ENHANCING PRODUCTIVITY AND CHEVON CONJUGATED LINOLEIC ACID (CLA) CONTENT IN GROWING GOATS USING PROBIOTICS TOGETHER WITH PLANT OILS

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A Thesis Submitted in Partial Fulfillment of the Requirements for the Degree of Doctor of Philosophy in Animal Production Technology

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การปรับปรุงประสิทธิภาพการผลิตและการสะสม conjugated linoleic acid (CLA) ในเนื้อแพะระยะกำลังเจริญเติบโตโดยใช้โปรไบโอติคร่วมกับน้ำมันพืช

นายหยง ฮาน

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรดุษฎีบัณฑิต
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Suranaree University of Technology has approved this thesis submitted in partial fulfillment of the requirements for the Degree of Doctor of Philosophy.

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PROBIOTICS / SOYBEAN OIL / SUNFLOWER OIL / GROWING GOATS / CLA

The aim of present study was to investigate the effects of dietary supplemental probiotics, soybean and sunflower oil, and a combination of probiotics plus soybean oil or sunflower oil on growth performance, rumen metabolism, plasma CLA content, carcass and meat quality, and meat CLA content of stall-fed growing goats fed with whole plant corn silage. The study was conducted by 3 affiliated experiments.

Experiment 1: Twenty-four crossbred (Thai native x Anglo-Nubian) growing goats that weighed 14.2±2.3 kg, aged about 6 months, were allocated to 4 treatments according to Randomized Complete Block Design (RCBD) with 6 goats in each treatment. The treatments consisted of 0, 2.5, 5.0 and 7.5 g/h/d supplementation of probiotics. The results indicated a significant improvement of ADG ($P<0.05$), stabilization of rumen pH, a significant increase of NDF digestibility and rumen viable microbes ($P<0.05$), and a significant increase of plasma CLA. In addition, this experiment verified that 2.5, and 5.0 g/h/d probiotics attained better results in stall-fed growing goats fed with whole plant corn silage.

Experiment 2: Thirty growing crossbred (Thai native x Anglo-Nubian) goats, aged about 6 months, weighed 14.8±2.5 kg, were allocated to 5 treatments according to RCBD with 6 goats in each treatment. The treatments were the control, 2.5, 5.0% soybean oil, and 2.5, 5.0% sunflower oil. The results showed that ADG and feed efficiencies significantly increased (soybean oil: $P<0.01$; sunflower oil: $P<0.05$); NH_3-N significantly reduced (soybean oil: $P<0.01$; sunflower oil: $P<0.05$); N absorption and retention increased ($P<0.05$); CLA content significantly enhanced ($P<0.01$). This experiment testified that the administration of soybean oil in diet of stall-fed growing goats fed with whole plant corn silage achieved better results than that of sunflower oil.

Experiment 3: The thirty goats that were used in Experiment 2 were prepared for this experiment with a 5-week adjustment. The animals were allocated to 5 treatments according to factorial arrangement on RCBD with 6 goats in each treatment. The treatments were the control, 2 levels of soybean oil (2.5 and 5.0%), and 2 levels of probiotics (2.5 and 5.0 g/h/d). The results showed that the ADG and feed efficiency increased significantly ($P<0.05$) with the supplementation of plant oils and probiotics. There was a distinct interaction between the supplementation of soybean oil and probiotics on the increase of ADG ($P=0.07$) and feed conversion ($P=0.04$). There was a significant synergized effect on nitrogen absorption ($P=0.07$) and total VFA ($P=0.05$) for soybean oil and probiotics supplementation. The plasma CLA increased significantly ($P<0.01$). There was a significant synergized impact between soybean oil and probiotics on the increase of CLA isomers in plasma. The meat

quality was improved. The meat C18:c9, t11 CLA increased 100 to 139.6% ($P<0.01$); the C18:t10, c12 CLA increased 100 to 300% ($P<0.01$). A significant synergized effect between soybean oil and probiotics on meat CLA isomers was found ($P<0.05$).

The overall results showed that administration of 2.5 and 5.0 g/h/d probiotics in diet of growing goats fed with whole plant corn silage improved animals' growth performance and feed conversion ($P<0.05$), optimized rumen metabolism, and increased plasma CLA content ($P<0.01$). The supplementation of 2.5 and 5.0% soybean oil or sunflower oil increased growing goats' ADG and feed efficiency ($P<0.05$) without negative impact on rumen metabolism, and significantly increased plasma CLA ($P<0.01$). The supplementation of soybean oil together with probiotics significantly improved animals' growth performance and feed conversion ($P<0.05$), optimized rumen metabolism, and increased plasma CLA content ($P<0.01$). The combined supplementation of soybean oil and probiotics enhanced carcass and meat quality ($P<0.05$), and significantly increased the meat CLA content ($P<0.01$).

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หยง ฮาน : การปรับปรุงประสิทธิภาพการผลิตและการสะสม conjugated linoleic acid (CLA) ในเนื้อแพะระยะกำลังเจริญเติบโตโดยใช้โปรไบโอติคร่วมกับน้ำมันพืช (ENHANCING PRODUCTIVITY AND CHEVON CONJUGATED LINOLEIC ACID (CLA) CONTENT IN GROWING GOATS USING PROBIOTICS TOGETHER WITH PLANT OILS) อาจารย์ที่ปรึกษา : ผู้ช่วยศาสตราจารย์ ดร.ปราโมทย์ แพงคำ, 244 หน้า

การศึกษาในครั้งนี้มีวัตถุประสงค์เพื่อศึกษาผลของการเสริมโปรไบโอติคน้ำมันถั่วเหลืองและน้ำมันทานตะวันและการใช้ร่วมกันระหว่างโปรไบโอติคน้ำมันถั่วเหลืองและน้ำมันทานตะวันต่อประสิทธิภาพของการเจริญเติบโต เมแทบอลิซึมในรูเมน conjugated linoleic acid (CLA) ในพลาสมาในแพะที่ได้รับต้นข้าวโพดหมักเป็นอาหาร โดยการศึกษาประกอบด้วย 3 การทดลอง

การทดลองที่ 1 ใช้แพะเนื้อพันธุ์ลูกผสม (พื้นเมืองไทยและแองโกลนูเบียน) จำนวน 24 ตัว น้ำหนักเฉลี่ย 14.2±2.3 กก. อายุประมาณ 6 เดือน ใช้แผนการทดลองแบบ Randomized Complete Block Design (RCBD) แบ่งแพะออกเป็น 4 กลุ่มๆ ละ 6 ตัว โดยเสริมโปรไบโอติค 0, 2.5, 5.0 และ 7.5 กรัม/ตัว/วัน ผลการศึกษาพบว่า อัตราการเจริญเติบโตเพิ่มขึ้นอย่างมีนัยสำคัญทางสถิติ ($P<0.05$) การย่อยได้ของ neutral detergent fiber (NDF) จุลินทรีย์ในรูเมนและ CLA ในพลาสมาสูงขึ้นอย่างมีนัยสำคัญทางสถิติ ($P<0.05$) ส่วนความเป็นกรดด่างในรูเมนไม่มีความแตกต่างกันทางสถิติ จากผลการทดลองสามารถสรุปได้ว่าระดับของโปรไบโอติคที่เหมาะสมในอาหารแพะที่ได้รับต้นข้าวโพดหมัก เป็นอาหารหยาบอยู่ระหว่าง 2.5 ถึง 5.0 กรัม/ตัว/วัน

การทดลองที่ 2 ใช้แพะเนื้อพันธุ์ลูกผสม (พื้นเมืองไทยและแองโกลนูเบียน) จำนวน 30 ตัว อายุประมาณ 6 เดือน น้ำหนักเฉลี่ย 14.8±2.5 กก. ใช้แผนการทดลองแบบ RCBD ประกอบด้วย 5 กลุ่มทดลอง กลุ่มทดลองละ 6 ตัว โดยอาหารทดลองประกอบด้วย กลุ่มควบคุม เสริมน้ำมันถั่วเหลือง 2.5% และ 5.0% และเสริมน้ำมันทานตะวัน 2.5% และ 5.0 % ผลการศึกษาพบว่า อัตราการเจริญเติบโตเพิ่มขึ้นในกลุ่มที่เสริมน้ำมันถั่วเหลือง ($P<0.01$) และกลุ่มที่เสริมน้ำมันทานตะวัน ($P<0.05$) ส่วนแอมโมเนียไนโตรเจนในของเหลวจากรูเมนลดลงในกลุ่มที่เสริมน้ำมันถั่วเหลือง ($P<0.01$) และน้ำมันทานตะวัน ($P<0.05$) การดูดซึมและการกักเก็บไนโตรเจน และ CLA ในพลาสมาเพิ่มขึ้นอย่างมีนัยสำคัญทางสถิติ ($P<0.05$) ในกลุ่มที่เสริมน้ำมันทั้งสองชนิด ในการทดลองนี้พิสูจน์ได้ว่า การเสริมน้ำมันถั่วเหลืองสามารถปรับปรุงประสิทธิภาพในการผลิตแพะเนื้อที่ได้รับต้นข้าวโพดที่หมักได้ดีกว่าการเสริมน้ำมันทานตะวัน

การทดลองที่ 3 ใช้แพะเนื้อชุดเดียวกับการทดลองที่ 2 โดยปรับสัตว์ก่อนการทดลอง 5 สัปดาห์ แบ่งสัตว์ออกเป็น 5 กลุ่ม จัดกลุ่มทดลองโดย factorial in RCBD แต่ละกลุ่มประกอบด้วย

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แพะ 6 ตัว กลุ่มการทดลองประกอบด้วยการเสริมน้ำมันถั่วเหลือง 2 ระดับ (2.5 %และ 5.0 %) และการเสริมโปรไบโอติค 2 ระดับ (2.5 และ 5.0 กรัม/ตัว/วัน) ผลการศึกษาพบว่า อัตราการเจริญเติบโตและประสิทธิภาพการใช้อาหารเพิ่มขึ้น (P<0.05) ในกลุ่มที่มีการเสริมน้ำมันพืชและโปรไบโอติคและยังพบว่ามีปฏิกิริยาร่วม (P=0.07) ระหว่างปัจจัยการเสริมน้ำมันพืชและโปรไบโอติค ในการเพิ่มขึ้นของอัตราการเจริญเติบโต (P=0.07) และประสิทธิภาพการใช้อาหาร (P=0.04) การดูดซึมของไนโตรเจน (P=0.07) กรดไขมันระเหยได้รวม (P<0.05) และ CLA ในพลาสมา (P=0.01)
การใช้น้ำมันถั่วเหลืองร่วมกับโปรไบโอติคมีผลในการปรับปรุงระดับของ CLA ในพลาสมาและสามารถปรับปรุงคุณภาพซาก C18:c9, t11 CLA ในเนื้อเพิ่มขึ้น 100 ถึง 140% (P<0.01) และ C18:t10, c12 CLA เพิ่มขึ้น 100 ถึง 300% (P<0.01) การใช้น้ำมันถั่วเหลืองร่วมกับโปรไบโอติคมีผลต่อการเพิ่ม CLA ในเนื้อ (P<0.05)

โดยภาพรวมจากการศึกษาในครั้งนี้แสดงให้เห็นว่า ในการเลี้ยงแพะเนื้อพันธุ์ลูกผสมที่ได้รับต้นข้าวโพดหมักเป็นอาหารหลักและเสริมด้วยโปรไบโอติคที่ระดับ 2.5 และ 5.0 กรัม/ตัว/วัน สามารถปรับปรุงประสิทธิภาพการผลิตและประสิทธิภาพการใช้อาหารได้ (P<0.05) และยังทำให้เกิดกระบวนการหมักในรูเมนอย่างเหมาะสม และระดับ CLA ในพลาสมาเพิ่มขึ้น (P<0.01) ในขณะที่อีกการทดลองเป็นการเสริมด้วยน้ำมันพืชสองชนิดคือน้ำมันถั่วเหลืองและน้ำมันทานตะวันที่ระดับ 2.5 และ 5.0% พบว่า สามารถเพิ่มอัตราการเจริญเติบโตและประสิทธิภาพการใช้อาหารโดยไม่ทำให้เกิดผลด้านลบต่อกระบวนการหมักในรูเมนและยังทำให้ระดับ CLA (P<0.01) ในพลาสมาเพิ่มขึ้น การเสริมน้ำมันถั่วเหลืองร่วมกับโปรไบโอติคพบว่าสามารถปรับปรุงประสิทธิภาพในการเจริญเติบโต ประสิทธิภาพในการใช้อาหาร เมแทบอลิซึมในรูเมนและระดับ CLA ในพลาสมา (P<0.05) และผลของการเสริมน้ำมันถั่วเหลืองร่วมกับโปรไบโอติคยังช่วยปรับปรุงคุณภาพซาก (P<0.05) และเพิ่มระดับ CLA ในเนื้ออย่างมีนัยสำคัญทางสถิติ (P<0.01)

สาขาวิชาเทคโนโลยีการผลิตสัตว์
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ลายมือชื่อนักศึกษา_____
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LIST OF ABBREVIATIONS

CLA	=	Conjugated linoleic acid
VFA	=	Volatile fatty acid
ADG	=	Average daily gain
CF	=	Crude fiber
CP	=	Crude protein
DM	=	Dry matter
ADF	=	Acid detergent fiber
NDF	=	Neutral detergent fiber
SFA	=	Saturated fatty acid
UFA	=	Unsaturated fatty acid
RCBD	=	Randomized Complete Block Design
OM	=	Organic matter
TDMI	=	Total dry matter intake
TSFA	=	Total saturated fatty acid
PUSFA	=	Poly-unsaturated fatty acid
MUSFA	=	Mono-unsaturated fatty acid
TVFA	=	Total volatile fatty acid
NEFA	=	Non-essential fatty acid
NRC	=	National Research Council

CHAPTER I

INTRODUCTION

1.1 Rationale of the Study

1.1.1 Correlation between goats and degradation of grasslands

The degradation of world's grasslands has been inferred from present condition (Suttie et al., 2005). China, for instance, 50.24% of total amount of grassland has degraded or is degrading, the degradation of grassland is expanding with terrific speed, viz., 20,000 square kilometres per year (Jiang and Gao, 2007), and Chinese is suffering from the consequent disasters such as sand storm, debris flow, and so on. Since goats can eat the very short grass, and even dig the grass root out by their forequarters for eating, the over grazing of goats is responsible to worsening of the grassland degradation (Wang, 2007). Nevertheless, since the goat products have a favorite image, the number of goats has increased globally, even in countries with high and intermediate incomes, despite the changes in agriculture due to industrialization, globalization, and technological advances in developed countries (McMillin and Brock, 2005). The increasing number of goats certainly aggravates the grazing, and takes a bad turn of the degradation of grassland. The grazing system also contributes to the destroyed environment for the higher nitrate contamination of surface and groundwater, pathogens contamination, and also methane emissions (Siegford et al.,

governments seriously encourage raising goats with stall-feeding. Thus, an ecology and sustained stall-feeding technique that in line with the expectations of the farmers and at the same time respecting animal welfare and environmental protection for goats farming is pressingly needed to assure that the products can meet the consumers' requirements of being high- quality, safe, tasty, and wholesome.

