactobacilli* spp. and compared with germfree control. They found that the activities of alkaline phophatase, phosphodiesterase and thymidine kinase were the same whether or not the animals contained the bacteria. However, Collington et al. (1990), reported that Probios®, the lactic acid bacteria, had a significant effect on the development of sucrase, lactase and tripeptidase activities in the pig before weaning.

Probiotic was the live microbial effect on microbial balance as mentioned before. There were a number of researches involving the probiotic effect on the concentration of SCFAs in the intestinal content of the animal. Nousiainen and Suomi (1991) found that probiotic had no effect on the concentration of organic acids in the intestinal content of the pig. Kirchgessner et al. (1993) studied the effect of *Bacillus cereus* as a probiotic in the pig. They found that the concentration of lactate and volatile fatty acids in the intestinal contents was mostly diminished. Furthermore the concentrations of acetic acid and propionic acid in the caecal contents were decreased in the groups supplemented with *Bacillus cereus*.

Short chain fatty acids (SCFAs) are the products of microbial fermentation of unabsorbed starch and fiber. The three main acids are acetate, propionate and butyrate. They are important anions in the colonic lumen, affecting colonic mucosal blood flow, ileal motility, caecal mucin secretion, colonocyte cellular differentiation and mucosal cell proliferation. Approximately 95-99% of SCFAs produced by bacterial fermentation is rapidly absorbed from the colonic lumen. SCFAs absorption is coupled with Na^+ absorption, probably by Na^+- H^+ exchange. SCFAs act as an anti-diarrhoeal agent by stimulating sodium and water absorption (Scheppach, 1994).

It is suggested that SCFAs can affect cell proliferation. Sakata and Engelhardt (1983) studied the stimulatory effect of SCFA on epithelial cell proliferation in the large intestine of the rat. SCFAs [acetate 75, propionate 35, butyrate 20 (mM)] introduced intraluminally, increased the mitotic index and labeling index of the large intestinal epithelial cells of the rat.

Prebiotic

The term prebiotic has been coined to describe the dietary carbohydrates, which are able to stimulate, specifically, the growth of potentially beneficial bacteria at the expense of the more harmful pathogenic microorganisms (Gibson and Roberfroid, 1995). Gibson and Roberfroid (1995) explained that prebiotics were non-digestible food ingredients that beneficially affected the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon that could improve host health.

Lactulose (4-O-β-D-galactopyranosyl)-D-fructose, a disaccharide analogue, is neither digested nor absorbed in the small intestine. It passes relatively unchanged to the colon where it serves as energy source for the carbohydrate-splitting intestinal bacteria. Salminen and Salminen (1997) proposed that lactulose and LAB had an influence on constipation and diarrhea as shown in Figure 2.

Nousiainen and Suomi (1991) have reported the use of prebiotic in animal feed as feed supplement. They found that lactulose and/or lactitol did not affect intestinal parameters when compared with those of the control. However, Bovee-Oudenhoven et al. (1997) found that lactulose fermentation in the rats fed on lactulose diet lowered the intestinal pH and increased the lactic acid concentration of the intestinal contents.

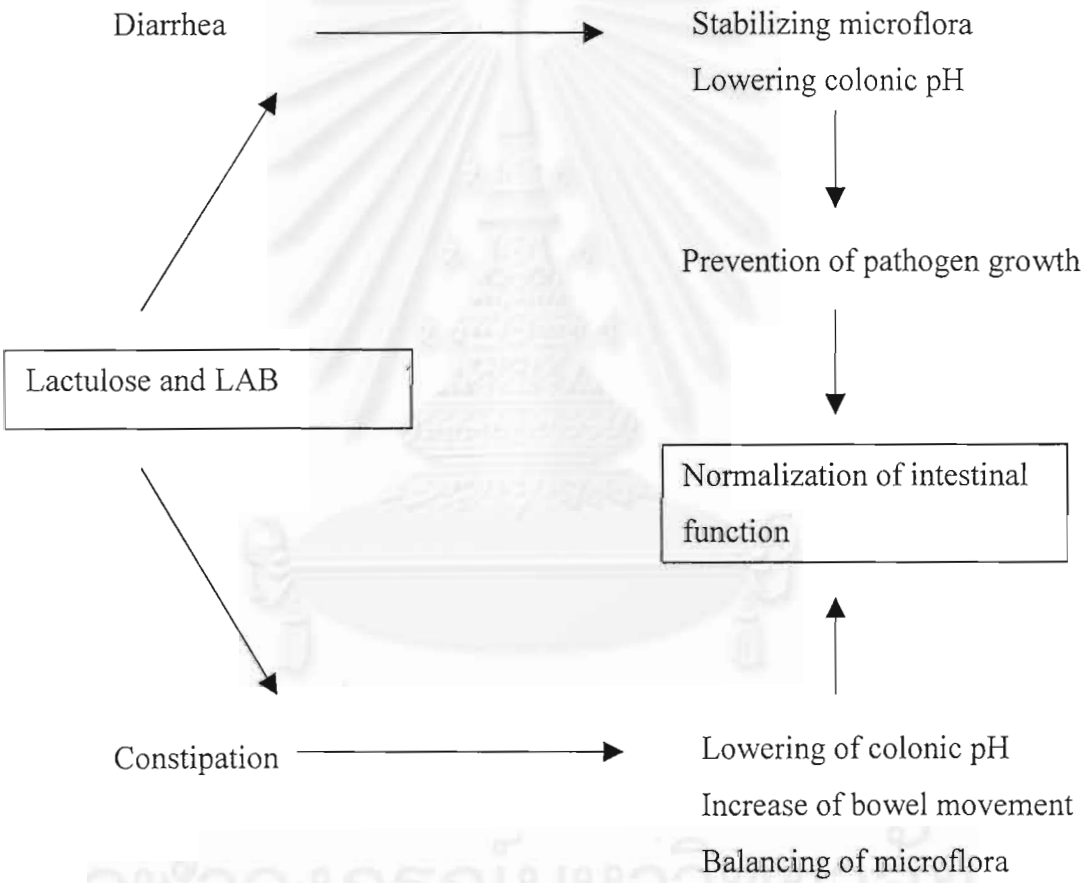


Figure 2. Suggested mechanisms for the influence of lactulose and lactic acid bacteria on constipation and diarrhea (Salminen and Salminen, 1997).

CHAPTER III
MATERIALS AND METHODS

Animals

One hundred and fifty, three-week old, weanling male Wistar rats were used in the study. They were housed in five-tier stainless steel cages with three rats per cage. The rats were fed *ad lib* on commercial pellets feed (CP rat chow) and tap water. For 5 days run-in period, the rats were orally administered with 1 milliliter (ml) of distilled water. The rat was restrained using left hand and the feeding tube was directly put into the esophagus. The oral administration was repeated if the rats regurgitated.

Experimental procedure

The study was divided into two experiments.

Experiment 1. This experiment was designed for rats in conventional condition, 75 rats were allocated into 5 groups of 15 rats (5 replicates of 3 rats each). They were orally fed via feeding tube according to the experimental design.

Group 1.Control group (C). Rats were given 1 ml of distilled water.

Group 2.Antibiotic group (T). Rats were given Tylosin tartrate (Olic Co. Ltd.) containing 0.1 milligram (mg) tylosin base diluted in 1 ml of water.

Group 3.Probiotic group (P). Rats were given 50 mg All-Lac®100X (Alltech Biotechnology Center, Nicholasville, KY) diluted in 1 ml of water.

Group 4.Lactulose group (L). Rats were given 1 ml of Hepalac®(Berlin Pharmaceutical Industry Co. Ltd.) containing 667 mg of lactulose.

Group 5.Probiotic and Lactulose group (PL). Rats were given 50 mg All-Lac® 100X diluted in 1 ml of Hepalac®.

Experiment 2. Seventy-five rats were used. Rats were allocated to five groups with 15 rats per group as in the experiment 1. To create the microbial imbalance condition in the gut of the rats, all rats were orally administered with hemolytic *E.coli*. The hemolytic *E.coli* from the lung of the pig (courtesy of Department of Medicine, Faculty of Veterinary Science, Chulalongkorn University) 10^8 colony-forming-units (CFU)/ml were given to the rat 1 ml once a day for 5 days during the run-in period.

All animals in the both experiments were fed on commercial diet and were given each treatment using feeding tube once daily at 8.00-9.00 AM for 14 days of the experimental period.

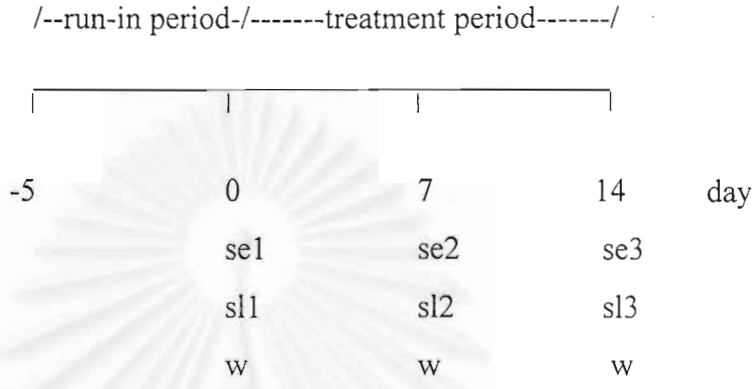
Table 1. The description of the treatments in the both experiments.

Treatments	Description
1. Control (C)	Water 1 ml
2. Antibiotic (T)	Tylosin tartrate 0.1 mg/ml
3. Probiotic (P)	All-lac® 50 mg/ml
4. Lactulose (L)	Hepalac® 1 ml (667 mg of lactulose/ml)
5.Probiotic and Lactulose (PL)	All-lac® 50 mg and Hepalac® 1 ml

All-lac® = 10^{10} *Lactobacillus acidophilus* and 10^{10} *Streptococcus faecium*

Protocol of the experiment

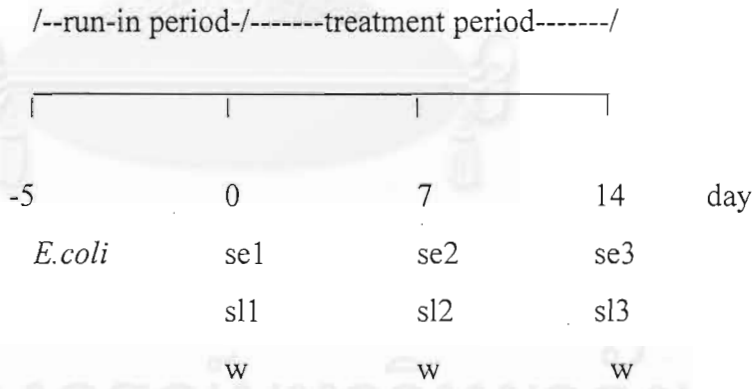
Experiment 1



se = sampling for enzyme activity, sl = sampling for intestinal contents, w = weighing

Figure 3. Diagram of the experiment 1.