1.1.2 Corn silage as roughage for stall-feeding growing goats

There is no doubt that grazing is less capital intensive as well as less labor intensive for animal husbandry. Nevertheless, ecology and sustained development become the highlight of the goats farming recently. It is claimed that stall-feeding of growing goats to mitigate over or heavy grazing is highly necessary in many countries. Stall-feeding growing goats, first and foremost, a high quality of forage that is suitable for this feeding system should be considerately selected. It is believed that succulent fresh grass is optimal and come first, however, it is highly limited by season and other factors e.g. cutting and carrying. Corn silage is popular forage for ruminants because it is high in energy and digestibility and also easily adapted to mechanization for making and feeding (Howell, 1993). Furthermore, Corn silage's high palatability and high productivity per hectare characteristics make it certainly be a desirable forage source particularly where there is marginal availability of land for growing feed (Moreira et al., 2006). On the other hand, residues and by-products (particularly the stalk with some green fresh leaves) of corn can be preserved as silage by adding appropriate amount of water or additives (Schoonhoven et al., 2006). As the amount of corn stalk is very huge in the area where corn is cultivated, the corn stalk can be ensiled as silage and supplied to ruminants, which is ecology and sustained steps to make full use of corn residues. Several years ago, Sormunen-Cristian et al. (2001) demonstrated that

ewes' performances and lambs' growth were consistently better for silage rather than hay; these results may show that corn silage can improve the growing goats' productivity. According to the above reviews and the present fact of degradation and desertification of grassland, corn silage is not only considered as the best roughage for ruminants during periods of scarcity (Kunkle et al., 2006), but also it can be considered as a suitable, ecology and sustained rough source for stall-feeding goats.

1.1.3 Requirements of a consumer for chevon

At present, the consumers are willing to pay higher price for their favorite value-added products (USDA, 2004). Functional foods that provide health benefits beyond basic nutrition and have potential to lower the incidence of diet-related diseases (AFIC, 2004) have drawn great consumption appeal. It is a trend that many people prefer wholesome foods, so they pay more strict attention to the origination and qualities of food. Therefore to develop meat goat farming is urgently needed regarding chevon's ecological image, dietetic and health qualities, along with the tendency of consumers toward natural foods and healthier diets (Dubeuf et al., 2004). Besides of the consumers' natural and healthier food favorite, the features of the goat meat such as decreased fat, lower cholesterol, less sodium (McMillin, 2005), particularly its salubrious fatty acid profile and rich in conjugated linoleic acids (CLA) (12 mg/g fat, Wei, 2005) have made goat meat become value-added products, and meet the requirement of individual or niche markets (McMillin, 2005).

1.1.4 Foundations for utilization of probiotics and the rich in linoleic acid plants oil to improve goats' rumen metabolism and chevon CLA content

Since limitation of antibiotics in animal diet becomes a common sense,

probiotics are expected to play a role of improving animal's health and performance as a no social effects of pollution feed additive. The additional probiotics have a large impact on reduction of the incidence of infection, increase of the immune system function, prevention of microbial imbalances, decrease of production of lactate from carbohydrates, production of vitamins, and production of ammonia from amino acids that are not digested by the gastric juices. The application of *Saccharomyces. cerevisiae* has been proved successfully in beneficially modifying rumen fermentation (McDonald et al., 2002). Supplementation of probiotics such as yeast and *Lactobacillus acidophilus* on ruminants has attracted lots of researchers, nevertheless, the data has a density on dairy cows rather than goats (Giger-Reverdin et al., 2004). Hereby, it is necessary to conduct research to proof-test the effect of probiotics on the goats' performance and rumen metabolism.

On the other hand, CLA is produced naturally by the microflora that lives in the rumen of the ruminants through the digestion of dietary linoleic acid, it is readily absorbed by the animal from the rumen and ends up in milk, meat, and fat (John et al., 2007). Additional probiotics increase ruminal CLA production by modulation of rumen microbial balance. Supplementation of material that is rich in linoleic acid increases the production of CLA, and consequently causes increment of product CLA content. Hereby, supplementation of probiotics (*L. acidophilus* and *S. cerevisae*) and vegetable oil that is rich in linoleic acid for growing goats can be a practicable feeding strategy for enhancing the concentration and output of CLA in goat meat, while improving the goats' performance.

Nowadays, the conflicts that were resulted from the increasing number of goats, degradation of grassland, destroying of entironment, and wholesome

requirement of consumers are influential day by day. And some countries or governments are encouraging or pushing stall-feeding of goats. The ultimate objective of this study is to contrive a workable stall-feeding strategy to meet requirements of a large herd of big industry merge and high mechanization farm other than a small herd of a farmer, to produce high quality choven that meet the safe, tasty, and wholesome requirements of consumers by a ecological and sustainable way.

1.2 Research objectives

1.2.1 To study the effect of dietary supplemental probiotics on performance and chevon CLA content of growing goats.

1.2.2 To study the effect of dietary supplemental high linoleic acid plant oil on performance and chevon CLA content of growing goats.

1.2.3 To study the effect of a amalgamative dietary supplementation of probiotics and high linoleic acid content plant oils on performance and chevon CLA content of growing goats.

1.3 Research hypothesis

1.3.1 A dietary supplementation of probiotics (*S. cerevisia* and *L. acidophilus*) not only improves growing goats' performance, but also the chevon and plasma CLA content by influencing ruminal microbe metabolism.

1.3.2 A property dietary supplementation of high linoleic acid plant oil can increase CLA content of chevon and plasma.

1.3.3 The effect of probiotics (*S. cerevisiae* and *L. acidophilus*) and high linoleic acid plant oils on the growing goats' performance as well as chevon and plasma CLA content are cooperative with each other.

1.4 Scope and limitation of the study

Anglo-Nubian x Thai native crossbred growing goats were purchased from Pukthongchai district, Nakhon Ratchasima province of Thailand to carry out this study. The concentrate used in this study was supplied by the farm of Suranaree University of Technology (Muang district, Nakhon Ratchasima province of Thailand). The corn silage was purchased form Kornburee Cooperatives (Kornburee district, Nakhon Ratchasima province of Thailand). The probiotics was purchased from L.P.Feeds Tech Co., Led. (Bangkok, Thailand), it contains *L. acidophilus* 2.0 x 10^{12} cfu/g, *S. cerevisiae* 5.0 x 10^{11} cfu/g. The sunflower oil and soybean oil were purchased from Macro supermarket (Muang district, Nakhon Ratchasima province of Thailand).

1.5 Expected results

1.5.1 To optimize ruminal metabolism of growing goats, improve their productivity, enhance CLA content of the chevon by dietary supplementation of probiotics.

1.5.2 To increase the growing goats' productivity as well as CLA content of the chevon through supplementation of the rich in linoleic acid plant oils in the diet.

1.5.3 To get better results for affecting of a amalgamative dietary supplementation of probiotics and high linoleic acid plant oil growing goats' productivity as well as meat CLA content.

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CHAPTER II

REVIEW OF THE LITERATURE

2.1 Goats and entironment

Throughout developing countries, a goat makes a very valuable contribution such as meat, milk, fiber, and skins. Up to now, the goat had been domesticated by human being for 8,000 years (Yang et al., 2008). There are totally 570 goat breeds in the world. There exist about 146 goat breeds in Asia, 55 % of these breeds live in China, India and Pakistan (Devendra, 2005). The goat population in Asia is 390.4×10^6 (India; 35.2%, China; 29.3%, and Pakistan; 12.0%), it accounts for about 57% of the total world population of goats. The population of goat keeps increasing by an annual growth rate of 1.3% due to the increased price, expanded market (Figure 2.1) (McMillin and Brock, 2005), and also the excellent adaptability to various agro-climatic conditions (low availability of vegetation in arid areas, feeds rich in fiber and low in nitrogen, lack of water, and heat stress) (Morand-Fehr, 2005). Consequently, damage to the entironment is inevitable so long as there is no control on over-numbers and grazing, especially in situations where feeds are scarce. The damage to natural vegetation caused by goats is owing to that they like browing and can graze very short grass and even dig out grass roots for eating (Figure 2.2 and 2.3)

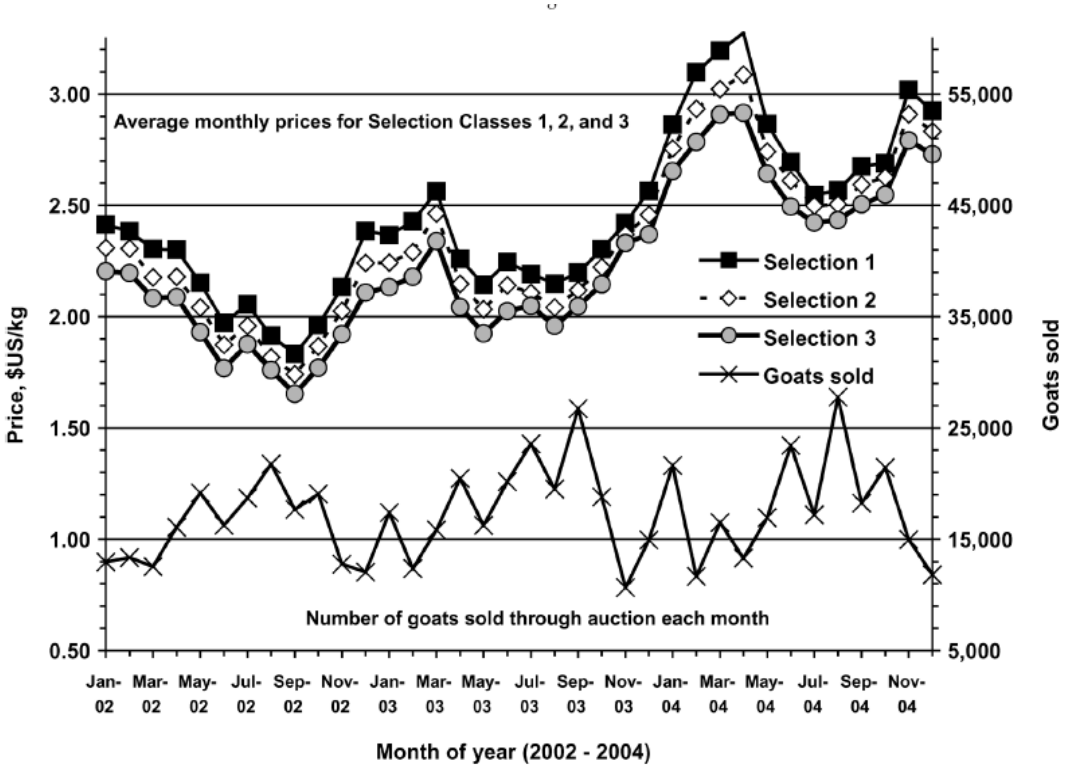

Figure 2.1 Average monthly prices and goats sold through Producers Auction, San Angelo, TX, 2002 through 2004 (Pinkerton and McMillin, 2005). Jan, Mar, Jul, Sep, and Nov = January, March, July, September, and November respectively. 02, 03, and 04 = 2002, 2003, and 2004 respectively.

Figure 2.2 Goats are browing (Source: http:// www. mth. pdx. Edu /fountain).

Figure 2.3 A goat is gnawing while digging with the left forelimb (Source: Julio, 2007).

Presently, deterioration of grassland increased with the passing of time in many parts of the world. In China, for instance, the degradation of grassland is expanding with a terrific rate, i.e., 20,000 square kilometers per year (Jiang and Gao, 2007). People have been suffering from the disasters such as dusty storms and debris flows that result from

the degradation and desertification of entironment. Since the grazing of goats affirmatively worsens the damage of natural vegetation, to some extent, the problem can be reduced by establishing improved pasture for goat grazing and/or housing them (Li and Walter, 2008). The former involves high capital investment and it is a long-term project of the governments' policies; the latter, however, is a feasible measure to put into practice. And many researchers suggested that stall-feeding of goats is an effectual means to mitigate the damage of grassland and entironment (Zhou and Wu, 2002; Wu and Yang, 2002; Wang et al., 2004). Housing the goat, problems then arise, including respecting of animal welfare, maintenance or enhancing of animal productivity and health, maintenance or enhancing of chevon flour and texture. In order to solve the problems above mentioned, the "green" feed additives such as probiotics and plant oil are adopted.

2.2 Supplementation of probiotics to goats

2.2.1 Brief introduction of probiotics

Antibiotics and other growing stimulants play an important role in animal agriculture, but they cause such disadvantages as antibiotic residues, disease-cross infection and increase of antibiotic-resistant microbial pathogens in animal products, which threat the human's health (Rial, 2000). Accordingly, the consumers nowadays, not only concern about price and quality but also safety and origin of animal products. Thereby, the purpose of using feed additives could not just focused on increase of animal productivity and put aside to lower the risk of carriage of human pathogens and to decrease excretion of polluting outputs like nitrogen-based compounds, and methane.

Probiotics is live single or mixed microbial which beneficially affects the host animal by improving its gastrointestinal microbial balance. Although there is no probiotics that can compete antibiotics with functions of growth stimulating and prevention or treatment of disease, as a nuisance free feed additive, probiotics can be equal to the role of improving animal performance. Probiotics increases daily gain and feed efficiency, enhances health and animal performance. More recently, there have been some claims that probiotics might have beneficial effects on decreasing the potential for ruminal acidosis, reducing the incidence of infection, stimulating the immune system, preventing microbial imbalances, decreasing production of lactate from carbohydrates, production of vitamins, production of ammonia from amino acids that are not digested by the gastric juices, deconjugating bile acids, and lowering the total body pool of cholesterol (Krehbiel et al., 2003; Leila, 2006).

2.2.2. Possible mechanisms and action modes of probiotics

There are many commercial probiotics nowadays. These products often contain *lactobacilli* with *L. acidophilus* being one of the most common microorganisms used, and some probiotics contain *Bifidobacterium*, *Enterococcus,* and *Bacillus*. Other commonly used microorganisms are yeast live cell or culture extracts that are based on various strains of *S. cerevisiae* (Krehbiel et al., 2003). In Europe, *S. cerevisiae* has been officially authorized as feed additives since 1996. The purpose for using probiotics feed additives is to prevent rumen flora disorders and disturbances, especially those associated with the consumption of high energy concentrates to sustain high productivity production system. There have been lots of research demonstrating that *L. acidophilus* in combination with fungal cultures were more efficacious for increasing milk production by lactating dairy cows (Komari et al., 1999; Block et al., 2000).

Furthermore, Draksler et al. (2004) found the *L. acidophilus* is resistant to pH 2.0 and bile salts (0.3%) and could be pre-selected as a probiotic for use in goat feed. Whereby, a commercial probiotics that contains *L. acidophilus* about 2.0×10^{12} cfu/g, *S. cerevisiae* about 5.0×10^{11} cfu/g was used in the present study. Thereinafter, the literature reviews will exclusively focus on the *L. acidophilus* and *S. cerevisiae* probiotics.

The utilization of probiotics mainly affects the gastric intestinal tract environment (Hajime et al., 2004). Some of the hypotheses on how probiotics benefits animals are shown as followed: a) production of antibacterial compounds (acids, bacteriocins, antibiotics); b) competition with undesirable organisms for colonization space and/or nutrients (Figure 2.4); c) production of nutrients (e.g. amino acids, vitamins) or other growing factors stimulatory to other microorganisms in the digestive tract; d) production and/or stimulation of enzymes; e) metabolism and/or detoxification of undesirable compounds; f) stimulation of immune response in host animal and g) production other growing factors stimulatory to the host animal (Yoon et al., 1995). The proposed modes of action associated with yeast culture include: a) removal of oxygen from the rumen environment (Figure 2.5); b) providing various growth factors, pro-vitamins, and/or micronutrients that help stimulate the growth of the ruminal bacteria; c) stimulating lactic acid utilizing bacteria (e.g. *Megasphaera elsdenii* and *Propionibacterium*), increasing the rumen nadir pH and d) a positive influence on ammonia uptake and results in increase of microbial protein production (Miller-Webster, 2002). As shown in Figure 6, supplementation of yeast improves the balance of rumen microbes, reduces the production of lactate, methane, and ammonia,

moderates rumen pH and VFA, increases digestion and microbial protein synthesis, consequently the animal's productive performance can be improved.