Experiment 2



se = sampling for enzyme activity, sl = sampling for intestinal contents, w = weighing

Figure 4. Diagram of the experiment 2.

Sample collection and Tissue preparation

In the both experiments, 5 rats from each group were randomly sampled on day 0, 7 and 14. The rats were anesthetized by intraperitoneal injection of sodium pentobarbitone at 60 mg / kilogram body weight (kg BW). The abdomen was opened and approximately 1 ml of blood sample were collected from renal vein into an eppendorf tube containing EDTA as an anticoagulant. Blood sample was determined for hematocrit (microcapillary tube), hemoglobin (cyanmethomoglobin) and white blood cell (counting chamber). The whole intestine from duodenum to rectum was removed (Figure 3.). The intestinal section from which the pancreatic loop terminated to a section at 15 cm before the ileocaecal junction was taken as the jejunal part. The jejunal part was separated into 2 parts, the upper half was the proximal jejunum (PJ) and the lower part was the distal jejunum (DJ). The ileal (I) part was taken from the intestinal section at 15 cm before the ileocaecal junction to the ileocaecal junction. The contents of PJ, DJ, I, caecum (CE) and colorectum (CR) were collected by gentle squeezing with thumb and finger into plastic tubes. The pH of the contents were immediately measured by dipping the pH paper (range 6.2-7.8) directly to the contents and then the contents were kept frozen at –70 degree celsius (°C) until analysis. SCFAs in the intestinal contents were analyzed by gas liquid chromatography. Each intestinal section was opened longitudinally, rinsed with ice cold saline and placed on foam pad. Mucosal samples were scraped from the muscle layer using a glass slide, wrapped with tin foil and stored at –70°C until analysis. Mucosal scrapings were analyzed for disaccharidases activity (Ddahlquist, 1968), DNA contents (Burton, 1955; Giles and Mayers, 1965) and RNA contents (Flek and Begg, 1954).

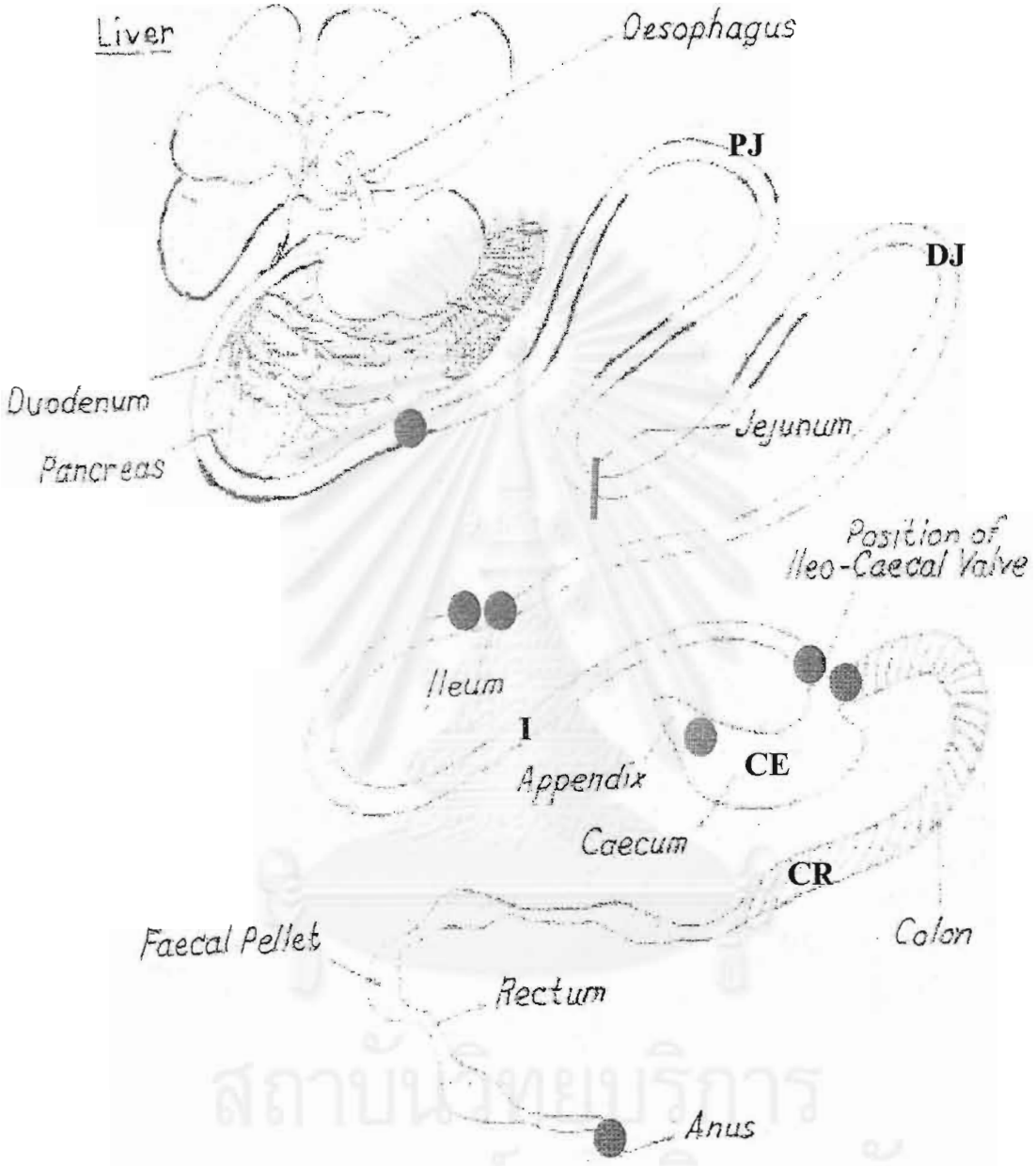


Figure 5. Alimentary canal of the rat.

Short-chain fatty acids determination

SCFAs of the rat intestinal contents were analyzed by the method modified by Erwin (1961). The intestinal content was separated into two parts. First, the proximal intestinal content included the contents of PJ, DJ and I. The distal intestinal content included the contents of CE and CR. Frozen rat intestinal content was thawed and diluted with distilled water (1 mg:1 μl) then centrifuged at 3,000 rpm for 10 minutes. The supernatant was removed for SCFAs determination. The internal standards used in this method were meta-valeric acid and standard volatile fatty acid with 25% metaphospholic acid. The volume of 0.4 ml of standard solution was mixed with 0.7 of the supernatant of the intestinal content to meet the volume of 1.1 ml. These samples were measured for the SCFA concentration by a gas chromatography equipped with a hydrogen flame ionization detector. The column treated with 1% (wt/wt) H_3PO_4 (20 m×4 mm (i.d.), 3mm (o.d.) packed with 10% AT-1200 (80-100 mesh), was used for analyzing. The concentration of individual SCFA was expressed as mmol/ml of the sample.

$$[SCFA/C_x]\ (mM) = \frac{[std\ C_x] \times (A\text{-}sample)C_x \times (A\text{-}standard)_{int\ std}}{(A\text{-}sample)_{int\ std} \times (A\text{-}standard)C_x} \times \frac{11}{7}$$

The determination of ribonucleic acid (RNA) and deoxyribonucleic acid (DNA) contents

Frozen mucosal scrappings were homogenized in 0.2 N perchloric acid (PCA) and all homogenized samples were centrifuged at 500 g for 10 minutes. The precipitate concentration both RNA and DNA fractions was washed in cold 0.2 N PCA and recentrifuged. The precipitate was solubilized for RNA in 3.0 ml of 0.3 N potassium hydroxide (KOH) and placed at 37°C for 60 minutes. Added Two ml of 10 % PCA into the tube and placed on ice for 10 minutes then centrifuged as descriped above (Berseth et al, 1983 ; Simmen et al, 1990). The supernatant was separated for the determination of RNA content by the method of Flek and Begg (1954), using ultraviolet absorption measurement. The two wavelengths (λ) (wavelengths of maximal and minimal absorption of RNA) were 260 and 232 nm. The content of rat intestinal mucosal RNA was calculated from the following formula:

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

DNA fraction in the residual pellet was solubilized in 10 % PCA and heated to 70°C for 20 minutes. The DNA content of the samples was determined by the Burton procedure as modified by Giles and Mayers (1965). Two ml 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 different at λ 595-700 nm were read against the blank in the without DNA solution.

Assay of disaccharidase activities

Frozen mucosal scrapings were homogenized with four parts of distilled water weight by volume (w/v). The samples were centrifuged at 500 g for 10 minutes to remove the large debris of the cells. An aliquot of the sample was adjusted with different dilution factor for the appropriate disaccharidase activity. Suitable dilutions for the different activities were as the following: maltase 1:50; sucrase 1:10; and lactase 1:5. The eppendorf tubes containing 10 microliters (μl) of the diluted enzyme solution were placed in a water bath at 37°C for a few minutes, 10 μl of the substrate-buffer dilution was added and mixed. After incubated in 37°C for 60 minutes, the microgram (μg) of glucose liberated in the dilution was determined by the glucose kit (Human Gesellschaft fur Biochemica und Diagnostica, Germany). The optical density was read at the wavelength (λ) of 500 against the blank compared with 10 milligrams percent (mg %) glucose standard. Results were expressed as specific activity (units per gram brush border protein). One unit (U) was defined as the activity that hydrolyzed 1 micromole (μmole) of the substrate per minute under the experiment condition. The disaccharidase activity was obtained by the following formula:

$$\frac{a \times d}{n \times 108} \quad \text{units/ml}$$

where a = μg glucose liberated in 60 minutes

d = dilution factor for enzyme solution

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

$$108 = \frac{10}{1000} \ \mu l \times 60 \text{ min} \times 180 \ \mu g \text{ glucose}$$

The brush border protein concentration was determined by Lowry method.

Calculation of the growth performance

The feed intake of rats was measured daily for 14 days. The rats were weighing at day 0, day 7 and day 14 of the experiment.

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

$$\text{Feed/Gain} = \frac{\text{Total feed intake}}{\text{Final BW} - \text{Initial BW}}$$

Statistic analysis

All data were presented as Means ± SD. The data were analyzed using one-way Analysis of Variance (ANOVA) to determine the effects of treatments and days of the treatment. If there were any significant effect, Duncan's New Multiple Range Test was used to compare the individual means.

CHAPTER IV
RESULTS

Experiment 1

Animal performance

For the overall period of the trial, rats in group C had significantly ($P<0.05$) higher feed intake than other groups (Table 2). There was no effect of any treatment on the live weights, average daily gain (ADG) and feed/gain in each period of the study.

Effect of treatments on blood pictures of the rats.

The effect of various treatments on hematocrit (Hct), hemoglobin (Hb) and white blood cell (WBC) count are shown in Table 3. There were no significant differences in blood pictures among each group of rats. The rats in PL group had significantly higher Hct ($P<0.05$) at day 14 than those at day 0. In addition, WBC counts were higher at day 7 and day 14 than day 0 ($P<0.05$).

Effect of treatments on pH of the intestinal contents in various parts of the small intestine of the rats.

The changes in pH of the intestinal contents at the PJ, DJ, I, CE and CR were measured (Table 4). There was no effect of treatments on intestinal pH. However, intestinal pH of the rats in PL group was higher in DJ and I at day 7 and day 14 than day 0.

Table 2 Feed intake, live weights, average daily gain and feed/gain of the rats.

Item	Treatment[1]				
	C	T	P	L	PL
Feed intake (g/rat/d)					
Day 0 - Day 7	15.3±0.6^{b}	14.3±1.2^{ab}	14.8±0.7^{ab}	13.8±0.8^{a}	14.0±0.9^{a}
Day 7 - Day 14	16.7±1.5^{b}	16.6±0.5^{b}	16.0±1.7^{b}	15.2±1.8^{ab}	14.0±0.9^{a}
Day 0 - Day 14	16.1±0.8^{c}	15.5 ±0.7^{bc}	15.5±1.2^{bc}	14.5±1.3^{ab}	13.9±0.6^{a}
Live weights (g/rat)					
Day 0	100.5±7.8	97.9±5.6	98.9±9.3	97.3±8.8	98.1±10.0
Day 7	154.3±10.7	151.0±9.1	154.8±16.0	148.3±9.6	153.0±12.9
Day 14	192.2±12.5	191.4±6.2	192.0±19.0	191.2±17.1	188.6±6.7
Average daily gain (g/rat/d)					
Day 0 - Day 7	8.0±1.1	7.6±0.7	8.0±1.0	7.6±0.7	7.7±1.0
Day 7 - Day 14	6.1±2.2	5.7±1.3	5.1±1.6	5.4±1.1	5.8±0.7
Day 0 - Day 14	7.0±0.6	6.6±0.4	6.7±0.7	6.7±0.6	6.5±0.7
Feed / gain					
Day 0 – Day 14	2.3±0.1	2.4±0.2	2.3±0.2	2.2±0.1	2.2±0.2

[1] Treatments were C: control; T: tylosin; P: probiotic; L: lactulose; PL: combination of P and L.

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

Table 3 Effect of treatments on blood pictures of the rats.

	Treatment[1]				
	C	T	P	L	PL
Hct (%)					
Day 0	39.8±4.7	39.0±5.1	43.0±4.2	40.6±4.6	40.0±2.5
Day 7	39.8±3.8	38.8±3.4	41.6±1.5	40.4±1.8	38.0±1.8
Day 14	45.8±4.8	44.8±5.5	44.2±5.9	44.6±5.0	47.0±1.6*
Hb (g/dl)					
Day 0	13.1±1.1	11.1±1.8	13.8±0.9	12.0±1.3	11.9±1.4
Day 7	12.3±1.7	11.9±1.2	12.6±0.8	12.5±0.7	11.5±0.4
Day 14	13.5±0.8	13.5±1.1	13.2±1.2	13.3±0.2	13.1±1.0
WBC ($\times 10^3/mm^3$)					
Day 0	3.3±1.0	3.5±0.4	3.6±1.4	3.6±1.2	4.4±1.0
Day 7	3.2±1.1	2.4±0.7	3.3±1.0	2.8±0.5	2.2±0.9*
Day 14	2.2±0.8	2.7±1.2	2.6±0.9	2.0±0.6*	2.5±0.9*

[1] Treatments were C: control; T: tylosin; P: probiotic; L: lactulose; PL: combination of P and L.

* Means in the same column differed significantly ($P<0.05$) from the control (day 0).

Table 4 Effect of treatments on the intestinal pH of the rats.

Intestinal part	Treatment[1]				
	C	T	P	L	PL
Proximal jejunum (PJ)					
Day 0	6.4±0.2	6.4±0.2	6.2±0.0	6.2±0.0	6.2±0.0
Day 7	6.3±0.1	6.4±0.2	6.3±0.1	6.5±0.2*	6.4±0.2
Day 14	6.4±0.2	6.3±0.1	6.4±0.2	6.4±0.2	6.4±0.2
Distal jejunum (DJ)					
Day 0	6.4±0.2	6.3±0.2	6.3±0.1	6.2±0.1	6.2±0.1
Day 7	6.4±0.1	6.4±0.2	6.5±0.2	6.6±0.1*	6.6±0.1*
Day 14	6.4±0.2	6.3±0.2	6.4±0.2	6.5±0.2*	6.4±0.1*
Ileum (I)					
Day 0	7.0±0.5	7.1±0.4	6.8±0.4	7.0±0.4	6.9±0.3
Day 7	7.4±0.2	7.3±0.4	7.4±0.1*	7.4±0.3	7.4±0.1*
Day 14	7.3±0.2	7.4±0.2	7.3±0.4	7.2±0.3	7.4±0.3*
Cecum (CE)					
Day 0	6.8±0.2	6.6±0.2	6.6±0.1	6.7±0.1	6.7±0.3
Day 7	6.6±0.1	6.8±0.3	6.9±0.3	6.6±0.2	6.6±0.0
Day 14	6.7±0.2	6.8±0.4	6.8±0.1	6.6±0.1	6.6±0.1
Colorectum (CR)					
Day 0	6.6±0.1	6.6±0.1	6.7±0.1	6.7±0.1	6.7±0.2
Day 7	6.6±0.1	6.8±0.3	6.8±0.3	6.6±0.1	6.5±0.2
Day 14	6.6±0.2	6.6±0.2	6.6±0.1	6.6±0.2	6.6±0.0

[1] Treatments were C: control; T: tylosin; P: probiotic; L: lactulose; PL: combination of P and L.

* Means in the same column differed significantly ($P<0.05$) from the control (day 0).

Effect of treatments on SCFAs concentrations in the rat intestines.

The concentrations of SCFAs in the small intestine are shown in Table 5. The concentrations of SCFAs in the small intestine were low and only acetate and butyrate were detected. P increased the concentration of acetate ($P<0.05$) at day 7 of the experiment (Figure 6).

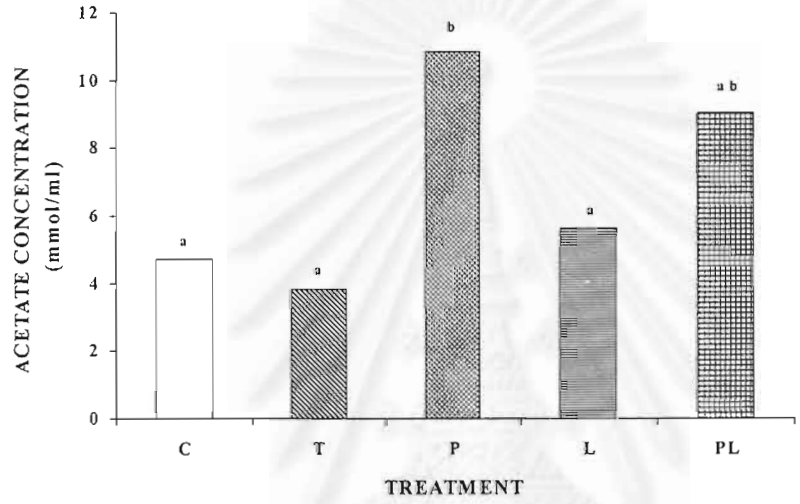


Figure 6. Effect of treatments on acetate concentration (mmol/ml) at day 7 in the small intestine of the rats.

The concentrations of SCFAs in the large intestine are shown in Table 6. The propionate in the large intestinal content of the rats in the PL group was significantly higher than those of the others ($P<0.05$) while the valerate concentration in the T, P and PL group were lower ($P<0.05$) than those of the other at day 14 (Figure 7). The concentrations of acetate were higher at day 14 than day 0 in all treatments.

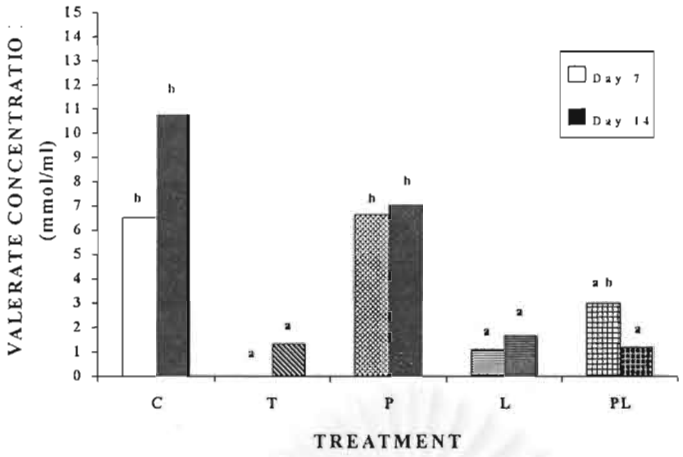


Figure 7. Effect of treatments on valerate concentration (mmol/ml) at day 7 and day14 in the large intestine of the rats.

Table 5 Effect of treatments and days on the short-chain fatty acid (SCFA) concentrations (mmol/ml) in the small intestine of the rats.

SCFA	Treatment[1]				
	C	T	P	L	PL
Acetate					
Day 0	4.28±2.93	1.15±1.45	3.96±1.70	4.02±2.73	4.40±4.08
Day 7	4.74±1.60[a]	3.84±4.33[a]	10.86±5.21[b*]	5.64±1.94[a]	9.04±3.03[ab]
Day 14	9.78±13.98	11.82±8.97	8.42±3.26	3.00±4.47	19.40±12.55[*]
Propionate*					
Day 0	NR	NR	NR	NR	NR
Day 7	NR	NR	NR	NR	NR
Day 14	NR	NR	NR	NR	NR
Butyrate					
Day 0	0.50±0.58	0.80±0.29	0.72±0.97	0.94±1.41	0.66±0.70
Day 7	0.50±0.54	1.18±1.25	1.80±0.85	1.22±0.83	2.34±1.15
Day 14	1.76±1.09[*]	2.38±1.31	0.94±0.76	1.16±1.11	2.43±2.75
Valerate*					
Day 0	NR	NR	NR	NR	NR
Day 7	NR	NR	NR	NR	NR
Day 14	NR	NR	NR	NR	NR

[1] Treatments were C: control; T: tylosin; P: probiotic; L: lactulose; PL: combination of P and L.