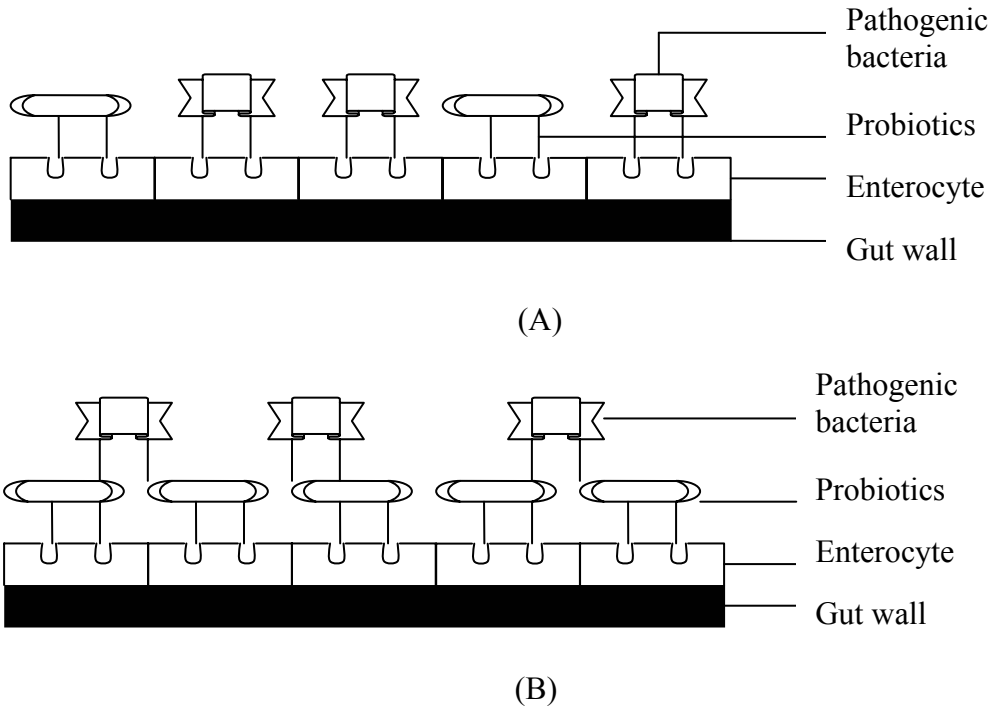

Figure 2.4 (A) A mixed population of probiotics with substantial attachment of pathogenic bacteria, (B) competitive exclusion of pathogens due to preferential attachment of probiotics (Adapted from McDonald et al., 2002).

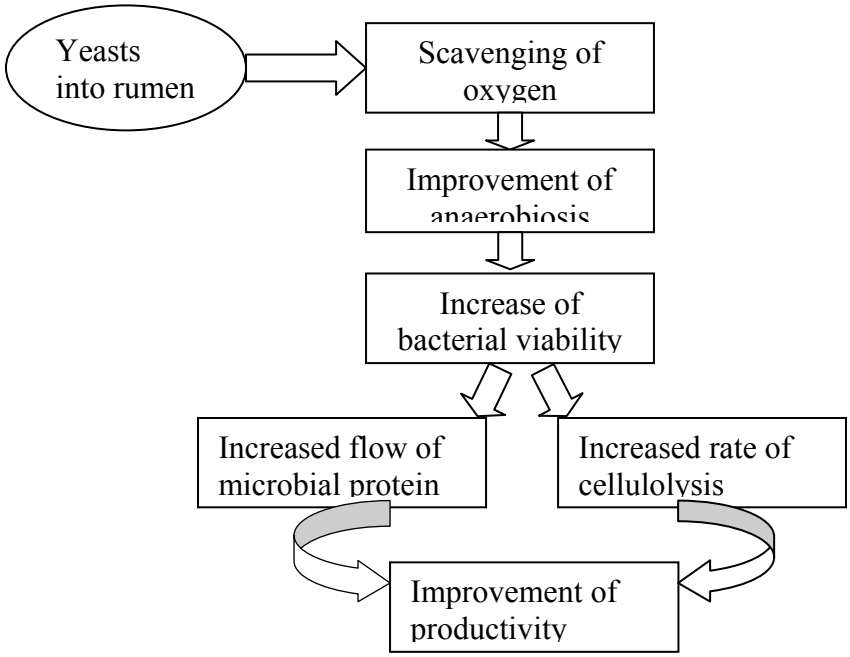

Figure 2.5 Oxygen scavenging hypothesis mode of yeasts (Adapted form Yoon and Stern, 1995)

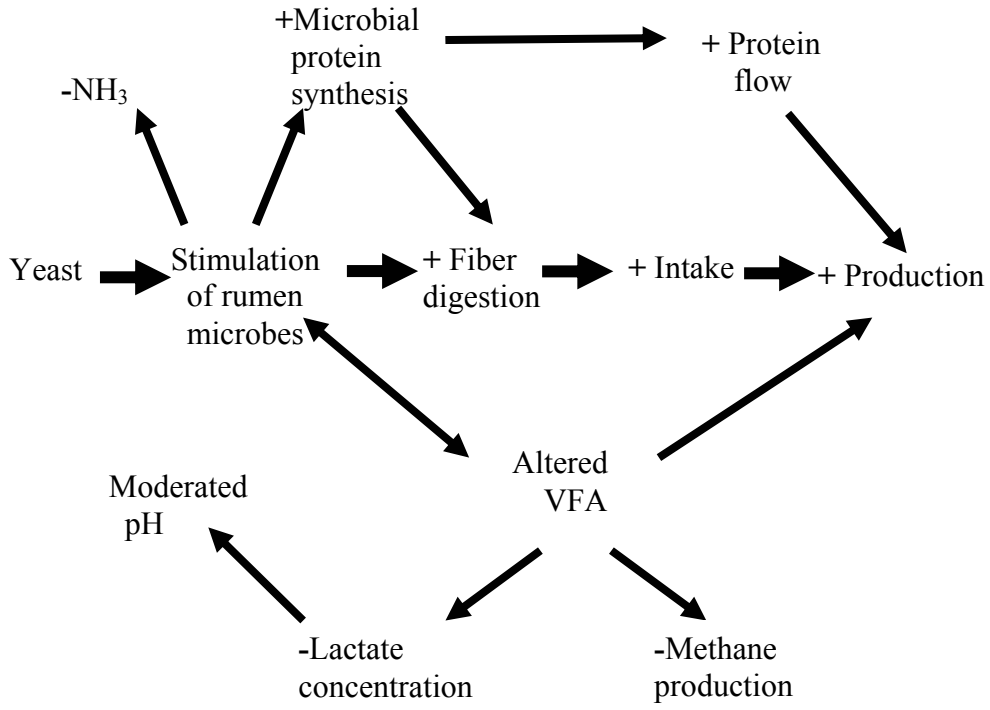

Figure 2.6 Possible modes of actions of yeast on ruminal fermentation (Dawson and Hopkins, 1991).

2.2.3 Effects of yeast (*S. cerevisiae*) on goat rumen fermentation

In vitro studies, *S. cerevisiae* was able to outcompete lactate-producing bacterial species (e. g., *Streptococcus bovis*) for the utilization of sugar, and this consequently limited the amount of lactate produced (Chaucheyras et al., 1996). On the other hand, the stimulation of growth and metabolism of lactate-utilizing bacteria, such as *Megasphaera elsdenii* or *Seknomonas ruminantium* was observed in the presence of different live yeasts (Newbold et al., 1996； Chaucheyras et al., 1996). As shown in Table 2.1, in the Pure Culture Studies, both of *S. cerevisioe* culture and its filter-sterilized filtrate increased lactate uptake by whole cells of *S. ruminantium* (Scott et al., 1992). The reduce of rumen lactate, accordingly, maintains rumen pH at values that is compatible with an efficient rumen function, as shown by higher fibrolytic activities (cellulases, hemicellulases) in the rumen (Figure 2.7).

Table 2.1 Effects of *S. cerevisioe* culture and its filter-sterilized filtrate on lactate uptake by whole cells of *Seknomonas ruminantium* (nmol/mg of protein per min).

Concentration	**Mean**	**SD**
S. cerevisioe culture (g/L)		
0	1.0	0.3
0.5	1.9	0.8
1.0	1.9	0.4
2.5	2.8	0.2
5.0	3.9	0.1
10.0	3.2	0.1
50.0	1.8	0.4
filter-sterilized filtrate of *S. cerevisioe* (µl/ml)		
0	0.6	0.1
10.0	3.5	0.2
25.0	5.2	0.7
50.0	3.5	0.2
100.0	2.5	0.6

Adapted from Scott and David (1992)

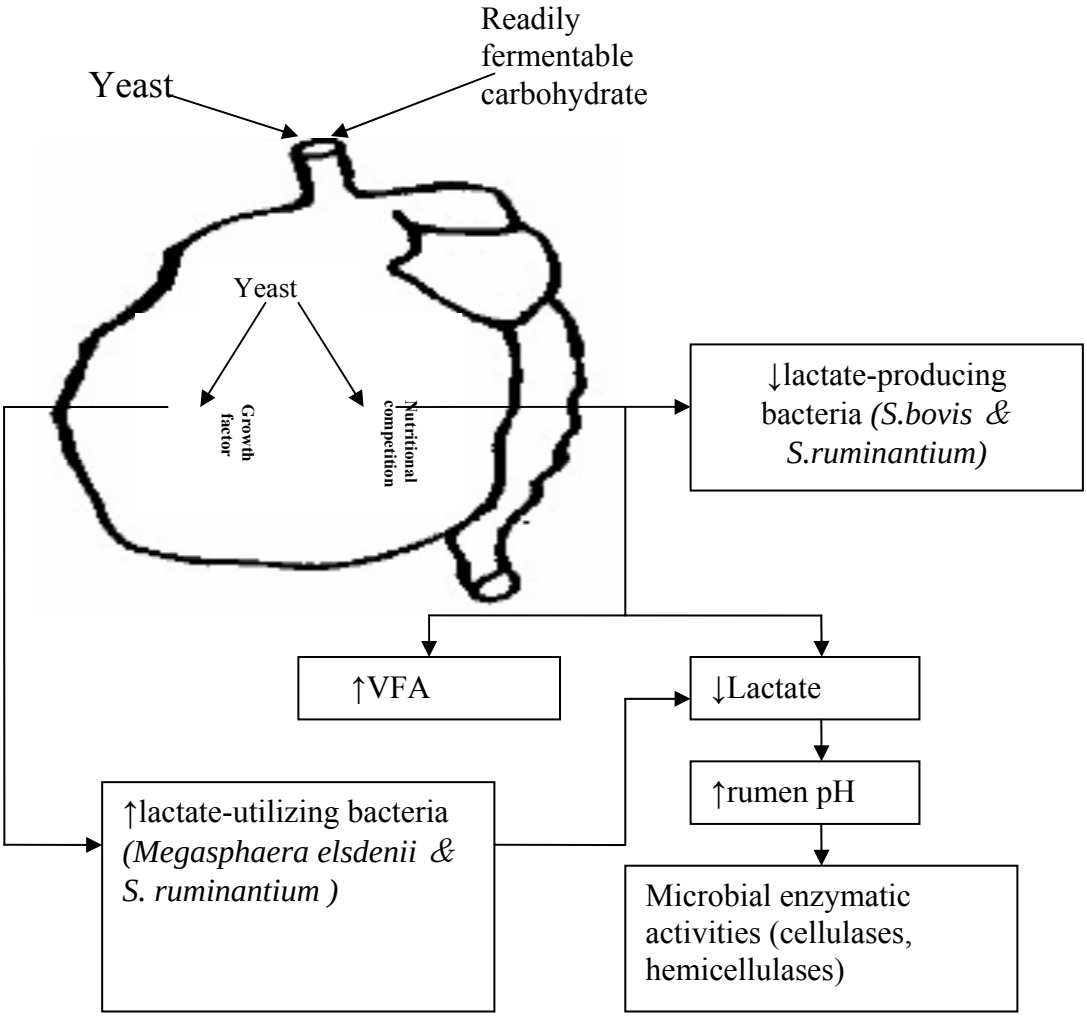

Figure 2.7 Mode of action of active dry yeast (ADY) on lactate metabolism and rumen pH. (Adapted from Fonty and Chaucheyras, 2006).

A lot of studies in sheep and dairy cows have clearly demonstrated that to use live yeasts as probiotics to limit lactate accumulation in the rumen was a feasible way (Williams et al., 1991; Jouany et al., 1998). The results that were found out from a study with fistulated sheep demonstrated that *S. cerevisiae* could be efficient to stabilize ruminal pH by stimulating ciliate *Entodiniomorphid* protozoa, which are known to engulf starch granules very rapidly and thus compete effectively with

amylolytic bacteria for their substrate. Moreover, the starch was fermented to VFA other than lactate by protozoa, which differed from amylolytic bacteria. In addition, ciliate *Entodiniomorphid* protozoa its own is capable of consuming lactate and thus may play an essential role in the prevention of lactate accumulation (Fonty et al., 2006). *In vivo* studies, as shown in Table 2.2, a lot of findings have stated that the yeast probiotics did not affect goats' rumen pH value with any significance (Han et al., 2008; Jiang et al., 2008; Fadel Elseed and Abusamra, 2007; Kumagai et al., 2004; Giger-Reverdin et al., 2004; Dawson et al., 1990). However, it stabilized pH in a range that is compatible with the optimal ruminal ecologic dominance.

Table 2.2 Effect of yeast probiotics on ruminal pH of goats.

Probiotics (g/d/goat)	**0**	**2.5**	**5.0**	**SEM**	**Effect**	**References**
pH	6.22	6.28	6.26	0.30	ns	Fadel Elseed and Abusamra (2007)
pH	6.65	6.75	6.50	0.40	ns	Han et al. (2008)
pH	6.09		6.15		ns	Giger-Reverdin et al. (2004)
Exp.1 (hay feeding)						
Hours after feeding	**0**	**1**	**3**	**6**	**9**	Kumagai et al. (2004)
Control	6.78	6.71	6.54	6.65	NA	
Treatment	6.82	6.66	6.56	6.76	NA	
Exp. 2 (high concentrate feeding)						
Control	5.84	5.75	5.54	5.49	5.58	
Treatment	5.74	5.75	5.52	5.50	5.46	
SEM	0.08	0.08	0.07	0.07	0.12	
Effect	ns	ns	ns	ns	ns	

NA, not analyzed; ns, not significant

Chaucheyras et al. (1995) demonstrated that the addition of yeast cells to a vitamin-deficient medium stimulated the germination of a rumen fungal strain of *Neocallimastix frontalis* zoospores and increased the colonization of *Neocallimastix frontalis* fugal on plant cell, and thereby increased cellulose degradation. The effectiveness of some yeast strains to stimulate growth or/and activity of fibrolytic bacteria has also been pointed out (Dawson et al., 1990; Harrison et al., 1988). E.g.,

Chaucheyras-Durand et al. (2001) carried out experiment with lambs that were fitted with a rumen cannula, their findings were that *S. Cerevisiae I-1077* had effect on establishing fibrolytic bacteria such as *Fibrobacter succinogenes*, *Ruminococcus albus* and *Ruminococcus flavefaciens* in the rumen (Figure 2.8), on degradation of a lignocellulosic substrate, on the main polysaccharide depolymerase and glycoside hydrolase activities of particle-associated microorganisms (Table 2.3), and on the development of the rumen digestive function. Recently, Feng et al. (2008) also reported that the yeast culture increased the activities of xylanase and pectinase. In fact most of ruminal microorganisms are highly sensitive to oxygen, the beneficial effect of yeast which increases fibre degrading bacteria is resulted from the capacity of yeast cells to scavenge oxygen and create more favorable ecological conditions for growth and activities of the anaerobic autochtonous microflora (Fonty et al., 2006) (Figure 2.5).