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

[*] Means in the same column differed significantly (P<0.05) from the control (day 0).

NR The values were not remarkable.

Table 6 Effect of treatments and days on the short-chain fatty acid (SCFA) concentrations (mmol/ml) in the large intestine of the rats.

SCFA	Treatment[1]				
	C	T	P	L	PL
Acetate					
Day 0	36.23±14.54	41.70±18.51	38.00±10.14	46.00±17.30	31.16±13.44
Day 7	52.08±29.47	35.92±23.72	46.25±24.05	39.36±13.12	65.48±4.37*
Day 14	106.48±3.11*	87.78±35.30*	95.70±30.77*	100.24±24.33*	83.57±25.92*
Propionate					
Day 0	16.13±6.49	13.30±4.06	13.20±3.67	17.66±7.80	9.88±3.74
Day 7	18.50±6.91[a]	12.16±4.07[a]	15.94±11.59[a]	15.54±4.47[a]	29.22±5.02[b]*
Day 14	14.56±4.47	14.16±1.35	15.56±5.04	15.82±4.62	11.70±4.33
Butyrate					
Day 0	8.97±3.35	8.18±1.04	7.14±2.06	10.25±1.60	7.83±2.00
Day 7	12.92±7.7	6.70±3.96	12.74±9.44	12.52±4.69	17.56±2.39*
Day 14	12.74±5.74	9.30±4.23	20.20±6.64*	13.48±6.79	12.03±8.72
Valerate					
Day 0	4.93±5.70	3.95±2.71	3.80±3.66	4.14±3.95	4.22±2.95
Day 7	6.54±4.16[b]	0.00[a]	6.66±3.99[b]	1.08±1.51[a]	3.00±4.16[ab]
Day 14	10.74±4.65[b]	1.34±3.00[a]	7.04±4.36[b]	1.68±2.31[a]	1.17±2.02[a]

[1] Treatments were C: control; T: tylosin; P: probiotic; L: lactulose; PL: combination of P and L.

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

* Means in the same column differed significantly ($P<0.05$) from the control (day 0).

Effect of treatments on the concentrations of the intestinal mucosal DNA of the rats.

The rat intestinal mucosa at the PJ, DJ, I, CE and CR were analyzed for the concentration of DNA (Table 7). The total DNA in PJ, DJ, I, CE and CR at day 14 of rats received lactulose were significantly higher ($P<0.05$) than those in other groups. The colorectal DNA of the rat in P group were lower ($P<0.05$) than the control at day 7 and day 14 (Figure 8).

Effect of treatments on the concentrations of the intestinal mucosal RNA of the rats.

The rat intestinal mucosa at the PJ, DJ, I, CE and CR were analyzed for the concentration of RNA (Table 8). There was no significant effect of treatments on total RNA concentrations in PJ, DJ, I, CE and CR.

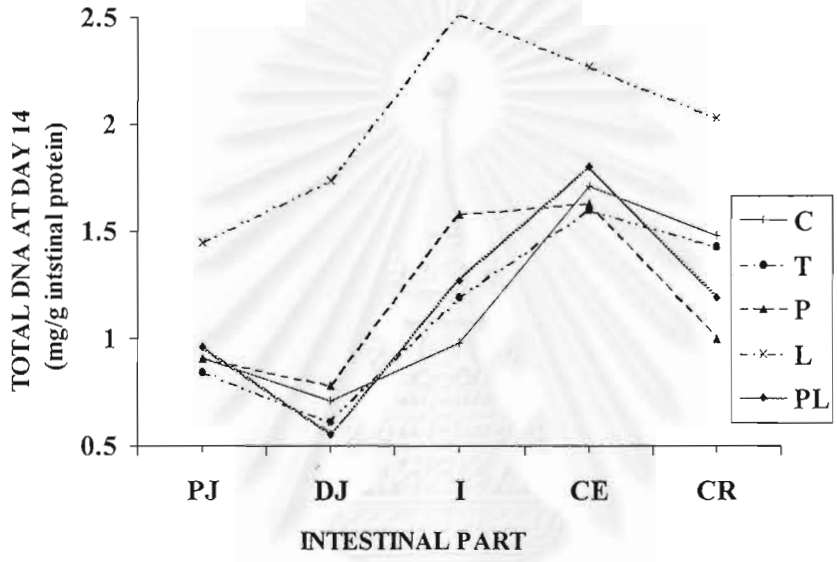


Figure 8. Concentrations of rats intestinal mucosal DNA at day 14 of the experiment.

Table 7 Effect of treatments and days on the concentrations of intestinal mucosal DNA of the rats (mg/g intestinal mucosal protein).

Intestinal part	Treatment[1]				
	C	T	P	L	PL
Proximal jejunum (PJ)					
Day 0	1.36±0.28	1.26±0.17	1.46±0.25	1.48±0.28	1.37±0.10
Day 7	0.72±0.06	0.94±0.15	0.95±0.26*	0.81±0.31	1.02±0.18
Day 14	0.91±0.22^{a*}	0.84±0.23^{a*}	0.91±0.29^{a}	1.45±0.17^{b}	0.96±0.29^{a}
Distal jejunum (DJ)					
Day 0	1.10±0.21	1.24±0.26	1.21±0.41	1.21±0.12	1.20±0.20
Day 7	1.03±0.16	0.93±0.09	1.06±0.16	1.06±0.20	1.17±0.11
Day 14	0.71±0.23^{a}	0.61±0.23^{a*}	0.78±0.10^{a}	1.74±0.21^{b*}	0.55±0.10^{a*}
Ileum (I)					
Day 0	1.76±0.35	1.69±0.23	1.68±0.30	1.86±0.29	1.84±0.26
Day 7	1.80±0.12^{b}	1.47±0.33^{b}	1.55±0.21^{b}	0.98±0.26^{a*}	1.79±0.26^{b}
Day 14	0.98±0.15^{a*}	1.19±0.21^{ab}	1.58±0.30^{b}	2.51±0.20^{c*}	1.27±0.30^{ab*}
Cecum (CE)					
Day 0	2.43±0.31	2.65±0.37	2.65±0.31	2.65±0.25	2.45±0.48
Day 7	2.56±0.30*	2.36±0.24	2.47±0.22	2.26±0.30	1.91±0.15
Day 14	1.71±0.08^{a*}	1.60±0.10^{a*}	1.63±0.07^{a*}	2.27±0.12^{b}	1.80±0.19^{a}
Colorectum (CR)					
Day 0	1.15±0.01	1.18±0.43	1.28±0.35	1.67±0.38	1.58±0.29
Day 7	1.58±0.26^{c}	1.12±0.20^{ab}	0.90±0.23^{a}	1.51±0.27^{bc}	1.06±0.02^{a}
Day 14	1.48±0.26^{b}	1.43±0.36^{b}	1.00±0.14^{a}	2.03±0.04^{c}	1.19±0.22^{ab}

[1] Treatments were C: control; T: tylosin; P: probiotic; L: lactulose; PL: combination of P and L.

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

* Means in the same column differed significantly ($P<0.05$) from the control (day 0).

Table 8 Effect of treatments and days on the concentrations of intestinal mucosal RNA of the rats (mg/g intestinal mucosal protein).

Intestinal part	Treatment[1]				
	C	T	P	L	PL
Proximal jejunum (PJ)					
Day 0	0.87±0.18	0.81±0.07	1.01±0.16	1.08±0.27	0.94±0.28
Day 7	1.13±0.20	0.93±0.19	1.44±0.16	1.15±0.26	1.01±0.15
Day 14	0.88±0.11	0.83±0.19	1.01±0.29	0.89±0.15	0.90±0.24
Distal jejunum (DJ)					
Day 0	0.97±0.29	0.98±0.18	1.16±0.11	1.16±0.11	1.04±0.31
Day 7	1.18±0.13	1.16±0.32	1.33±0.60	1.08±0.19	0.97±0.14
Day 14	1.41±0.09	0.96±0.30	1.15±0.18	1.13±0.21	1.16±0.21
Ileum (I)					
Day 0	1.14±0.33	1.01±0.24	1.16±0.04	1.18±0.38	1.07±0.21
Day 7	1.38±0.16	1.27±0.23	1.12±0.42	1.28±0.17	1.10±0.14
Day 14	1.48±0.29	1.03±0.28	1.06±0.05	1.26±0.39	1.07±0.19
Cecum (CE)					
Day 0	0.98±0.31	1.14±0.15	1.03±0.07	1.09±0.36	0.77±0.12
Day 7	0.95±0.13	0.87±0.12	0.87±0.16	0.83±0.12	0.92±0.21
Day 14	1.03±0.17	0.70±0.20	0.78±0.16	0.94±0.11	0.82±0.16
Colorectum (CR)					
Day 0	1.38±0.36	1.42±0.09	1.58±0.13	1.41±0.13	1.38±0.05
Day 7	1.16±0.25	1.48±0.25	0.88±0.18	1.34±0.32	1.52±0.15
Day 14	1.80±0.08	1.45±0.19	1.51±0.27	1.58±0.24	1.52±0.26

[1] Treatments were C: control; T: tylosin; P: probiotic; L: lactulose; PL: combination of P and L.

Means in the same column differed significantly ($P<0.05$) from the control (day 0).

Effect of treatments on the disaccharidase activities.

Maltase

The alterations in maltase activities in the small intestines are shown in Table 9. Maltase activities were high in both the proximal jejunum and the distal jejunum. The mean value of maltase activity in the ileum was approximately half of that in the jejunum. Maltase activities in PJ of rats in L group were significantly higher ($P<0.05$) than T and PL group but not differ from control. L and PL increased ($P<0.05$) maltase activities in I than control. The maltase activities in the PJ group increased ($P<0.05$) at day 7 and day 14 were higher than those in day 0.

Sucrase

The sucrase activities in mucosal tissue of the small intestine are depicted in Table 10. Likewise the maltase activities, the sucrase activities at day 7 and day 14 were significantly higher ($P<0.05$) than at day 0. Sucrase activities at day 14 in DJ and I of rats in T group were significantly lower ($P<0.05$) than the control. At day 14, the rats in the P group had lower sucrase activities in I than those in the control.

Lactase

The lactase activities in mucosal tissue of the small intestine are demonstrated in Table 11. Lactase activities at day 7 in DJ of rats in T, P, L and PL were significantly higher ($P<0.05$) than control. The rats in the T and P group had significant higher ($P<0.05$) lactase activities in the ileum than those in other groups.

Table 9 Effect of treatments and days on the maltase activities (unit) in the mucosal tissue of the small intestines of the rats.