Based on the above reviews, there seems to be potential for the use of yeasts to optimize the microbial degradation of lignocellulosic materials. And theoretically, the more favorable ecological conditions for growth and activities of the anaerobic autochtonous microflora that is created by yeast probiotics should consequently with increasing digestibility of diet crude fiber (CF), crude protein (CP), dry mater (DM), ether extract, and so forth. *In vivo* studies, Kumagai et al. (2004) observed that in the condition of both of oat hay and high concentrate feeding, the presence of yeast propiotics tended to increase the digestibility of CP, CF, and organic cell wall. Han et al., (2008) pointed out that DM ($P<0.01$), organic matter (OM) ($P<0.05$), and NDF ($P<0.05$) digestibility was increased significantly with probiotics, CP digestibility showed an obvious increasing tendency. Some of others' studies also agreed with these findings (Fadel Elseed et al., 2007; Feng et al., 2008).

Table 2.3 Depolymerase and glycoside hydrolase specific activities (nmol.min–1.mg–1 protein) of particle-associated bacteria in the rumen of lambs from control and yeast treatment groups.

Items	**Control**	**Yeast**
CMCase	1018.9 ± 763.83	825.9 ± 300.8
Avicelase	108.9 ± 166.3	122.0 ± 111.0
Xylanase	15,358.0 ± 5, 126.3	22,481.0 ± 6,881.5
b-galactosidase	3,132.1 ± 1264.0	6,885.5 ± 1,795.3**
b-glucosidase	2,003.3 ± 439.6	4,123.3 ± 1,225.3**
b-xylosidase	1,998.1 ± 762.8	2,614.7 ± 599.4
b-cellobiohydrolase	1,136.8 ± 727.3	1,409.3 ± 420.5

Adapted from Chaucheyras and Fonty (2001) ; ** P<0.05

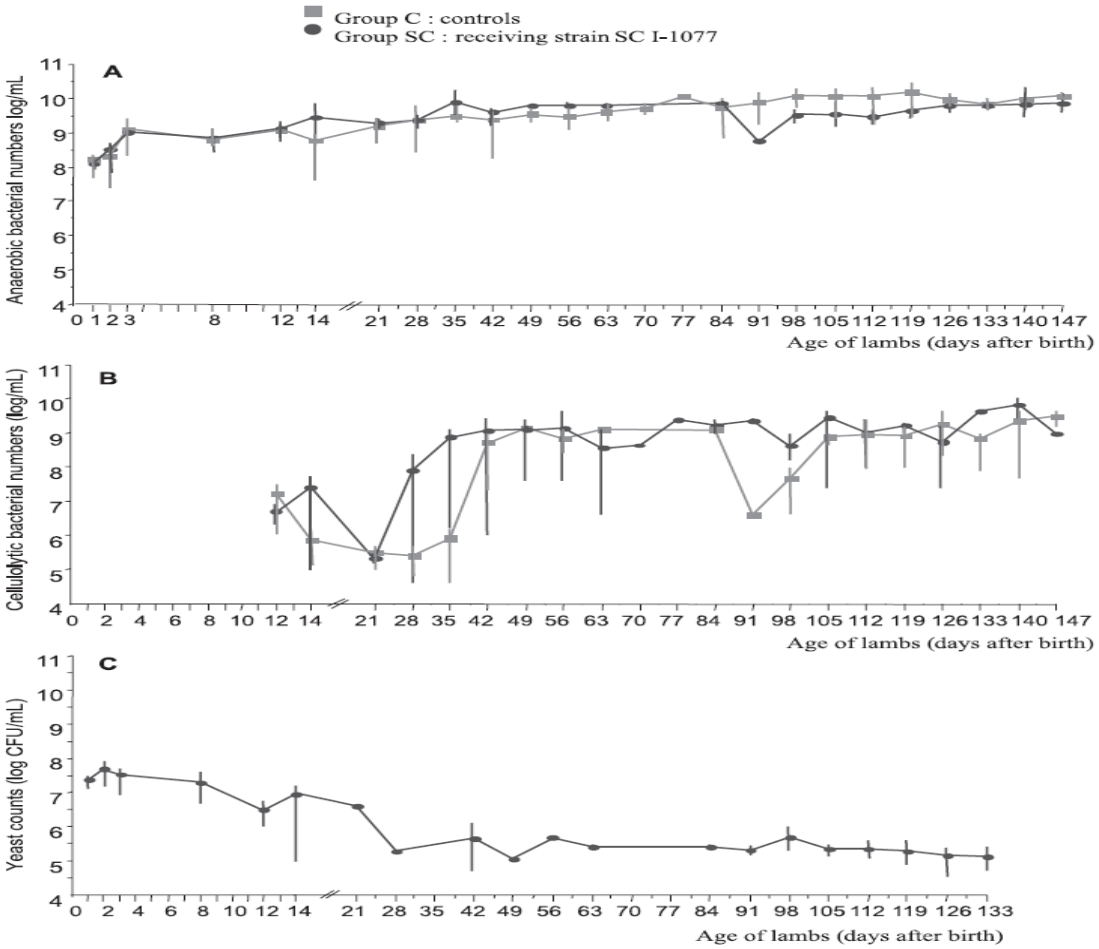

Figure 2.8 (A) Establishment of total anaerobic bacteria (log.mL–1 of rumen contents) in the rumen of lambs; (B) establishment of cellulolytic bacteria (log.mL–1 of rumen contents) in the rumen of lambs; (C) yeast counts (log CFU.mL–1) in the rumen of lambs SC. Results are expressed as mean log.mL–1 and bars show the range between the lowest and the highest log values. (Source: Chaucheyras and Fonty, 2001).

In vitro studies, the yeast probiotics has beneficial effects on growth and H_2-utilisation of acetogenic bacteria were observed (Chaucheyras et al., 1995b; Chaucheyras-Durand et al., 1997), and since the acetogenic bacteria, which produce acetate from CO_2 and H_2, the acetic centesimal proportion and/or total VFA that

produced in the rumen should appear to increase. However, in an *in vivo* experiment that carried out in lambs (Chaucheyras et al., 2001), even though total VFA was significantly higher in the *S. cerevisiae* group during the 20–50 d period, no any significant effect was observed on the centesimal composition of the major VFA mixture (acetate, propionate, and butyrate) excepted that of acetate tended to increase. Han et al. (2008) also detected a significant increase of total VFA in probiotics supplemental group, and in the meantime, no any significant effect was observed on the centesimal composition of the major VFA mixture as well as the ratio of C2:C3. However, some studies (Giger-Reverdin et al., 2004; Fadel Elseed et al., 2007; Jiang ea tl., 2008) suggested that *S. cerevisiae* probiotics did not increase rumen total VFA of goats significantly, even though a clear increased tendency was found (Table 2.2). In previous studies, a lot of research also found the increase of VFA (Arambel et al., 1987; Dawson et al., 1990; Martin et al., 1989).

Table 2.4 VFA profiles of rumen fluid from lambs and goats receiving *S. cerevisiae* (mmol/L).

Items	**Control**	**SC**			**Effect**	**References**
Total VFA	18.0±5.0	31.1±8.4			*	Chaucheyras
Acetate (%)	76.3±10.1	81.3±3.3			ns	et al. (2001)
Propionate (%)	16.3±4.2	14.3±2.6			ns	
Butyrate (%)	4.3±6.0	3.5±2.4			ns	
Probiotics (g/d)	**0**	**2.5**	**5.0**	**SEM**	**Effect**	
Post-morning feeding 0 h						Han et al.
Total VFA	46.9[b]	59.0[a]	55.1[a]	1.4	*	(2008)
Acetate (%)	67.7	67.8	66.9	1.5	ns	
Propionate (%)	23.0	22.8	24.0	0.8	ns	
Butyrate (%)	8.6	8.7	8.5	0.9	ns	
C_2:C_3	2.9	3.0	2.8	0.1	ns	
Post-morning feeding 4 h						
Total VFA	52.0[b]	66.7[a]	56.8[b]	1.0	*	
Acetate (%)	65.5	67.3	67.5	1.0	ns	
Propionate (%)	22.7	22.2	23.0	0.6	ns	
Butyrate (%)	9.4	9.6	9.3	0.6	ns	
C_2:C_3	2.9	2.9	2.8	0.1	ns	
VFA(mml/dL)	9.4	11.0	10.6	0.6	ns	Fadel Elseed
						et al. (2007)

SC= *S. cerevisiae*; * P<0.05; ns no significance

2.2.4 Effects of *L. acidophilus* on goat rumen fermentation

Supplementation of *L. acidophilus* has been shown to decrease in the area below subacute ruminal pH, to increase in ruminal propionate concentrations, to increase protozoal numbers, to change in viable bacterial counts, and to reduce shedding of pathogen (Krehbiel et al., 2003). The utilization of *Lactobacilli* provided important benefits to the host animals through the constitution of a healthier and more favorable gastro-enteric setting for digestive and absorption processes (Klaenhammer, 1998). In vitro, some people proposed that *L. acidophilus* can be used as probiotics feed additive to enhance starch utilization in cattle (Early et al., 2006). Overall, the previous study data of *L.* species fed to young ruminants have been proved to establish and maintain 'normal' intestinal microorganisms, and/or to alleviate metabolic disorder such as diarrhea and acidosis, rather than as a production stimulant. The literatures pertained to rumen fermentation and animal production (i.e., body gain, feed efficiency, milk yield and quality, and meat quality) are scare for *L. acidophilus*. And the *in vivo* studies were primarily related to dairy cows and calves. e.g., McGilliard and Stallings (1998) pointed out an increase of the milk yield in cows which received a diet containing lactobacilli and enzymes. What is more, Dell'Orto et al. (2000) reported an improvement in the dry matter intake, daily gain, and fecal score have been observed in calves during the pre-weaning period. And other research have also shown an improvement in the productive performances dairy cows (Savoini et al., 2000). Nevertheless, as goats as concerned, much less data were available.

In 2004, Chiofalo et al. complete their studies on twenty growing Maltese goat kids to evaluate the effect of some lactobacilli on body growth and on the metabolic-nutritional status in the animals. They observed the presence of lactobacilli

significantly increased body weight (P<0.001) (Figure 2.9), and in confirmation of their expectation, the lactobacilli treatment group had higher anamorphosis (P<0.05) and body proportion (P<0.01) indices (Table 2.5). Regarding to the parameters concerning energetic metabolism, lactobacilli treatment group can significantly lower the levels for non-essential fatty acid (NEFA) (P<0.001) and for triglycerides (P<0.05). And among the parameters of protein metabolism, the urea of the lactobacilli treatment group showed significant lower levels (7.65 vs. 8.83 (mmol/L), P<0.05). Their results testified to the better metabolic activity, growing performances, and productive efficiency of the supplementation of lactobacilli in goat kids.

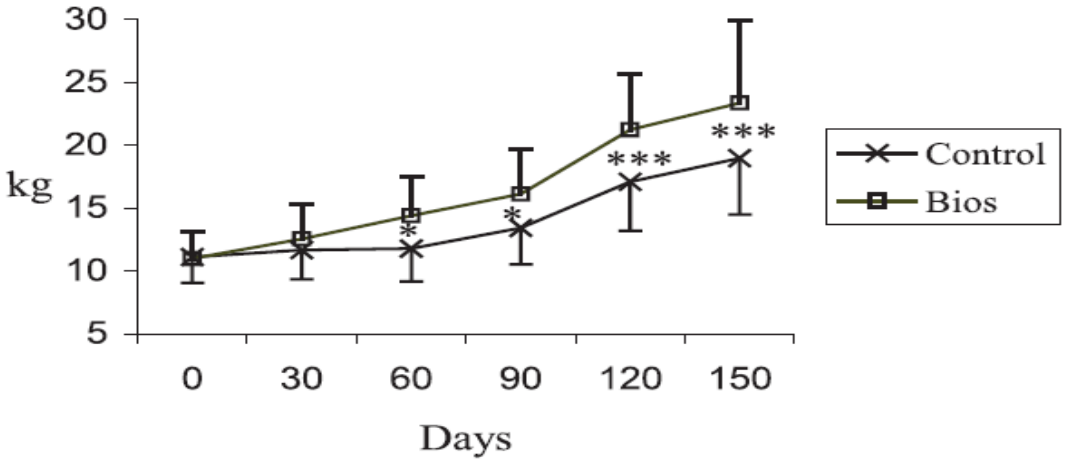

Figure 2.9 Trend of body weight (mean ± SD). * P<0.05; *** P<0.001. (Source: Chiofalo et al., 2004)

Table 2.5 Body measurements and biometric indices at the end of the trial.

	Control	**Lactobacilli**	***P***
Body Weight (kg)	18.97 ± 0.80	23.37 ± 0.84	< 0.001
Circumference of chest (cm)	62.09 ± 1.13	66.60 ± 1.18	0.007
Height at withers (cm)	54.27 ± 0.77	57.20 ± 0.812	0.0103
Anamorphosis index	71.21 ± 2.02	77.71 ± 2.12	0.028
Body proportion index	34.85 ± 1.30	40.77 ± 1.36	0.002

Adapted from Chiofalo et al. (2004)

2.3 Supplementation of soybean and sunflower oil to goats

Soybean oil and sunflower oil both contain high levels of polyunsaturated fatty acids (PUFA) (60.8 and 69%, respectively), with a PUFA: saturated fat ratio of 4.0 for soybean oil and 6.4 for sunflower oil (Meydani et al., 1991). Soybean oil is the first vegetable oil in the world, it contains about 51% linoleic acid (Wikipedia, 2007). Sunflower is the fourth largest source of vegetable oil, and the high linoleic acid sunflower oil contains 63%～70% linoleic acid normally (Jasso et al., 2002). The major fatty acids components of soybean and sunflower oil are shown in Table 2.8.

Table 2.6 The major fatty acids components of soybean and sunflower oil (%).

	Linoleic acid	**Palmitic acid**	**Oleic acid**	**Stearic acid**	**References**
Soybean oil	51	10	23	4	Wikipedia
Sunflower oil	48-74	4-9	14-40	1-7	(2007)
Sunflower oil	68.2		18.6	4.7	Chow (2000)
Soybean oil	43.3		21.2	5.3	

For the supplementation of soybean and sunflower oil to ruminants, the data is rare in recent years. Furthermore, most of the topics were dealt with cattles and the results were shown with positive advantages. For example, Gülşen et al. (2006) who suggested that increasing levels (3, 6, and 9%) of sunflower and soybean oil linear increases pH, did not affect NH_3-N concentration, and depressed ruminal fermentation in cattles. Eweedah et al. (1997) stated that fullfat soybean and sunflower seed did not impact the apparent digestibility of dry matter, organic matter, N-free extract and crude protein as well as nutritive value in Holstein bulls (179-203 kg) fed corn silage. However, the increasing fat level tended to decrease digestibility of crude fiber, acid detergent fiber (ADF) and neutral detergent fiber (NDF), increased the proportions of C18:1, C18:2 and C18:3 in adipose fat tissue, and decreased the proportion of C16:0. Mir et al. (2002) suggested that sunflower oil improved ADG ($p=0.011$), feed conversion efficiency ($p=0.06$), and conjugated linoleic acid (CLA) concentrations by 339% ($p=0.01$) of steers. Some other evidences have indicated that soybean and sunflower oil decreased milk fat yield and content ($P<0.05$), as well as the milk fat short and medium chain fatty acids ($P<0.05$), but increased concentration of long chain fatty acids and particularly CLA by 61% in milk fat ($P<0.05$) in dairy cows (Oldemiro et al., 2005). As far as sheep concerned, Zhang et al. (2005) used 4 fistulated sheep to investigate impact of different levels (0%, 2%, 4%, and 6%) of soybean oil on profile of rumimal cis9, trans11-CLA, C18:1 trans-11, and other fatty acids, the results demonstrated that with the increasing levels of soybean oil, the increases of ruminal cis9, trans11-CLA (0.13, 0.26, 0.42, and 0.59 mg/g), C18:1trans-11 (1.27, 3.95, 8.78, and 13.48 mg/g), C18:0, C18:1cis-9, C18:2cis-9,12, SFA, UFA, MUFA, and PUFA (mg/g) were highly significant ($P<0.01$). The content of cis9, trans11-CLA and C18:1trans-11 positively correlated to the levels of soybean oil ($P<0.01$).

In terms of goats' performance, very few papers addressed the effects of soybean and sunflower oil. Still, it has been shown that milk from Saanen goats that received sunflower oil presented the higher CLA concentrations; however, goats treated with soybean oil had the higher monounsaturated and polyunsatured fatty acids (PUFA) concentrations and the lower concentration of saturated fatty acids, excepted for the similar (3.90, 4.24) n−6/n−3 ratios. It was testified that nutritional milk quality can be improved by adding soybean and sunflower oil (Matsushita et al., 2007). For digestibility and rumen fermentation, Rogério et al. (2005) have verified that presence of soybean oil in goats' diet decreased the intakes of dry matter (%BW and g/kg $BW^{0.75}$), neutral detergent fiber (NDF) and non-fibrous carbohydrates; decreased the digestibility of NDF in contrasted to increase the digestibilities of CP, EE, and total digestible nutrients content (TDN); increased the pH differed from decreasing of the acetate: propionate ratio in the ruminal fluid. And when focusing on plasma fatty acid of goat, Yeom et al. (2003) demonstrated that soybean oil significantly ($P<0.05$) elevated the linoleic acid (C18:2n-6) content by 9.3% on the contrary to decrease C14:0, C17:0 , C18:1n-9, C18:3n-3, and C20:5n-3 by highly significance (Table 2.9).

Table 2.7 Plasma fatty acid composition (gram fatty acid methyl ester/100 g fatty acid methyl esters) of goats fed additional soybean oil.

Fatty acid	Control	S.E.	Soybean bean oil	S.E.	P-value
C14:0	0.7	0.04	0.4	0.04	<0.001
C15:0	0.4	0.03	0.2	0.04	0.034
C16:0	13.1	0.17	12.7	0.65	0.541
C16:1	0.9	0.09	1.2	0.29	0.356
C17:0	0.9	0.05	0.4	0.02	<0.001
C17:1	0.7	0.08	0.2	0.06	0.002
C18:0	21.1	0.59	22.9	2.48	0.414
C18:1n-9	18.9	1.05	12.6	0.63	<0.001
C18:1n-7	2.4	0.12	4.8	1.53	0.154
C18:2n-6	19.8	0.73	29.1	1.66	<0.001
C18:3n-6	0.6	0.07	0.3	0.09	0.134
C18:3n-3	2.2	0.08	1.3	0.06	<0.001
C20:3n-6	0.3	0.02	0.2	0.04	0.070
C20:4n-6	5.6	0.43	3.7	0.20	0.001
C20:5n-3	2.6	0.22	1.3	0.10	<0.001
C22:4n-6	0.4	0.20	0.4	0.16	0.809
C22:5n-3	3.0	0.28	1.8	0.13	0.006
C22:6n-3	1.4	0.26	0.8	0.08	0.040
Unknown	5.0	0.46	5.7	0.93	0.552

Adapted from Yeom et al. (2003)

2.4 Brief review on conjugated linoleic acid

Conjugated linoleic acid is a collective term for geometrical and positional conjugated dienoic isomers of linoleic acid (Ip et al., 1994), and it is characterized as two double bonds separated by a signal bone at various carbon positions. The following 17 isomers: t12, t14; t11, t13; t10, t12; t9, t11; t8, t10; t7, t9; t7, c9; t6, t8; c12, t14; t11, c13; c11, t13; c10, t12; c9, t11; c8, t10; c7, t9; c9, c11; and c11, c13 have hitherto been reported (Bauman et al., 2000; Lobb and Chow, 2000). However, amongst of them, the c9, t11 and t10, c12 are the primary CLA contented in ruminant products, and it is these two CLA isomers that show biological importance and play crucial role on human health, and therefore, they have been studied in detail. If there is no note, the ensuing will discuss CLA, namely the cis-9, trans-11 and trans-10, cis-12. The structures of cis-9, trans-11 and trans-10, cis-12 CLA is shown in Figure 2.10. Since 1988 when CLA was reported to be of impact on anti-carcinogenesis (An, 2006), it has been attracting the interest of many researchers, and several other health promoting effects such as immonumodulation, anti-atherosclerosis, anti-diabetes, and shifting the partitioning of energy towards protein instead of fat deposition had been demonstrated in animals (Webb et al., 2005). From *In vivo* studies, sufficient data has been tested that CLA can prevent adverse effects caused by immune stimulation in chicks, mice and rats, and has been shown to decrease the ratio of low density lipoprotein cholesterol to high density lipoprotein cholesterol in rabbits fed with an atherogenic diet. CLA also has been shown to reduce body fat in mouse, rat, chick and pig models, and to be effective in treating skin lesions when included in the diet (Reinhardt et al., 2004). In this year, Kanwar et al. (2008) just completed their study on mice, and found that feeding of cis-9, trans-11 CLA and

veccinic acid (VA) enriched milk fat led to marked suppression of airway inflammation as evidenced by reductions in eosinophilia and lymphocytosis in the airways. And compared with feeding of normal milk fat and control diet, the enriched milk fat significantly reduced circulating allergen-specific IgE and IgG1 levels, concurred by reducing in bronchoalveolar lavage fluid of IL-5 and CCL11. The treatment significantly inhibited changes in the airway including airway epithelial cell hypertrophy, goblet cell metaplasia and mucus hypersecretion.

A general deduction is that the concentration of CLA isomers in human plasma responds to increasing in daily intake of the isomers in dietary sources. Burdge et al. (2005) have performed studies to confirm that consumption of naturally CLA enriched dairy products in amounts similar to habitual intakes of these foods increased the c9,t11 CLA content of plasma and cellular lipids. They clearly elucidated in detail that when supplying CLA-enriched dairy products (control: 0.17 g c9,t11 CLA/d; 0.31 g trans-vaccenic acid (tVA)/d, treatment: 1.43 g c9,t11 CLA/d; 4.71 g tVA/d) to people who aged 34-60 years for 7 weeks, the c9,t11 CLA concentration increased substantially in plasma phosphatidylcholine (38%; $P=0.035$), triacylglycerol (22%; $P<0.0001$) and cholesteryl esters (205%; $P<0.0001$), and also in peripheral blood mononuclear cells (238%; $P<0.0001$). What is more, some other research which have started off on human have shown that CLA supplementation (3-4 grams/day) promoted a loss of body fat (0.9-1.8 kg) in overweight subjects over 12 weeks, and reduces abdominal fat (by about 1 inch) in obese men (Miner et al., 2001). Likewise, Hunter (2000) found that human receiving 3 g/d CLA reduced body fat and increased body mass but left body weight to be untouched. Berven et al. (2000) and Blankson et

al. (2000) also reported that human receiving 3 - 4 g/d CLA for 100 d got reduction in both body weight and body fat. As such, the study of Zambell et al. (2000) stressed that CLA have shown benefits on loss of fat and body weight for using doses of 3.4g and 4.2g per day. In short, data consistently supports that a dose of 3-4.2 g/d CLA for human is responsible for loss of fat and body weight. It is a pity that there is no *in vivo* research published up to now to show the impact of CLA on other aspects such as inhibit tumor, anti-atherosclerosis, anti-diabetes and so forth on human being. The mechanisms whereby these healthy benefits are kept unknown, but some presumed theories are that CLA reduces cell proliferation, alters various components of the cell cycle, and induces apoptosis (Belury, 2002). Stressing on human cancer studies, some researchers have found an inverse association between the level of CLA in the diet and the risk of developing cancer in breast adipose tissue (Durgam and Fernandes, 1997; Thompson et al., 1997; Visonneau et al., 1997; Bougnoux et al., 1999).

It is the aforementioned nutritional benefits of CLA that have become the driving forces for so many people to research on it. However, information is still lacking on the nadir effectual does of CLA for humans, nevertheless, it is estimated from animal studies that a daily intake of 3 g/d may be effective for cancer prevention. Whereas, the average estimated CLA intake of human has been reported to range from 0.35 to 1 g/d (Alonso et al., 2003), it is much less that the nadir effectual level. It has been a common knowledge that CLA resource for human being principally is ruminant products, therefore, strive towards higher production of CLA content in ruminant products, and whereby to increase the daily intake of consumers is significant.

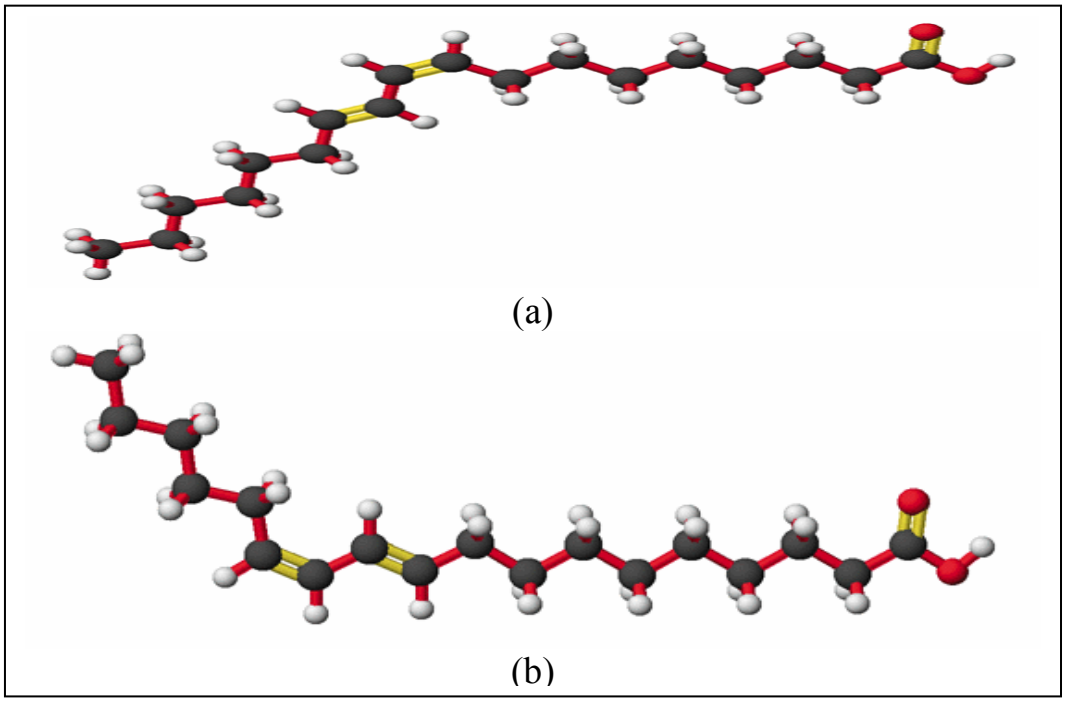

Figure 2.10 The structures of cis-9, trans-11(a) and trans-10, cis-12 (b) CLA (Adapted Steinhart, 1996).

2.5 Biosynthesis of CLA

John et al. (2007) incubated the ruminal digesta of sheep with linoleic acid anaerobically and observed a rapid declining of the linoleic acid (LA) concentration simultaneously with the increasing accumulation of CLA isomers that were composed of cis9, trans11 CLA to be the most abundant isomer, and followed by trans-10,cis-12-CLA (Figure 2.11). Theretofore, many people pointed out that the biohydrogenation of dietary unsaturated fatty acids that leading to the biosynthesis of CLA in the rumen is from LA, linolenic acid (LNA), however, in the body tissue and organs it is from t-11 C18:1 (TVA) on the catalysis of Δ9desaturase (Khanal and Dhiman, 2004; Collomb et al., 2004; Griinari et al.,2000). Certainly, it is noteworthy to understand the mechanisms involved in the biosynthesis of CLA from LA and LNA present in

rumen since it will allows us to design feeding strategies for enhancing the content of CLA in ruminant products, and whereby, the consumer can derive the potential health benefits from it.

It is shown clearly in Figure 2.12, the biohydrogenation of LA and LNA occurs in a similar manner. The first reaction in the biohydrogenation is the isomerization at carbon-12 double bond position, and the double bond is migrated to carbon-11 position forming c-9, t-11 CLA for LA or c-9, t-11, c-15 C18:3 for LNA. This step embroils a complicated reaction: the first is the H on C-11 of LA is removed by hydrogen abstraction to leave a radical that is thermodynamically less favorable than a conjugated double bond system with the radical located on C-13; the follow is the movement of the double bond from carbon atoms 12 and 13 proceeds by reasons of thermodynamic stability, and a hydrogen atom is then abstracted from water to complete the reaction (John et al., 2007). After the double bond is migrated to carbon-11 position, the followed step is a rapid hydrogenation of cis- 9 double bond and form TVA for LA or t-11, c-15 C18:2 for LNA. Both these steps are carried out with the catalysis of a particulate enzyme bound to the bacterial cell membrane that is called as linoleic acid isomerase (EC 5.3.1.5) (Griinari and Bauman, 1999). Successively for LNA, the c-15 double bond of t-11, c-15 C18:2 is hydrogenated to form TVA, or is migrated to carbon-13 position to form t-11, c-13 CLA. The isomerization of LA to CLA occurs via an ionic reaction, whereby it is initiated via hydride transfer from C-11 to the N5 of bound flavin adenine dinucleotide, followed by electron migration resulting in the formation of a carbocation and reintroduction of a hydride on C-9 of the fatty acid. Such a mechanism does not involve an exchange with water, consistent with the low incorporation of 2H from deuterium oxide in the present experiments with *P. acnes* (John et al., 2007). On the other hand, it was proposed that the

formation c-9, t-11 CLA from LA by lactic acid bacteria involves a hydration-dehydration mechanism that vias a 10-hydroxy, cis-12-18:1 intermediate (Ogawa et al., 2005).

Regarding biosynthesis of t-10, c-12 CLA, it involves bacterial c-9, t-10 isomerase with the formation the double bonds as the first step in the process ((Khanal and Dhiman, 2004). The t-10, c-12 isomer was formed from LA exclusively, which is not in the same case with c-9, t-11 isomer of CLA. The cloning, crystallization, and structural analysis of the isomerase catalyzing the formation of trans-10,cis-12-CLA by *Propionibacterium acnes* has revealed the geometry of fatty acid binding to the enzyme and demonstrated a mode of action that involves hydride abstraction by enzyme-bound flavin adenine dinucleotide and the involvement of specific aromatic amino acid residues (Liavonchanka et al., 2006).