Intestinal part	Treatment[1]				
	C	T	P	L	PL
Proximal jejunum (PJ)					
Day 0	154.0±36.0	146.1±15.3	173.1±8.4	192.2±49.3	148.0±18.9
Day 7	295.0±3.0*	328.8±24.9*	360.8±28.4*	329.0±22.6*	370.8±44.6*
Day 14	312.9±12.6^{ab}*	266.0±51.5^{a}*	289.8±24.1^{ab}*	357.9±21.9^{b}*	278.1±23.5^{a}*
Distal jejunum (DJ)					
Day 0	174.5±44.3	134.1±8.1	148.8±34.7	141.5±5.4	140.4±18.3
Day 7	280.3±15.9	349.7±47.6*	383.3±19.0*	355.0±40.7*	317.2±22.9*
Day 14	246.5±6.2	287.7±33.2*	239.3±15.7*	284.4±54.5*	222.8±21.3
Ileum (I)					
Day 0	80.6±22.7	79.3±30.7	86.1±33.8	90.0±18.2	79.3±33.0
Day 7	137.5±20.0^{a}	157.6±6.8^{ab}*	173.3±40.6^{abc}	212.9±21.5^{c}*	183.6±5.0^{bc}*
Day 14	113.8±29.0	144.8±36.1*	157.4±45.6	99.3±16.3	106.5±7.6

[1] Treatments were C: control; T: tylosin; P: probiotic; L: lactulose; PL: combination of P and L.

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

* Means in the same column differed significantly ($P<0.05$) from the control (day 0).

Table 10 Effect of treatments and days on the sucrase activities (unit) in the mucosal tissue of the small intestines of the rats.

Intestinal part	Treatment[1]				
	C	T	P	L	PL
Proximal jejunum (PJ)					
Day 0	49.5±6.3	48.4±0.7	57.6±3.5	60.5±11.8	52.0±5.3
Day 7	107.2±11.0*	107.0±6.2*	119.7±30.2	115.8±16.3*	113.0±6.8*
Day 14	95.7±18.2*	79.0±8.9*	99.0±21.6	93.3±8.8*	96.4±13.9*
Distal jejunum (DJ)					
Day 0	34.9±9.6	36.6±6.6	45.6±7.6	36.4±2.4	41.6±9.1
Day 7	81.7±14.6*	88.3±5.8*	78.5±12.9*	82.4±4.3*	75.2±15.4*
Day 14	53.1±6.0^{bc}	32.0±7.5^{a}	40.0±4.1^{ab}	54.7±9.4^{c*}	47.8±6.5^{bc}
Ileum (I)					
Day 0	19.1±2.4	26.5±3.4	22.1±3.7	21.6±5.2	23.1±1.9
Day 7	27.4±3.9*	30.2±1.4	36.8±5.0*	32.3±3.7*	32.1±7.6
Day 14	22.8±1.1^{b}	14.2±1.2^{a*}	14.9±3.9^{a}	25.6±1.7^{b*}	21.1±5.4^{ab}

[1] Treatments were C: control; T: tylosin; P: probiotic; L: lactulose; PL: combination of P and L.

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

* Means in the same column differed significantly ($P<0.05$) from the control (day 0).

Table 11 Effect of treatments and days on the lactase activities (unit) in the mucosal tissue of the small intestines of the rats.

Intestinal part	Treatment[1]				
	C	T	P	L	PL
Proximal jejunum (PJ)					
Day 0	5.6±3.0	5.0±0.5	8.1±1.2	5.6±2.5	6.1±2.0
Day 7	19.9±1.8*	21.4±3.5*	21.3±3.5*	23.0±7.3*	23.2±2.8*
Day 14	22.9±3.3^{ab}*	20.8±5.2^{a}*	28.2±3.0^{b}*	26.0±1.9^{ab}*	19.7±0.8^{a}*
Distal jejunum (DJ)					
Day 0	2.4±1.5	2.2±0.4	3.0±1.0	1.8±0.5	2.0±0.4
Day 7	12.2±1.2^{a}*	23.9±2.9^{b}*	23.4±2.4^{b}*	23.7±1.8^{b}*	20.5±2.2^{b}*
Day 14	13.1±1.2*	13.7±0.5*	13.4±5.0*	13.5±4.0*	9.8±0.3*
Ileum (I)					
Day 0	2.1±1.1	2.2±0.7	1.9±0.6	2.1±1.2	1.6±0.6
Day 7	2.2±0.7^{a}	3.2±0.2^{b}	3.0±0.5^{b}	1.9±0.1^{a}	1.6±0.1^{a}
Day 14	2.6±0.1	1.7±0.5	2.4±0.6	2.2±0.1	2.0±0.3

[1] Treatments were C: control; T: tylosin; P: probiotic; L: lactulose; PL: combination of P and L.

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

* Means in the same column differed significantly ($P<0.05$) from the control (day 0).

Experiment 2

Animal performance

At the end of the trial, rats in the C group had significantly ($P<0.05$) higher feed intake than rats in group L but did not significantly different from the other groups (Table 12). There were treatment effects on the live weight of the infected rat at day 7 of the experiment, the rats in group PL had lower BW than those of the rats in group C and T. ADG of the experimental rats was altered only during the first week of the experiment. Average daily gain of the rats in group T was higher than those of the rats in groups P and PL ($P<0.05$). There was no treatment effect on feed/gain of the experimental rats.

Effect of treatments on blood pictures of the rats administered with *E.coli* suspension.

The effect of various treatments on Hct, Hb and WBC count are shown in Table 13. There were no significant differences in Hct and Hb among each group of the rats. WBC counts at day 7 of the rats in the P and PL group were significantly lower ($P<0.05$) than control.

Effect of treatments on the pH of the intestinal contents in various parts of the small intestine of the rats administered with *E.coli* suspension.

The pH of the proximal jejunal content at day 7 in P and L group were lower ($P<0.05$) than control, while cecal contents of rats in P, L and PL group were significantly lower ($P<0.05$) than T but not differ from control (Table 14).

Table 12 Feed intake, live weights, average daily gain and feed/gain of the rats administered with *E.coli* suspension.

Item	Treatment[1]				
	C	T	P	L	PL
Feed intake (g/d/rat)					
Day 0 - Day 7	21.0±1.7^{b}	20.2±1.3^{b}	18.8±0.7^{a}	17.8±1.2^{a}	17.8±0.5^{a}
Day 7 - Day 14	20.3±0.6	20.7±4.1	20.0±2.0	19.3±2.1	19.8±1.6
Day 0 - Day 14	20.7±0.9^{b}	20.4±2.4^{ab}	19.4±1.1^{ab}	18.5±1.3^{a}	19.0±1.1^{ab}
Live weights (g)					
Day 0	130.2±11.3	127.9±13.2	130.1±12.6	127.5±15.2	123.9±11.2
Day 7	186.3±12.8^{b}	184.8±12.3^{b}	172.7±15.9^{ab}	176.2±16.9^{ab}	168.0±8.9^{a}
Day 14	244.8±9.8	242.8±23.8	232.0±12.1	240.8±30.1	233.2±14.3
Average daily gain (g/d/rat)					
Day 0 - Day 7	6.7±2.5^{ab}	8.1±1.1^{b}	6.4±1.1^{a}	6.8±1.0^{ab}	6.4±0.5^{a}
Day 7 - Day 14	8.6±1.2	8.2±1.6	8.7±0.8	7.8±2.2	7.8±2.1
Day 0 - Day 14	7.9±0.4	7.8±1.2	7.5±0.7	7.3±1.6	7.4±1.0
Feed / gain					
Day 0 – Day 14	2.2±0.8	2.6±0.5	2.6±0.2	2.6±0.6	2.6±0.3

[1] Treatments were C: control; T: tylosin; P: probiotic; L: lactulose; PL: combination of P and L.

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

Table 13 Effect of treatments on blood pictures of the rats administered with *E.coli* suspension.

	Treatment[1]				
	C	T	P	L	PL
Hct (%)					
Day 0	37.6±3.8	38.0±4.2	42.3±0.1	42.3±0.1	41.8±3.3
Day 7	43.8±4.5	41.8±1.8	39.0±6.1	45.8±2.8	42.0±6.2
Day 14	42.5±5.8	45.4±2.1*	44.4±2.8	45.3±1.7	46.0±0.8
Hb (g/dl)					
Day 0	11.6±0.6	12.2±0.1	12.9±0.7	13.0±1.2	12.6±1.4
Day 7	12.8±1.0	12.7±0.5	11.2±0.5*	12.5±1.4	11.1±0.6*
Day 14	12.4±2.0	13.4±0.5*	12.5±0.5	13.7±1.1	13.5±0.4
WBC ($\times 10^3/mm^3$)					
Day 0	3.2±0.6	2.8±0.8	3.1±0.8	3.4±0.4	3.3±1.5
Day 7	4.5±0.3[b*]	4.4±0.5[b*]	2.6± 0.9[a]	4.1±0.9[b]	3.0±0.5[a]
Day 14	3.3± 0.7	3.0±1.3	3.3±0.7	3.5±0.9	3.7±1.5

[1] Treatments were C: control; T: tylosin; P: probiotic; L: lactulose; PL: combination of P and L.

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

* Means in the same column differed significantly (P<0.05) from the control (day 0).

Table14 Effect of treatments on the intestinal pH of the rats administered with *E.coli* suspension.

Intestinal part	Treatment[1]				
	C	T	P	L	PL
Proximal jejunum (PJ)					
Day 0	6.4±0.2	6.4±0.2	6.5±0.1	6.6±0.1	6.5±0.2
Day 7	6.4±0.2^{b}	6.5±0.1^{b}	6.1±0.1^{a*}	6.0±0.2^{a*}	6.4±0.2^{b}
Day 14	6.3±0.1	6.2±0.0	6.3±0.1*	6.2±0.0*	6.2±0.2
Disral jejunum (DJ)					
Day 0	6.6±0.1	6.6±0.2	6.6±0.2	6.8±0.2	6.6±0.1
Day 7	6.5±0.2	6.5±0.3	6.2±0.2*	6.2±0.3	6.5±0.2
Day 14	6.2±0.0*	6.3±0.2	6.2±0.0*	6.5±0.6	6.3±0.2*
Ileum (I)					
Day 0	7.6±0.2	7.6±0.1	7.6±0.0	7.6±0.3	7.5±0.1
Day 7	7.3±0.3	7.4±0.1	7.2±0.4	7.2±0.5	7.1±0.6
Day 14	6.8±0.7*	7.2±0.2*	7.3±0.4	6.8±0.7*	6.7±0.3
Cecum (CE)					
Day 0	7.3±0.1	7.0±0.2	6.8±0.5	6.9±0.3	6.9±0.2
Day 7	6.7±0.4^{ab*}	6.8±0.4^{b}	6.3±0.2^{a*}	6.1±0.1^{a*}	6.3±0.1^{a*}
Day 14	6.2±0.1*	6.3±0.2*	6.3±0.1*	6.3±0.2*	6.3±0.1*
Colorectum (CR)					
Day 0	7.1±0.4	7.1±0.5	6.6±0.2	6.7±0.4	6.8±0.2
Day 7	6.5±0.1*	6.7±0.2	6.2±0.0*	6.3±0.4	6.4±0.0*
Day 14	6.2±0.0*	6.2±0.0*	6.1±0.1*	6.2±0.1*	6.3±0.1*

[1] Treatments were C: control; T: tylosin; P: probiotic; L: lactulose; PL: combination of P and L.