For the oleic acid presents in the rumen (Figure 2.12), it is isomerized to several trans C18:1 (C18:1 t (6-16)), including TVA during its biohydrogenation to stearic acid (Mosley et al., 2002). The TVA may have implications for the endogenous synthesis of CLA, and the 18:1 trans 7 is supposed to form trans7, cis9 CLA in tissues with the catalysis of Δ9desaturase. The aforementioned review shows that the TVA is a common intermediate during the biohydrogenation of LA, LNA, and oleic acid (Harfoot and Hazlewood, 1988). In addition, the TVA is by far the only precursor reported for synthesis of CLA in tissue and organ, and this process could not happen without the presence of Δ9desaturase. The endogenous synthesis processes of C18:1 fatty acyl CoA and c-9, t-11 isomer of CLA were shown in figure 2.13 and 2.14. Δ9desaturase is a key enzyme in the synthesis of desaturated fatty acyl-CoAs. It is an integral and intrinsic membrane protein in the endoplasmic reticulum (Figure 15), and it can be induced more than 50-fold by dietary manipulations (Ozols, 1997). Some

research confirmed that the activity and mRNA abundance ofΔ9-desaturase are higher in animals' livers and mammary glands, however, there is no hitherto literature to explore the activity and mRNA abundance in the tissues of ruminants.

Given these findings of Δ9desaturase catalyzing TVA to c-9, t-11 isomer of CLA endogenously, Griinari and Bauman (1999) came to the conclusion that ruminal synthesis of CLA was only marginal and could not account for the amount of CLA present in milk and meat from ruminants. Its major source is the endogenous conversion of TVA by Δ9desaturase in the mammary glands and tissues. Based on ratios of TVA to c-9, t-11 Lock and Garnsworthy (2002) estimated the endogenous synthesis of CLA to be more than 80% of the total. The findings of other researchers, such as Griinari et al. (2000), Corl et al. (2001), and Mosley et al. (2002) were highly in accordance with that of Lock and Garnsworthy (2002). And these results were testified to by the aforetime observation of Poulson, (2001) that showed a high correlation ($r = 0.84$) between tissue fat concentrations of CLA and trans-C18:1.

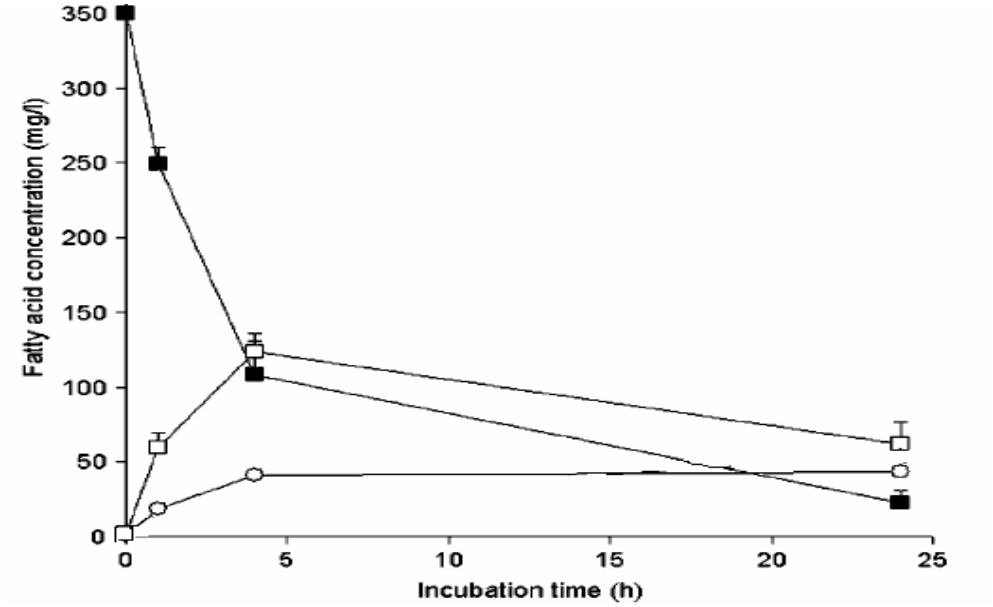

Figure 2.11 Synthesis of conjugated linoleic acid (CLA) isomers from linoleic acid (LA) during 24 h incubations with strained ruminal digesta of sheep (Adapted from John et al., 2007).

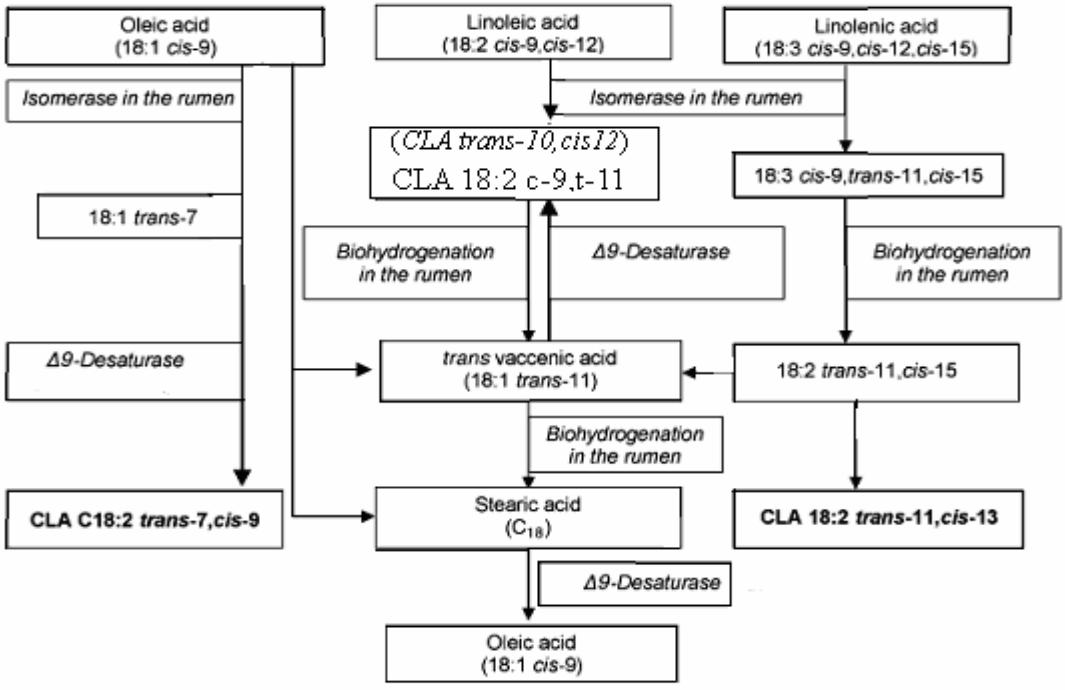

Figure 2.12 Synthesis of CLA in the ruminant. (Adapted from Collomb et al., 2004)

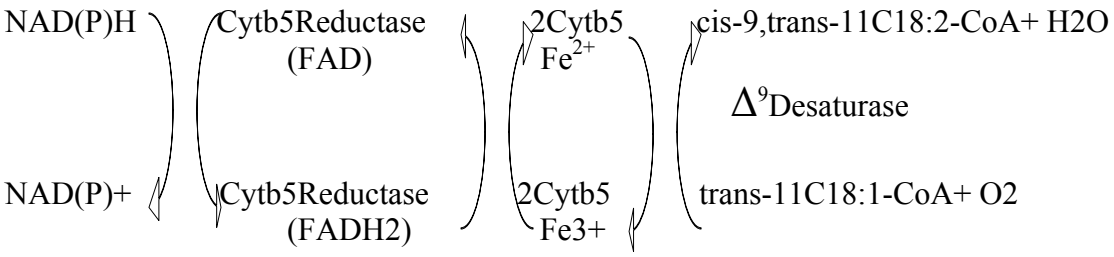

Figure 2.13 Biosynthesis of cis-9, trans-11C18:2 CLA in tissue and organ (Adapted from Khanal and Dhiman, 2004).

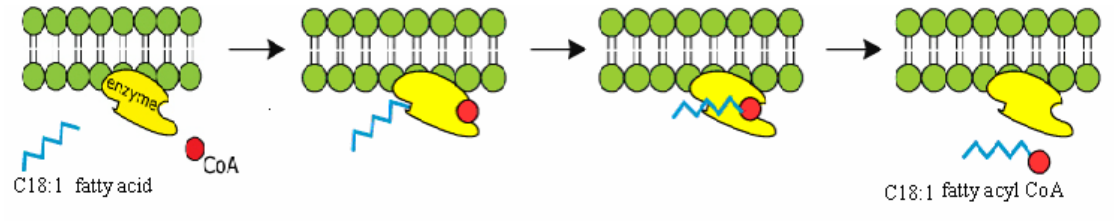

Figure 2.14 Biosynthesis of C18:1 fatty acyl CoA (Adapted from Kemp and Watkins, 2008)

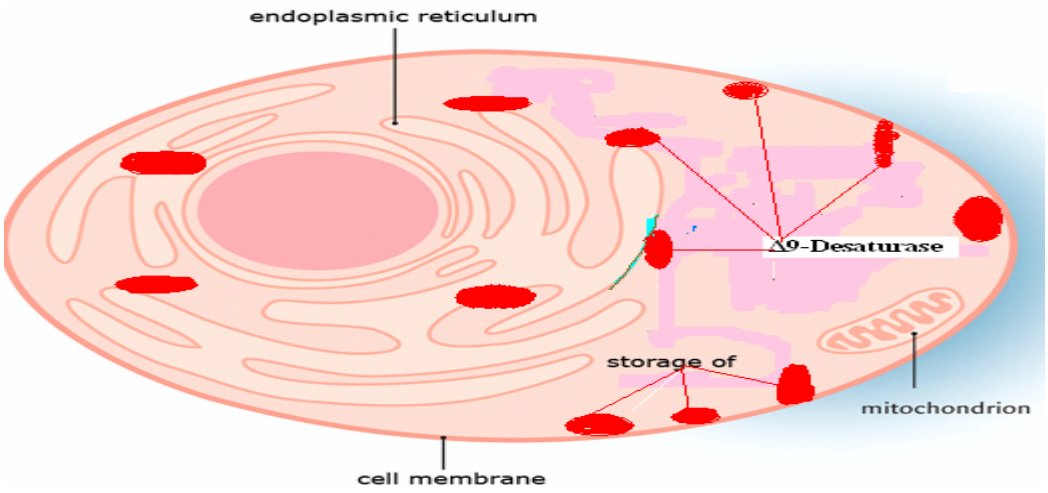

Figure 2.15 Biosynthesis and storage of Δ^9desaturase in endoplasmic reticulum (Adapted from Kemp and Watkins, 2008).

2.6 Effects of probiotics and enriched LA vegetable oil on CLA content of ruminant meats

2.6.1 Effects of probiotics (*S. cerevisiae* and *L. acidophilus*) on CLA content of ruminant meats

There is no research that demonstrates the supplemental *S. cerevisiae* probiotics to elevate the CLA content in ruminants products up to now. Thereto, Korniluk et al. (2007) reported that addition of Se-yeast to the diets of rats that enriched in CLA isomers increased the yield of CLA isomers accumulation in the spleens and pancreas in comparison with those fed the diet enriched in only CLA isomers. This finding may show that the addition of Se-yeast has some positive effects on enhancing of CLA isomers.

Topics that deal with effects of *L. acidophilus* probiotics on CLA are sufficient. CLA in *L.* cultures are clearly stressed as early as1988 by Fairbank et al. Later Pariza and Yang (1999) developed a method for production of cis-9, trans-11 CLA by utilizing of *L.* The ability of converting LA to CLA of probiotic compounds had been approved by many people (Kishino et al., 2002; Julia et al., 2006). And theretofore, the CLA-forming ability of different bacteria has been evaluated and the results demonstrated that *L. acidophilus* was the most effective in increasing the CLA content in a skim milk medium containing LA, and the addition of LA (1000 or 5000 mg/ml) significantly enhance CLA formation (Table 2.10), but not the increasing levels and incubation time. However, they found, on the contrary, the dose of 5000 mg/ml LA significantly depleted the CLA formation by *L. acidophilus* and so for it was reduced by the dose of 1000 mg/ml on the contrary (Lin et al., 1999). One year later, Lin (2000) made a further study and observed that inoculation of *L. acidophilus* into 60 g/L sweeteners and 10 g/L sodium chloride-treated skim milk medium under aerobic conditions for 24 h incubation was most effective in promoting c9,t11-CLA

formation. Besides Lin, (2000), Alonso et al. (2003) also found significant increases ($P<0.05$) in total amounts of CLA for *L. acidophilus* in broth containing added LA in comparison with the incubation of no LA. Their results were shown in Figure 2.16, and they agreed with the fact that 24 h incubation was most effective in promoting c9, t11-CLA formation for the *L. acidophilus* production of CLA (Lin et al., 2000). Contrasted with Lin (2000), they observed that the production of CLA increased significantly with the increasing levels of LA when the level was lower than 0.02%, but when the levels of LA was up to 0.05%, the production of CLA unexpectedly was lower than that of 0.02% level. In recent years, Ming and Shuting (2006) used *L. acidophilus 1.1854* for CLA production by employment of whole milk and alfalfa seed oil that contained LA about 40% as substrate. They observed a sharp increase in LA conversion from 0 to 50% with the level of additional alfalfa seed oil in the substrate increased from 0 to 0.05%, in the contrast, a descending conversion of CLA was followed when the level of additional alfalfa seed oil in the substrate continual increased from 0.05% to 0.9% (Figure 2.17). Except the above stated, we can find many other studies in this field that got the findings insist to each other (Alonso, 2003; Lin, 2006) extensively. Obviously, the previous studies proof-tested that the production of CLA by *L. acidophilus* from LA has an optimal level, it increased with the increasing level below the optimal level, the other way round beyond the optimal level. Furthermore, Ogawa et al. (2001) emphasized that *L. acidophilus* converts LA to CLA under microaerobic conditions other than aerobic conditions, their results are shown clearly in figure 2.18. The findings of Ogawa et al. provide a reliable basis in theory and practice for employment of a blend of yeast (*S. cerevisiae*) and *L. acidophilus* to optimize the production of CLA in the rumen, since the presence of yeast scavenges oxygen in the rumen.

Table 2.8 Effects of increasing addition of LA on c9, t11-CLA (mg/ml) formation of *L. acidophilus* bacterial.

LA (mg/ml)	Incubation time (h)		
	0	**24**	**48**
0	18.0	18.5	17.5
1000	23.0[b]	105.5[a]	106.5[a]
5000	25.0[b]	73.5[a]	68.5[a]

Adapted from Lin et al. (1999); means with different superscripts are significantly different ($p > 0.05$).

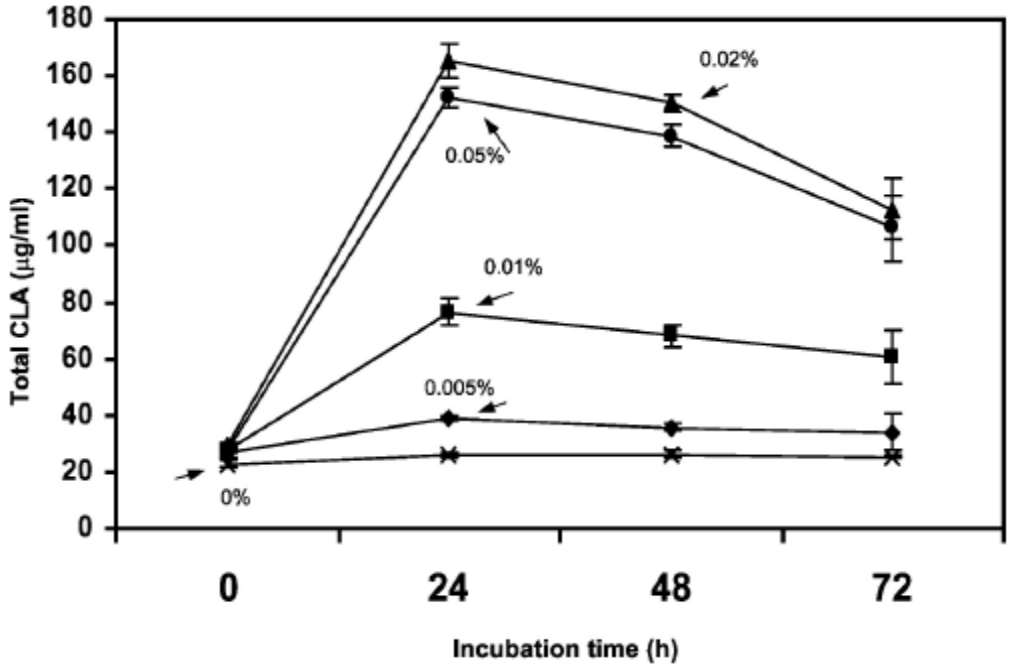

Figure 2.16 Production of total conjugated linoleic acid (CLA) by *L. acidophilus* (L1) in MRS broth supplemented with different level of linoleic acid (Source: Ming and Shuting, 2006)

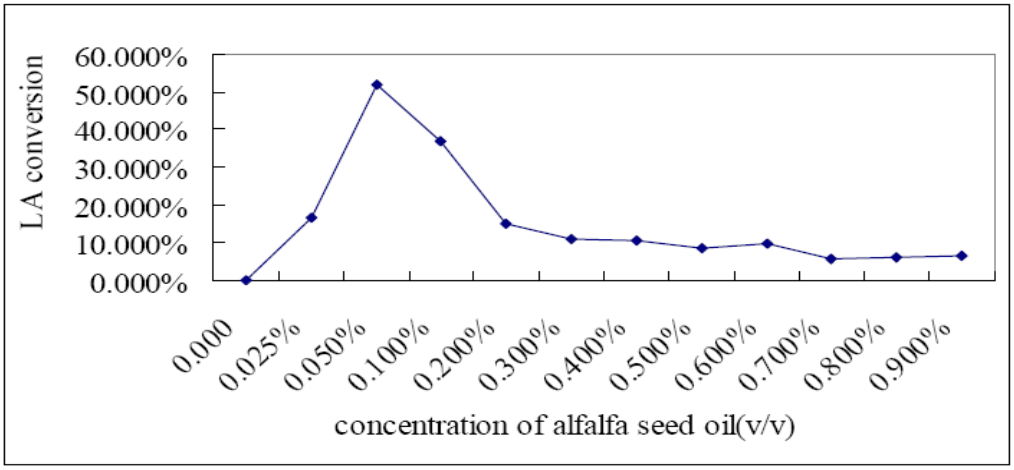

Figure 2.17 CLA production by *L. acidophilus 1.1854* in medium with different levels of alfalfa seed oil (Source: Ming and Shuting, 2006).

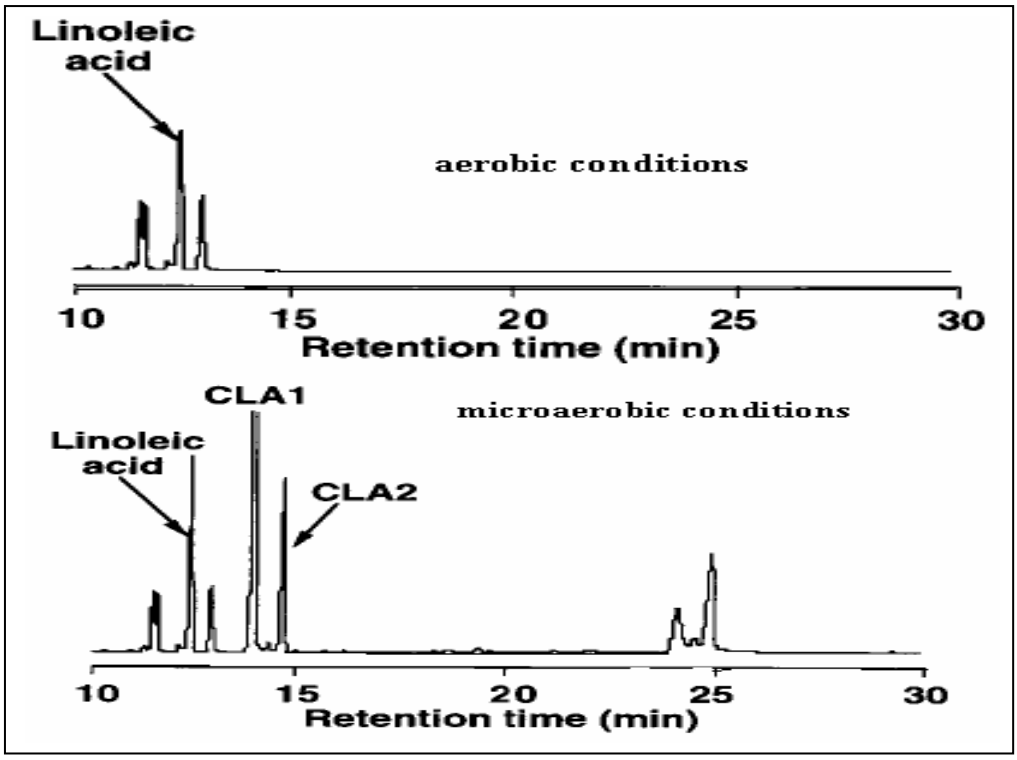

Figure 2.18 GC chromatography of fatty acids produced by washed cells of *L. acidophilus* under aerobic and microaerobic conditions (Adapted from Ogawa et al., 2005).

2.6.2 Effects of enriched linoleic acid vegetable oil on CLA content of ruminant meats

Since linoleic acid is the key precursor for initiating the biohydrogenation process and promoting the formation of CLA was revealed (Kim et al., 2002), many researchers are interested in increasing milk CLA content by addition of enriched linoleic acid vegetable oil. So then, the references on feeding plant seed oils, such as sunflower and soybean that are rich in C18:2 and C18:3 FA to increased CLA content in dairy products are sufficient. E.g. Kelly et al. (1998) found the sunflower oil resulted in higher CLA concentration in milk fat. Similarly, Dhiman et al. (2000) stated that feeding diets containing soybean oil (4%) resulted in approximately a four-fold increase in CLA content of milk fat (2.08%) over the control (0.50% of milk fat). In other studies, Dayani et al. (2004) reported that soybean and sunflower seed cube increased CLA yield in the milk of dairy cows without affecting yields of other milk components. Recently, Yin et al., (2008) stressed that the addition of sunflower oil to the diet caused higher CLA content in rumen fluid and milk fat in comparison with rape seed and linseed oils. Regarding to a goat, high linoleic acid sunflower seed and oil, or high oleic acid sunflower oil also increased milk fat CLA. However, feeding the seeds from soybean to goats fed a low forage diet (30:70 forage to concentrate ratio) did not increase goat milk fat CLA (Chilliard et al., 2003), this may be due to the increment of linoleic acid through the soybean seeds is not so sufficient to increase the milk fat CLA. The mechanism related to the observation of increased milk CLA for additional soybean and sunflower oil supposed to be the direct increasing of CLA and TVA in the rumen, in addition to C18:2 causes an inhibits for the final reduction of TVA to get its increased accumulation in the rumen and consequently increased

accumulation of TVA in the body, which causes CLA elevated since the TVA is the precursor for the endogenous synthesis of c9, t11 CLA (Tilak et al., 2005).

Although there are a vast amount of literatures available about the sunflower and soybean oil enhancing CLA content of milk, the number of research trials are limited when focusing on affecting of the CLA content of meat, particularly on goat meat, for there have been no data at all by far. To take an overview of the findings, it can be summarized that the supplementing beef cattle diets with C18:2 or C18:3- rich plant oils such as soybean and sunflower has yielded varied results as far as increasing the CLA content of ruminant meats. And CLA content of beef derived from addition of soybean oil raging from 0.28 to 0.73% of fat (Griswold et al., 2003; Madron et al., 2002; Beaulieu et al., 2002). This conclusion can be proof-tested by the successive studies. Engle et al., (2000), Beaulieu et al., (2002), and Dhiman et al., (1999) reported that addition of 4 to 6% (diet DM) soybean oil to beef cattle fed high grain diets either marginally increased or did not increase the c9, t11 CLA content of beef. Mir et al. (2002 and 2003) detailed that there was a small increase in the c9, t11 CLA content of fat from beef muscle when steers were fed 3 to 6% sunflower oil compared to beef from cattle fed no oil (0.35 vs. 0.25% (CLA in beef fat)). In the study of Mir et al. (2002), they reported that the feeding of 6% sunflower oil to cattle from Wagyu, Limousin x Wagyu, and Limousin breeds increased CLA (isomer not mentioned) content to 1.25% of fat in beef muscle compared to 0.28% in the control animals. The other study demonstrated the soybean and sunflower oil showing more promising effects on CLA content of beef than other plant oils (Tilak et al., 2005). With sunflower oil (70% linoleic acid) as the lipid source, esterified linoleic acid was almost as effective as free linoleic acid as a substrate for the formation of CLA by *L*..

lactis I-01. The possible reason is that *L.. lactis* strains showed a high tolerance to sunflower oil and also that biohydrogenation is efficient as a detoxification system for unsaturated long-chain FA (Kim and Liv, 2002). Li and Meng (2006) emphasized that when supplementing sunflower oil to the ruminants, the type of dietary fibers influenced ruminal fermentation traits, the biohydrogenation of unsaturated C18 fatty acids, and the profile of CLA. And they observed the lignified dietary fiber significantly increased the production of cis-9, trans-11 CLA and total CLA (sum of cis-9, trans-11 CLA, trans-10, cis-12 CLA, trans-9, trans-11 CLA, and cis-9, cis-11 CLA). The research of Szölloskei et al. (2005) elaborated that sunflower oil, soybean oil and fish oil, when supplemented to sheep, increased the cis-9, trans-11 CLA. And a significant increase in the amount of cis-9, trans-11 CLA for sunflower oil, soybean oil was found.

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CHAPTER III

EFFECTS OF SUPPLEMENTAL PROBIOTICS ON PERFORMANCES OF GROWNG GOATS FED WITH WHOLE PLANT CORN SILAGE

3.1 Abstract

This experiment was performed with the purpose of investigating effect of additional blend of *Saccharomyces cerevisiae* and *Lactobacillus acidophilus* probiotics on growth, ruminal metabolism, and plasma fatty acid profiles particularly conjugated linoleic acid (CLA) in growing goats fed corn silage, and selected the optimal levels of the probiotics for further study. Twenty-four growing crossbred (Thai native x Anglo-Nubian) goats that weighed (14.2 ± 2.3) kg, aged about 6 months, were purchased and allocated to 4 treatments according to Randomized Complete Block Design (RCBD) with 6 goats in each treatment. The blocks were made by weight into heavy, medium, and light goats and each of the treatments contained two goats from each of the blocks.

The results displayed that g/kg $W^{0.75}$ dry matter intake ($P<0.05$), ADG (g/d) ($P<0.01$), and feed conversion ($P<0.05$) were increased. At the same time digestibility of NDF ($P<0.05$), EE, ADF and CP ($P>0.05$) as well as that of DM and OM ($P>0.05$) also were increased. In the mean time, ruminal average pH unaffected, but the NH_3-N and also PUN ($P<0.05$), TVFA ($P>0.05$) were raised, but propionic proportion

(P<0.05) and butyric proportion (P>0.05) were reduced in concurrent with raise of acetic proportion and resultingly C2 : C3 ratio (P>0.05). Protozoal number (P>0.05) was depressed contrasted to heighten total viable bacterial number.

On plasma fatty acid profiles, total saturated fatty acids (P>0.05) was increased, and contrasted with decrease of C15:0 (P<0.01), C16:0 (P>0.05), and C18-C22 polyunsaturated fatty acids (P<0.05 or P<0.01). In addition, the experiment proved that the supplemented probiotics was in force for heightening CLA (P<0.01); for raising desirable fatty acids (P<0.05); for reducing ratio of PUFA: SFA (P>0.05) and for raising ratio of n6:n3 (P<0.05).

3.2 Introduction

Nowadays, public requirements for food quality and safety, environmental deterioration and pollution, together with animal welfare have become the keystone that should be considered in animal agriculture. In respect to these keystones, some governments have begun to formulate a rule or policy to aim at their individual practical situation. For example, to direct at the degradation of grassland, the Chinese government has been trying to push or encourage the goat husbandry turning back to housing. This has been stimulating the research interest on stall-feeding strategy and feed additives that are characterized by high bio-availability. It is obvious that the corn silage is appropriate to be employed in intensive and extensive goat industry for it can be free from the seasonal limitation and suitable for mechanization or highly-technological feeding. Amongst of high bio-availability nutraceuticals, probiotics are widely used in animal nutrition with purpose of inducing favorable changes in the activity of the digestive microflora (Chiofalo et al., 2004).

A probiotics was defined as a living single or mixed microbial which beneficially affects the host animal by improving its gastrointestinal microbial balance (Krehbiel et al., 2003). Despite the fact that there is no probiotics can compete antibiotics with functions of growth stimulating and prevention or treatment of diseases, but as a nuisance free feed additive, they are widely embroiled in *in vitro* or *in vivo* studies. In summation, the utilization of probiotics have mainly regarded the administration of yeast cultures partially strains of *S. cerevisiae* (Chaucheyras et al., 2001). Moreover, in parallelism yeast, *Lactobacilli* have drawn much study interest by the reason of providing the host animal healthier and more favorable gastro-enteric setting for digestive and absorption processes (Klaenhammer, 1998). There were abundant literatures to prove that among several *Lactobacilli* strains (*L. acidophilus*, *L. casei*, and *L. bifidus*), *L. acidophilus* was surely the most focalized one on productive performances, on the variation of intestinal flora and on the sanitary state of the host animals (Krause et al., 1995; Tannock et al., 1990).

Overall, the previous study data of *L.* species fed to young ruminants has proved to establish and maintain normal intestinal microorganisms, and/or to alleviate metabolic disorder such as diarrhea and acidosis, rather than as a production stimulant. The literatures related to effect of *L. acidophilus* on rumen fermentation and animal production (i.e., body gain, feed efficiency, milk yield and quality, and meat quality) are scarce. Lots of research demonstrated that *L. acidophilus* in combination with fungal cultures were more efficacious for increasing milk production in lactating dairy cows (Komari et al., 1999; Block et al., 2000). Furthermore, Draksler et al. (2004) found the *L. acidophilus* resistant to pH 2.0 and bile salts (0.3%) and could be pre-selected as a probiotics for use in goat feed.