$^{a, b, c}$ Means in the same row with different superscripts differed significantly ($P<0.05$).

* Means in the same column differed significantly ($P<0.05$) from the control (day 0).

Effect of treatments on SCFAs concentrations in the intestine of the rats administered with *E.coli* suspension.

The concentrations of SCFAs in the small intestine are shown in Table 15. Only acetate and butyrate were detected and there was difference among the treatments.

The concentrations of SCFAs in the large intestine are shown in Table 16. At day 7, PL increased ($P<0.05$) acetate, propionate, butyrate and valerate more than control. P significantly increased ($P<0.05$) acetate, propionate and butyrate, while L increased ($P<0.05$) propionate and butyrate when compare with control. Propionate, butyrate and valerate of group T were significantly lower ($P<0.05$) than control (Figure 9-12).

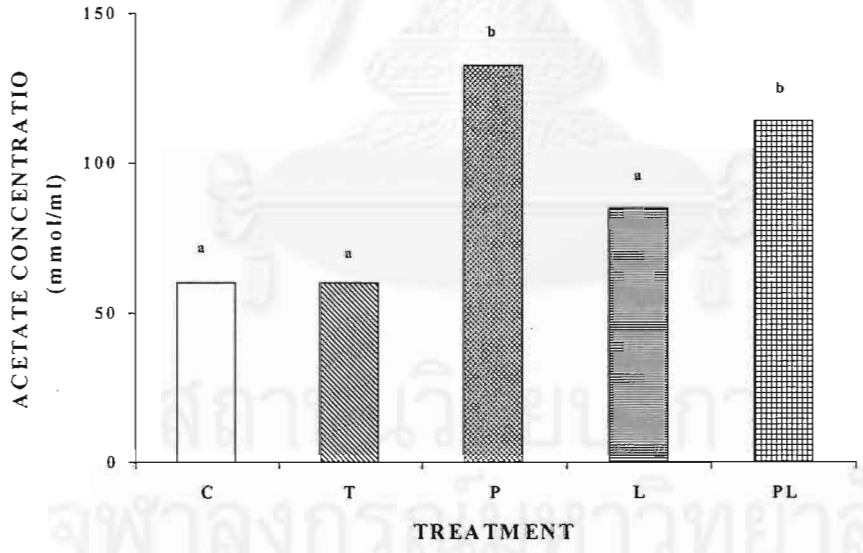


Figure 9. Effect of treatments on acetate concentration (mmol/ml) at day 7 in the large intestine of rats administered with *E.coli* suspension.

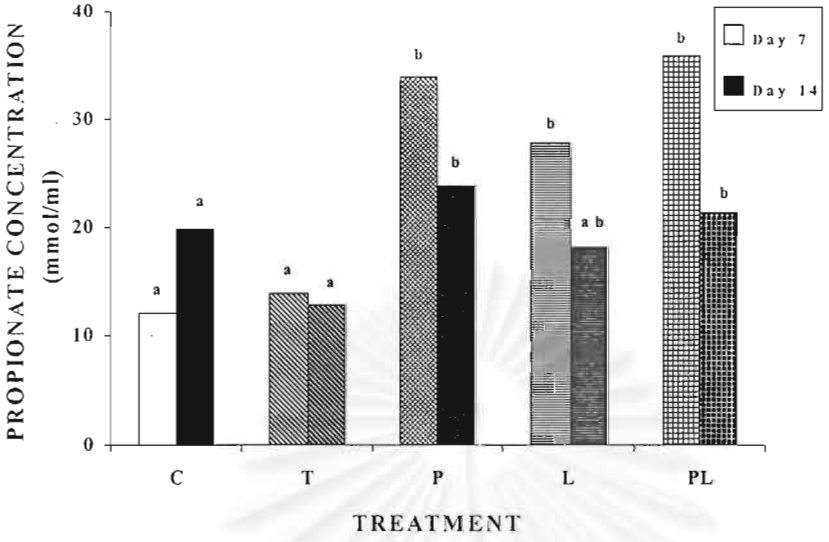


Figure 10. Effect of treatments on propionate concentration (mmol/ml) at day 7 and day 14 in the large intestine of rats administered with *E.coli* suspension.

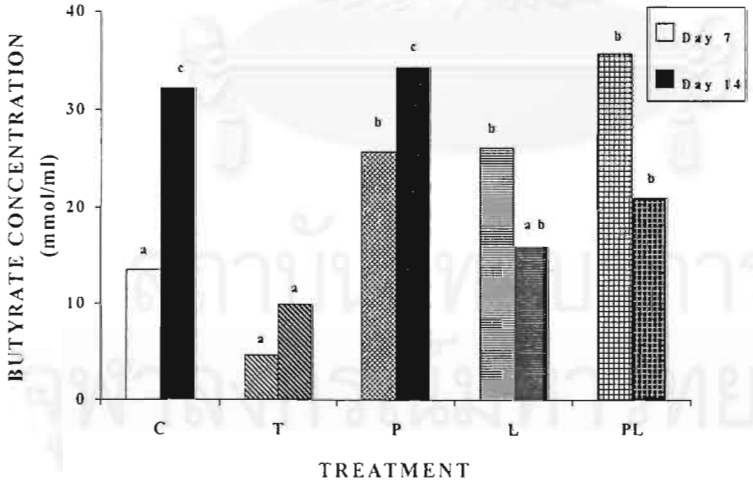


Figure 11. Effect of treatments on butyrate concentration (mmol/ml) at day 7 and day 14 in the large intestine of rats administered with *E.coli* suspension.

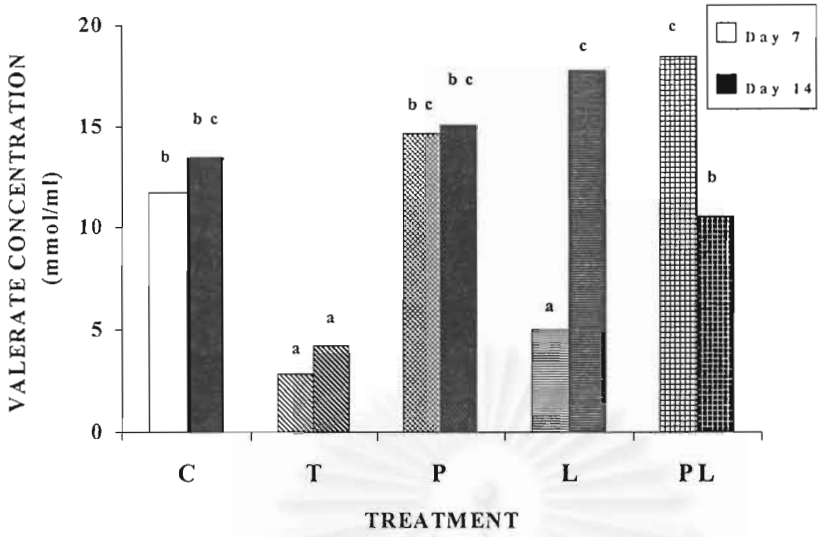


Figure 12. Effect of treatments on valerate concentration (mmol/ml) at day 7 and day 14 in the large intestine of rats administered with *E.coli* suspension.

Table 15 Effect of treatments and days on the short-chain fatty acid (SCFA) concentrations (mmol/ml) in the small intestine of the rats administered with *E.coli* suspension.

SCFA	Treatment[1]				
	C	T	P	L	PL
Acetate					
Day0	10.93±4.13	8.68±3.68	12.07±2.71	9.84±2.06	11.63±2.13
Day7	8.82±5.50	14.48±8.17	14.20±7.87	8.60±3.79	6.18±7.14
Day14	8.84±3.95	14.94±6.67	8.25±5.94	8.76±5.79	9.82±3.50
Propionate*					
Day0	NR	NR	NR	NR	NR
Day7	NR	NR	NR	NR	NR
Day14	NR	NR	NR	NR	NR
Butyrate					
Day0	1.87±0.51	1.88±0.81	2.23±0.93	2.45±0.47	2.50±0.67
Day7	1.98±0.93	3.54±2.62	2.15±1.46	1.66±0.81	2.48±1.94
Day14	3.32±3.73	2.16±0.59	2.58±1.39	1.62±1.09	2.64±1.91
Valerate*					
Day0	NR	NR	NR	NR	NR
Day7	NR	NR	NR	NR	NR
Day14	NR	NR	NR	NR	NR

[1] Treatments were C: control; T: tylosin; P: probiotic; L: lactulose; PL: combination of P and L.

NR The values were not remarkable.

Table 16 Effect of treatments and days on the short-chain fatty acid (SCFA) concentrations (mmol/ml) in the large intestine of the rats administered with *E.coli* suspension.

SCFA	Treatment[1]				
	C	T	P	L	PL
Acetate					
Day0	87.24±14.78	93.88±20.76	80.50±18.02	76.06±9.26	78.10±7.47
Day7	59.92±14.89^{a*}	59.98±21.70^{a}	132.58±26.66^{b*}	84.90±7.76^{a}	114.00±9.97^{b*}
Day14	84.94±18.66	78.25±11.90	80.05±16.75	75.14±10.38	67.46±12.91
Propionate					
Day0	28.27±5.65	27.38±0.70	22.10±7.95	23.55±5.80	22.43±3.47
Day7	12.03±2.09^{a*}	13.88±3.96^{a*}	33.90±6.55^{b*}	27.75±9.64^{b}	35.83±4.41^{b*}
Day14	19.76±3.74^{b*}	12.88±3.78^{a*}	23.78±2.34^{b}	18.10±6.54^{ab}	21.35±2.59^{b}
Butyrate					
Day0	16.80±6.56	19.54±2.81	14.18±6.56	15.24±6.53	13.30±1.08
Day7	13.53±1.54^{a}	4.60±1.54^{a*}	25.60±7.47^{b}	25.60±7.47^{b}	35.63±3.04^{b*}
Day14	32.20±5.91^{c*}	9.93±2.15^{a*}	34.23±12.26^{c*}	15.78±1.63^{ab}	20.78±2.60^{b*}
Valerate					
Day0	9.08±5.63	9.26±5.32	6.32±1.48	7.36±2.14	7.72±1.90
Day7	11.75±1.03^{b}	2.80±3.93^{a}	14.63±3.86^{bc*}	4.96±4.69^{a}	18.43±4.32^{c*}
Day14	13.43±3.21^{bc}	4.20±5.79^{a}	15.03±5.07^{bc*}	17.70±3.66^{c*}	10.58±1.78^{b}

[1] Treatments were C: control; T: tylosin; P: probiotic; L: lactulose; PL: combination of P and L.

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

[*] Means in the same column differed significantly ($P<0.05$) from the control (day 0).

Effect of treatments on the concentration of the intestinal mucosal DNA of the rats administered with *E.coli* suspension.

Intestinal mucosa of the rat infected with *E.coli* at the PJ, DJ, I, CE and CR were analyzed for the concentrations of DNA (Table 17). There were no significant effect of treatments on DNA concentration in PJ, DJ, CE and CR. In the ileum, total DNA in PL group at day 14 was significantly higher ($P<0.05$) than control. The distal jejunal DNA were lower at day 14 compared to day 0 in all treatments.

Effect of treatments on the concentrations of the intestinal mucosal RNA of the rats administered with *E.coli* suspension.

The intestinal mucosal RNA concentrations of the rat infected with *E.coli* are shown in Table 18. There was a significant effect of treatment in the I segment at day 7, the RNA concentrations in group L was lower than those in groups C, T and P ($P <0.05$). In CR, total RNA at day7 of PL group was higher ($P<0.05$) than others and higher than day 0, while total RNA in other groups were not different from day 0.

Table 17 Effect of treatments and days on the concentrations of intestinal mucosal DNA (mg/g intestinal mucosal protein) of the rats administered with *E.coli* suspension.

Intestinal part	Treatment[1]				
	C	T	P	L	PL
Proximal jejunum (PJ)					
Day 0	1.25±0.31	1.24±0.15	1.07±0.53	1.32±0.09	1.26±0.28
Day 7	1.22±0.38	0.97±0.23	1.13±0.27	0.94±0.44	1.31±0.24
Day 14	0.40±0.16*	0.68±0.21*	0.83±0.48	0.48±0.12*	0.92±0.14
Distal jejunum (DJ)					
Day 0	1.15±0.38	1.21±0.16	1.04±0.11	1.21±0.41	1.26±0.28
Day 7	0.62±0.30	0.34±0.16*	0.77±0.42	0.80±0.31	0.88±0.18
Day 14	0.20±0.10*	0.34±0.28*	0.32±0.24*	0.14±0.04*	0.51±0.43*
Ileum (I)					
Day 0	1.67±0.67	1.74±0.37	1.24±0.73	1.57±0.18	1.54±0.19
Day 7	1.29±0.32	0.90±0.52*	1.45±0.36	1.09±0.58	1.41±0.45
Day 14	0.52±0.17^{ab*}	0.43±0.38^{a*}	1.01±0.20^{bc}	0.80±0.19^{ab*}	1.32±0.37^{c}
Cecum (CE)					
Day 0	1.48±0.02	1.30±0.38	1.15±0.26	1.46±0.30	1.45±0.19
Day 7	1.13±0.65	2.14±0.61	2.20±0.51	1.77±0.55	2.02±0.26*
Day 14	1.95±0.72	1.98±0.62	1.77±0.93	1.91±0.34	1.04±0.21
Colorectum (CR)					
Day 0	1.37±0.34	1.33±0.51	1.21±0.28	1.40±0.03	1.25±0.21
Day 7	1.52±0.33	1.30±0.25	1.32±0.22	1.16±0.40	1.63±0.37
Day 14	1.27±0.40	1.27±0.43	1.14±0.35	1.09±0.36	1.10±0.37

[1] Treatments were C: control; T: tylosin; P: probiotic; L: lactulose; PL: combination of P and L.

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

* Means in the same column differed significantly (P<0.05) from the control (day 0).

Table 18 Effect of treatments and days on the concentrations of intestinal mucosal RNA (mg/g intestinal mucosal protein) of the rats administered with *E.coli* suspension.

Intestinal part	Treatment[1]				
	C	T	P	L	PL
Proximal jejunum (PJ)					
Day 0	1.09±0.04	0.89±0.09	1.07±0.06	0.92±0.16	0.97±0.27
Day 7	0.95±0.24	1.05±0.03	1.03±0.03	1.04±0.43	0.93±0.04
Day 14	0.84±0.37	1.02±0.24	0.85±0.17*	0.85±0.10	0.81±0.21
Distal jejunum (DJ)					
Day 0	1.09±0.18	1.07±0.15	1.19±0.09	0.99±0.37	0.89±0.17
Day 7	1.34±0.59	1.33±0.36	1.53±0.27	1.31±0.41	1.31±0.73
Day 14	1.26±0.49	0.99±0.18	1.08±0.46	1.10±0.14	1.03±0.08
Ileum (I)					
Day 0	1.03±0.32	1.20±0.31	0.96±0.38	0.93±0.26	1.22±0.12
Day 7	1.49±0.10^{b}	1.56±0.26^{b}	1.53±0.35^{b}	1.07±0.16^{a}	1.18±0.20^{ab}
Day 14	1.16±0.22^{ab}	1.57±0.22^{b}	1.34±0.31^{ab}	0.92±0.13^{a}	1.06±0.25^{a}
Cecum (CE)					
Day 0	0.98±0.35	1.04±0.15	0.85±0.25	0.89±.0.07	0.89±0.10
Day 7	0.87±0.21	0.86±0.17	0.87±0.21	0.87±0.18	0.87±0.17
Day 14	0.91±0.22	0.90±0.25	0.87±0.13	0.92±0.36	1.02±0.38
Colorectum (CR)					
Day 0	0.97±0.30	0.87±0.23	1.01±0.39	0.90±0.18	0.95±0.15
Day 7	0.93±0.15^{a}	1.16±0.14^{a}	1.24±0.35^{a}	1.32±0.27^{a}	1.94±0.37^{b*}
Day 14	1.22±0.47	1.11±0.16	1.12±0.62	1.33±0.26	1.11±0.34

[1] Treatments were C: control; T: tylosin; P: probiotic; L: lactulose; PL: combination of P and L.

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

* Means in the same column differed significantly (P<0.05) from the control (day 0).

Effect of treatments on the disaccharidase activities.

Maltase

The alterations in maltase activity in the small intestine are shown in Table 19. Maltase activities were high in both proximal jejunum and distal jejunum. The mean values of maltase activity in the ileum were approximately half of those in the jejunum. At day 7, maltase activities in I were significantly lower ($P<0.05$) in L and PL group.

Sucrase

The sucrase activities in mucosal tissue of the small intestine are depicted in Table 20. Sucrase activities in DJ at day 7 were significantly higher ($P<0.05$) than other groups.

Lactase

The lactase activities in mucosal tissue of the small intestine are demonstrated in Table 21. No significant effect of treatments on lactase activity was observed.

Table 19 Effect of treatments and days on the maltase activities (unit) in the mucosal tissue of the small intestine of the rats administered with *E.coli* suspension.

Intestinal part	Treatment[1]				
	C	T	P	L	PL
Proximal jejunum (PJ)					
Day 0	395.0±161.1	432.7±89.9	445.1±95.1	461.6±163.6	458.8±52.0
Day 7	424.6±153.6	363.2±45.0	381.5±52.9	355.8±96.0	367.1±48.1
Day 14	386.0±34.0	468.1±46.2	432.2±96.4	361.8±31.1	357.9±102.5
Distal jejunum (DJ)					
Day 0	376.3±60.0	408.8±22.8	480.7±74.5	352.4±89.9	376.3±89.7
Day 7	396.4±25.6	386.2±42.3	348.1±10.7	340.4±56.4	404.3±61.9
Day 14	376.1±36.0	394.3±137.1	421.3±118.0	448.3±75.2	401.5±78.5
Ileum (I)					
Day 0	200.6±33.7	225.0±49.6	225.8±68.5	207.5±27.0	232.4±23.3
Day 7	258.8±48.1^{b}	218.0±26.6^{ab}	257.1±22.9^{b}	201.8±27.7^{a}	193.1±21.6^{a}
Day 14	193.3±59.5	182.6±32.8	210.2±11.5	190.2±37.4	183.8±50.5

[1] Treatments were C: control; T: tylosin; P: probiotic; L: lactulose; PL: combination of P and L.

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

Table 20 Effect of treatments and days on the sucrase activities (unit) in the mucosal tissue of the small intestine of the rats administered with *E.coli* suspension.

Intestinal part	Treatment[1]				
	C	T	P	L	PL
Proximal jejunum (PJ)					
Day 0	124.3±31.3	106.9±7.8	131.2±27.0	102.5±15.3	106.8±16.5
Day 7	68.5±31.8	85.0±12.3	74.0±10.0*	55.6±29.1	80.9±10.2
Day 14	69.2±11.8	91.8±38.4	128.3±23.9	85.3±11.7	92.5±41.5
Distal jejunum (DJ)					
Day 0	79.3±0.5	88.4±15.6	74.9±41.3	80.0±9.1	81.0±20.6
Day 7	44.8±18.6[a]	83.6±21.8[b]	43.3±8.7[a]	47.0±20.0[a]*	64.4±2.9[ab]
Day 14	79.6±25.6	62.7±20.3	76.2±10.0	73.0±11.3	65.4±28.1
Ileum (I)*					
Day 0	-	-	-	-	-
Day 7	-	-	-	-	-
Day 14	-	-	-	-	-

[1] Treatments were C: control; T: tylosin; P: probiotic; L: lactulose; PL: combination of P and L.

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

* Means in the same column differed significantly ($P<0.05$) from the control (day 0).

* The activity was not detected due to sample loss.

Table 21 Effect of treatments and days on the lactase activities (unit) in the mucosal tissue of the small intestine of the rats administered with *E.coli* suspension.

Intestinal part	Treatment[1]				
	C	T	P	L	PL
Proximal jejunum (PJ)					
Day 0	22.6±6.8	26.6±8.2	24.0±8.0	18.1±5.0	23.6±4.6
Day 7	19.0±3.5	18.8±1.7	11.6±1.6*	11.3±4.3	16.2±5.6
Day 14	18.2±6.0	24.2±12.9	23.5±6.0	19.9±5.5	20.2±5.6
Distal jejunum (DJ)					
Day 0	15.8±8.2	20.7±2.9	20.8±6.6	17.2±7.1	21.4±4.4
Day 7	12.1±6.0	15.5±6.4	8.7±4.4	8.2±4.0*	9.7±4.2
Day 14	16.1±3.2	18.7±4.1	13.8±6.2	15.1±3.7*	14.3±4.1
Ileum (I)					
Day 0	2.4±0.6	1.7±0.3	1.9±0.9	2.1±0.9	2.3±0.6
Day 7	3.4±1.3	3.5±2.0	2.8±1.1	2.3±0.4	2.3±0.4
Day 14	2.0±0.3	2.4±1.2	2.5±1.1	1.5±0.4	2.5±0.7

[1] Treatments were C: control; T: tylosin; P: probiotic; L: lactulose; PL: combination of P and L.
Means in the same column differed significantly ($P<0.05$) from the control (day 0).

CHAPTER V
DISCUSSION

Effect of tylosin (T), probiotic (P), lactulose (L) and combination of lactulose and probiotic (PL) on various parameters of rats.

T, P, L and PL were administered to the experimental rats to examine their effects on various parameters compare to those of the control rats. In experiment 1, the rats were reared in a conventional condition. All rats were healthy and there was no sign of the GI upsets. Experiment 2 was set to study the effect of treatments on the rats that were induced to have an imbalance of the GI microbiota. The rats were administered with hemolytic *E.coli* (10^8 CFU per ml) once daily for 5 consecutive days before the treatment were conducted. It was found that all rats had no diarrhea and only soft feces were seen. Due to the different batch of rats used, the BW of rats at the beginning in the experiment 2 (128 g) was higher than those in the experiment 1 (98 g). This difference influenced the feed intake of the rats and caused higher average growth performance for instances, the ADG.

Effect on the growth performance of the rats.

The result showed that oral administration of T, P or L to the rats did not affect the growth of the rats. In addition it was found that feed intake was reduced by given L and PL. The reduction might be due to the sticky solution of lactulose thus interfered with normal ingestion of feed. Previous reports showed that there was no effect of probiotic on growth of the animal (Hale and Newton, 1979; Pollmann et al., 1980; Tortuero, et al., 1995). Heijnen (1993) showed that final body weight and feed intake of rats fed on the lactulose supplemented diet was lower than the control rats. Beames (1969) found that the use of tylosin as feed additive in rat meal reduced body

weight of the rat. There was no clinical digestive upset in all rats in this experiment. Thus, the administration of antibiotic, probiotic or lactulose to healthy rat did not help to increase growth performance. For probiotic, the beneficial effect is maximized if it can be applied as soon as possible after birth to establish microbiota environment (Tournut, 1994). Implantation of exogenous bacteria after microbial establishment in the gut was difficult to achive. It is possible that the rats were all in good environment and were given all treatments for only 2 weeks. Therefore the changes in growth performance of each treatment were not different from control.

The rats received L had feed intake more than those given C. In the first week, live weight of rats in the PL group was lower than those in the C and the T. The ADG of rat in the T group was much more than those in the P and the PL. The lactulose solution might be the factor that lowered the growth performance of the rats due to the sticky solution of lactulose causing lower feed intake.

Effect on the blood pictures.

The blood pictures were not affected by different treatmets. It was found that there was no treatment effect on blood pictures in experiments 1 (Table 3). In the experiment 2 (Table 13), administration of *E.coli* suspension did not cause changes in Hct and Hb. After administration the treatments for 7 days, it was found that P and PL decreased WBC when compared with the control but those values were still in normal range of the rats. It is possible that the amount and time of giving *E.coli* suspension was too little to alter the function of the intestinal tract as well as blood pictures. Moreover, the hemolytic *E.coli* used in experiment 2 was isolated from the lung of pigs, which was the crude extract. Therefore it is possible that the hemolytic *E.coli* used in experiment 2 did not have a deleterious effect on the intestinal tract compared to those used in Macias et al. (1993). They reported that enterotoxigenic *E.coli* caused the mortility of the infected rats.

Effect on pH of intestinal contents.

In the experiment 1, there was no effect of treatments on pH of the intestinal contents (Table 4). In experiment 2, it was found that P and L decreased pH of the PJ contents. P, L and PL decreased pH of the CE contents more than T but were not differed from those of the control (Table 14). Thomlinson (1981) showed that probiotic synthesized lactate and acetate with subsequent reduction in the intestinal pH. In the case of lactulose, it is the undigestible sugar that escaped digestion and absorption by the host. Thus the microflora in the large intestine could digest lactulose and yielded SCFAs which reduced the intestinal pH. Lactulose was combined with probiotic because lactulose is a substrate for LAB fermentation, therefore they worked synergistically and produced SCFAs. But in the present result showed no effect of PL on the intestinal pH.

The pH values in the present experiment were not quite accurate because they were measured by pH paper. The values detected were more or less subjective and had some error. In the other reports, the measurement using microelectrode pH meter produced more consistent data (Heijnen, et al., 1993).

Effect on SCFAs in the intestinal content.

Propionate and valerate were not found in the small intestine in both experiments (Table 5 and Table 15). Though acetate and butyrate were found, the concentrations were substantially lower than those in the large intestine. SCFAs in the intestine are produced from the bacterial fermentation of undigested food materials (Fooks et al., 1999). There are low bacterial concentrations (10^5 bacterial/ml contents) in the small intestine. The essential mechanism which maintains this relative sterility of the upper digestive tract is the gastro-intestinal transit and in particular the interdigestive migrating motor complex (Rambard, 1992). There are more bacterial population in the terminal ileum (10^8 /ml) than the proximal small intestine, with the appearance of enterobacter and strict anaerobes

(Rambard, 1992). Thus, less SCFAs concentrations were found in the small intestinal contents. However, in the experiment 1, it was found that the acetate concentrations of rats in the P group at day 7 were significantly higher than other groups. It is possible that probiotic benefit the host animal by produced acetate in the small intestine.

In the lower gut, there were high concentrations of SCFAs (Table 6 and Table 16). It was found that T lowered valerate concentration in the experiment 1 and lowered propionate, butyrate and valerate in the experiment 2. The SCFAs concentrations altered due to changes in microbiota population in the intestinal tract. Antibiotic suppressed not only microorganism which were harmful to the host, but the normal flora was affected also (Hay, 1987). Cherbut et al. (1991) showed that antibiotic reduced intestinal microflora of the rat, which was consistent with the reduction of SCFAs in the feces. Moreover, L decreased valerate concentrations more than the control. It is possible that L prominently stimulated the growth of LAB more than valeric acid-producing bacteria. In the experiment 2, at day 7 after the treatments were given, it was found that P increased acetate, propionate and butyrate in the lower gut of the rats more than those in the control. The probiotic is LAB that produces lactic acid and acetic acid from fermentation of carbohydrates. It is possible that P fermented undigestible carbohydrate in lower gut and produced those acids. In this experiment, it was found that L increased propionate and butyrate. Lactulose is the undigestible carbohydrate that was fermented by intestinal microbial and yielded SCFAs. It is possible that lactulose promote the growth of propionate producing-bacteria. The combination of probiotic and prebiotic (PL) had advantage to the animal by increasing acetate, propionate, butyrate and valerate in the lower gut after 7 day of administration.

Effect on the intestinal mucosal DNA and RNA.

Total DNA and RNA concentrations in the intestinal mucosa represented the tissue growth and protein synthesis, respectively. In experiment 1, lactulose increased DNA concentration in PJ, DJ, I, CE and CR, (Table 7). This finding was similar to the work performed by Lupton et at. (1995) who studied the effect of dietary modification on the colonic epithelial cell cycle in rats. They reported that the percentage of cells in S shape (actively synthesizing DNA) was highest in the rats given lactulose. Johnson et al. (1988) indicated that ingestion of the complex and viscous polysaccharides (such as guar gum) could affect the crypt cell production rate. There were other reports showed that SCFAs stimulated epithelial cell proliferation (Sakata and Engelhardt, 1983; Sakata, 1987). This indicated that the fermentable fiber might induce changes in the mucosa via SCFAs production. Butyrate alone or combination of SCFAs (acetate, propionate and butyrate) raised parameters of intestinal mucosal cellularity: mucosal weight, protein, RNA and DNA (Kripke et al., 1989 reviewed by Sceppard, 1994). In contrast to L, it was found that P lowered DNA concentrations in the CR than C. It is possible that the bacteria formed a protective barrier on the gut wall, alleviating the luminal effect of toxins, and resulted in the decrease of the renewal rates of the enterocytes (Nousiainen, 1991) as reflected in the reduction of the DNA concentration. In experiment 2, it was found that PL increased total DNA concentrations in the ileum and total RNA concentrations in the colorectum. It is possible that PL stimulated the mucosal cellularity by SCFAs production.

Effect on the disaccharidase activities.

Hampson (1986) proposed that the animal at weaning showed a significant increase in the crypt depth and the increase in the complexity of villus morphology with a dramatic reduction in the villus height. These resulted in the reduction of the small intestinal absorptive area and the appearance of a less mature enterocyte

population. The more immature enterocytes resulted in the reduction of enzyme production. The supplementation of probiotic or antibiotic may be useful for the production of disaccharidase enzymes. Collinton et al. (1990) stated that inclusion of either probiotic or antibiotic had significant effects on the development of sucrase, lactase and tripeptidase. The present results showed that the administration of L and PL increased maltase activities in the ileum at day 7. T, P, L and PL increased lactase activities in the DJ while T and P increased lactase activities in the ileum. It is possible that the supplementation of tylosin, probiotic or lactulose affected the mucosal cell function as seen in the improvement of disaccharidase activity. Morover, it was found that maltase activities in the proximal jejunum changed in all treatment when the rats were older. Henning (1981) suggested that the activities of maltase and sucrase were low at birth and increased after birth while lactase was high at birth and declined after birth. In experiment 2, the supplement of probiotic, lactulose or probiotic combination with lactulose did not improve the disaccharidase activities of the rats administered with *E.coli* suspension.

In conclusion the supplementation of tylosin, probiotic or lactulose did not promote the growth and changes in blood pictures of the rats and rats administered with *E.coli* suspension. The administration of tylosin, probiotic or lactulose improved maltase and lactase activities of the rats in the experiment 1 but did not affect disaccharidase activities of the rats administered with *E.coli* suspension. The major effects of the supplementation were on the SCFAs concentrations in the lower gut of the experimental rats. Probiotic, lactulose and probiotic combined with lactulose affected the SCFAs concentration in the large bowel contents of the rats administered with *E.coli* suspension. They increased acetate, propionate, butyrate and valerate concentrations, while tylosin decreased SCFAs concentrations.

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BIOGRAPHY

Miss Tipmon Yaigate was born on April 7, 1971 in Saraburi, Thailand. She graduated from the Faculty of Agriculture, Kasetsart University. She received bachelor degree of Science of the Agriculture in 1992. She works at Tubkwang Research Station in Saraburi province.