Conjugated linoleic acid (CLA) is a collective term used to describe one or more positional and geometric isomers of linoleic acid with conjugated double bonds (Ip et al., 1994), and it is characterized as two double bonds separated by a signal bone at various carbon positions. CLA has been reported for wide range of beneficial effects such as anticarcinogenic, antiatherogenic, antidiabetic and immune stimulatory. They have also been shown to alter partitioning and lipid metabolism, and reduce body fat in a number of different animal species (Bauman et al., 2000; Lobb and Chow, 2000). Ruminant products are the predominant CLA resource. Whereby, it is fantabulous and interesting to work at enhancing of CLA concentration in ruminant products with the aim to meet the effectual level for human being.

One study had shown that addition of Se-yeast to the diets of rats has positive effects on enhancing of CLA isomers (Korniluk et al., 2007). On the other hand, since CLA in *L.* cultures was found in 1988, the ability of converting linoleic acid to CLA of *L. acidophilus* had been well documented (Kishino et al., 2002; Julia et al., 2006).

Thereupon, the present study was carried out to investigate the addition of a commercial probiotics that contains *L. acidophilus* 2.0 x 10^{12} cfu/g, and *S. cerevisiae* 5.0 x 10^{11} cfu/g impacted on growing goats' growth and rumen fermentation performance, and the highlight is on the plasma fatty acid profiles particularly the CLA content in the condition of feeding corn silage. Thereinafter, the term 'probiotics' would refer to this commercial probiotics unless it is specified.

3.3 Objectives

The present experiment was carried out to study the effect of additional *S. cerevisiae* and *L. acidophilus* probiotics on growth, ruminal metabolism, and plasma

fatty acid profiles particularly CLA in growing goats fed with corn silage, and selected the optimal levels of the probiotics for further study.

3.4 Materials and methods

3.4.1 Experimental design and treatment

Twenty-four growing crossbred (Thai native x Anglo-Nubian) goats that weighed 14.2 ± 2.3 kg, aged about 6 months, were purchased from Pukthongchai district, Nakhon Ratchasima province of Thailand to perform this experiment. The animals were allocated to 4 treatments according to Randomized Complete Block Design (RCBD) with six goats in each treatment. The blocks were made by weight into heavy, medium, and light goats and each of the treatments contained two goats from each of the blocks (Table 1). Before experiment, the animals were injected with Ivomic (Merial Ltd., Iselin, NJ) for anti-internal parasite, and housed in individual pens (0.9 x 1.4 m) where the animals could have an easy access to corn silage and fresh water *ad libitum*. What was more, the pens were cleaned and disinfected with Ciber solution prior to the housing of the animals. During the experiment, animals in different treatments received the whole plant corn silage plus concentrate basal diet and supplemented with 0, 2.5, 5, and 7.5 g/h/d probiotics (*L. acidophilus* about 2.0 x 10^{12} cfu/g, and *S. cerevisiae* about 5.0 x 1 0^{11} cfu/g). The additional probiotics was mixed evenly with concentrate prior to feeding, and offered to animals by half at 9:00 am and the other at 3:00 pm, respectively. The concentrate was supplied by 1.5% *percentage on* body weight for each goat to ensure that the dietary intakes of crude protein, growth net energy, and dry matter in accordance with the Nutrients Requirements of Goats (NRC, 1989) under the condition of maintenance plus lower

activity and 50 g/d weight gain. All animals accessed to the whole plant corn silage and clean water *ad libitum*. The experiment lasted 8 weeks, excepting 2 weeks for adjustment, 1 week for adaptation, and 1 week post-experiment for urinary and faecal samples collection.

Table 3.1 Lay-out of experimental treatments

Groups	Animals(n)	BW(kg)	Treatments
I (Control)	6	14.03±2.4	Basal diet + probiotics 0 g/d
II	6	14.87±2.9	Basal diet + probiotics 2.5 g/d
III	6	13.93±2.5	Basal diet + probiotics 5.0 g/d
IV	6	14.05±2.4	Basal diet + probiotics 5.0 g/d

Basal diet= whole plant corn silage plus concentrate.

3.4.2 Experimental material

The probiotics was purchased from L. P. Feeds Tech Co., Ltd (Bangkok, Thailand), containing *L. acidophilus* about 2.0 x 10^{12} cfu/g and *S. cerevisia* about 5.0 x 10^{11} cfu/g. The whole plant corn silage was purchased from Kornburee Cooperatives (Kornburee district, Nakhon Ratchasima province of Thailand). The pelleted concentrate was supplied by the farm of Suranaree University of Technology (Nakhon Ratchasima province of Thailand), and it was composed of cassava chip (12.0%), cassava pulp (31.5%), rice bran with germ (10.0%), defatted rice bran (10.0%), molasses (8.0%), palm kernel expeller meal (18.0%), rapeseed meal (4.0%), corn meal (4.0%), urea (1.8%), mineral (1.5%) (Containing Ca 14.5%, P 17%, NaCl 18%, Mg 10%, and carrier), and additional binder (0.2%).

3.4.3 Sampling

The daily offered and left concentrate and whole plant corn silage were weighed (the residues were removed) every morning before offering for the purpose of determination dry matter intake. Body weight of the animals were measured weekly prior to the morning feeding with the aim of evaluating the growing performances. The whole plant corn silage and concentrate were sampled weekly and dried at 60~65 °C in hot air oven for determination of dry matter (DM) composition, and followed by grounding through a 1 mm screen and then kept in tightly covered plastic containers to make a pool respectively for further proximate analysis. During the post-experiment week for urinary and faecal samples total collection, the all-day faece and urine (10% H_2SO_4 was used as a preserving reagent, 30 ml/container) were collected and the total amount was recorded down every morning (measured faece weight and urine volume). Subsequently, 15% of the total amounts was sub-sampled to make a pool respectively for each animal, and then was kept at -20 °C and in the end was dried prior to chemical composition analysis that aimed to determine digestibility and nitrogen balance. For ruminal fluid samples, they were withdrawn on the last day of the experiment through an esophageal stomach tube following 0, 3, and 6 h post-morning meal timing. The samples were strained through three layers of muslin cloth and then were followed by immediately measuring of pH with an OHS-3C pH meter. Thereafter, 1 ml of the samples were measured well and truly with a pipette into the tubes containing 9 ml 10% formalin (V:V=9:1) as a preserving reagent and then were closed tightly with screw caps that with butyl rubber lining for checking the counts of ruminal protozoa and bacteria. At the same time, 20 ml of the samples were measured and then put into small plastic bottles containing 5 ml 6 N

HCl as a preserving reagent, and then the bottles were closed tightly with screw caps that with butyl rubber lining for determination of ruminal ammonia N and volatile fatty acids. With that, all samples were kept at -20 °C until further analysis. The blood samples were collected from jugular veins into EDTA-containing vacuum tubes and were centrifuged at 2700 x r for 5 min to separate plasma from the cells within 20 minutes after sampling. Subsequently the plasma was collected, and then it was stored at −80 °C for subsequent analyses of blood urea nitrogen and fatty acid profiles.

3.4.4 Chemical analysis and calculation

The dry matter (DM) of feed (including residue) and feces samples were determined in triplicate by drying in a hot air oven at 60~65°C for 48 h, and the organic matter (OM), N (feed N, faecal N, and urinary N), and crude ash were determined according to the methods described in AOAC (1984). The neutral detergent fiber (NDF) compositions were determined by the method stated by Van Soest et al. (1991). The determination of ether extracts (EE) adopted a modified previous stated method (Yang Sheng, 1999; Nahm, 1992). The brief progress for modified method to analyze EE included weighing of 2-5 grams samples in duplicate into the constant weight filter paper containers, then they were dried until constant weight again, and then the 2050 SOXTEC Auto Control extraction apparatus was adopted to extract. The results were calculated by:

$$\% \text{ EE} = (①\% \text{ EE} + ② \% \text{ EE}) /2$$

$$① \% \text{ EE} = (T_1\text{-}T_2) /\text{SW} \times 100$$

Where: T_1 = constant weight of thimble before extraction, T_2 = constant weight of beaker after extraction, and SW = Weight of sample.

$$② \% \text{ EE} = (\text{B-A}) /(\text{B-C}) \times 100$$

Where: B = constant weight of filter paper container and sample before extraction, A= constant weight of filter paper container and sample after extraction, and C= constant weight of filter paper container.

An OHS-3C pH meter was used to measure the ruminal pH, and the counts of ruminal protozoa and bacteria were directly checked on a Tiefe Depth Profondeur by an electron microscope under 40-fold directly. The determination of apparent digestibility and nitrogen balance was done according to the equations of Schneider and Flatt (1975).

Plasma urea nitrogen (PUN) was determined using a Spectronic R Genesys 5 spectrophotometer; the principle is shown in Figure 3.1.

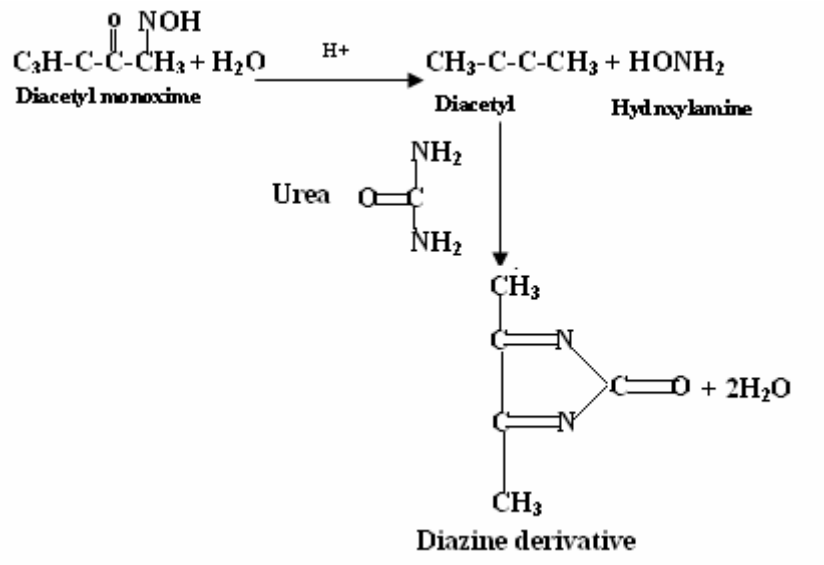

Figure 3.1 The principle of plasma urea nitrogen (PUN) determination (Adapted from Preston et al., 1964).

The brief progress for determination of PUN would be shown as followed.

Preparation of reagents:

1) Stock forric chloride-phosphoric acid regent

$FeCl_3.6H_2O$ 15 g
DI water 30 ml } + H_3PO_4 (85%) 300 ml, mixed evenly, adjusted to 450 ml with DI water and kept in brown bottle.

2) Acid reagent (preparation should be done shortly before use)

H_3PO_4 (85%) 150 ml + DI water 500 ml + Stock forric chloride-phosphoric acid regent 1 ml, mixed evenly and adjusted to 1000 ml with DI water.

3) Color reagent

Diacetyle monoxime 1.7 g
Thiosemicarbazide 0.3 g } mixed evenly and adjusted to 1000 ml with DI water, subsequently filtrated through waterman filter paper and kept in brown bottle.
DI water 100 ml

4) Stock PUN standard (mg/dl)

Urea 214.2 mg + 0.1N HCl 100 ml, mixed evenly and kept in brown bottle at 4 °C.

5) Analyzing

The standard was run in triplicate and adopting none but the r^2 over 0.98. The samples and reagents were measured into a 16 x 125 mm test tube with pipette, and the tubes were closed tightly with a screw cap that with butyl rubber lining, following mixed evenly, supervening by boiling at 80 °C until the color changed into pink and cooled them down to normal room temperature in cool water. Within 15 minutes after the preparation, the determination at 540-nanometer wavelength was done with employing of the blank to adjust the spectrophotometer to zero prior to it.

3.4.5 Preparation of samples for gas chromatography (GC) analysis

The ruminal fluid samples that used to determine total VFA and molar proportion of main VFA mix (acetate, propionate, and butyrate) were centrifuged at

3500 x r for 10 min at 4 °C to get rid of food particles and ruminal microbe, with that measured 1 ml supernatant into a 2 ml vial for gas chromatography (GC) analysis.

The preparation of plasma samples for GC analysis was done by using a modified method explained by Bondia-Pons et al. (2007). In short the procedures were:

a) Measured 2 ml plasma into a test tube that with a butyl rubber septa screw cap with pipette, and subsequent additions of 1 ml international acidinternal standard (heptadecanoic acid C17:0) (2 mg C17:0 dissolved into 1 ml hexane) and 2.5 ml 0.5 m sodium-methylate reagent (0.5 m NaOH dissolved into 1 L methanol);

b) Vigorous shaking and heated at 80 °C for 10 minutes;

c) Cooled down to normal room temperature in cool water and came on with a addition of 1 ml 40% BF_3, therewith vigorous shaking and reheated at 80 °C for 5 minutes;

d) Cooled down to room temperature in cool water and added 1ml hexane, with that vigorous shaking 1 minute, and added 1 ml saturated NaCl solution (26.47 g NaCl into 100 ml DI water at 25 °C) ;

e) Centrifuged for 10 minutes at 2200 r, and with what took 1 ml supernatant into a 2 ml vial for GC analysis.

3.4.6 Analysis of fatty acids by Gas chromatography (GC)

Total VFA and molar proportion of acetic, propionic, and butyric acids in ruminal fluid and fatty acid profile of plasma samples were determined by HP6890 gas chromatography (GC) (made in USA) that fitted with a Flame Ionization Detector (FID). In addition, a J&W 122~3232 column was applied for determination of VFA, whereas a 100 m x 0.25 mm fused silica capillary column (SP2560, Supelco Inc,

Bellefonte, PA, USA) for determination the plasma fatty acid profiles. The column temperature was fixed at 70 °C for 4 min, then it increased at 13 °C /min to 175 °C which lasted for 27 min. Continually it increased at 4 °C /min to 215 °C and kept for 31 min. Nitrogen was adopted as carrier gas with a 60 ml/min flow rate and the oven temperature was 250 °C. FID and injection temperature were fixed at 280 °C, and a 1μL injection was done with a 10-μL injector.

3.4.7 Body weight measurement

Body weights of testing animals were measured every Saturday morning before morning meal. The average daily gain (ADG) was calculated as:

$$ADG\,(g/d) = \frac{Total\ \ weekly\ gain\,(g)}{Number of\ weeks \times 7}$$

3.4.8 Data analysis

Data were analyzed using the General Linear Models procedure of SAS (SAS Inst. Inc., Cary, NC 1985) as a randomized complete block design. Variation due to blocks was extracted in the models employed for the analysis. The protected least significant differences method was used to determine differences among treatment means. Polynomial contrasts (linear, quadratic, and cubic effects) were used to evaluate the all effects. In addition, a non-parametric Mann-Whitney test was used to compare the count means of rumen protozoa also viable bacteria within groups. Differences were considered to be significant at $P<0.05$ (*), highly significant at $P<0.01$ (**), tendencies at $0.05<P>0.050$, and 'ns' was used to represent no significant difference.

3.4.9 Experimental site

The experiment was conducted on the farm of Suranaree University of Technology, whenas chemical analysis was performed at the center of Scientific and Technological Equipments of Suranaree University of Technology.

3.4.10 Duration

The experiment January 28, 2007 – April 22, 2007

3.5 Results

3.5.1 Diet composition

All animals received a diet composing of whole plant silage plus concentrate. The diet was adequate to meet the requirements of crude protein, growth net energy, and dry matter intakes of the goats under the condition of maintenance plus lower activity and 50 g/d weight gain Nutrients Requirements of Goats (NRC, 1989). As to the concentrate, it contained DM 88.8%, CP 13.4%, and NDF 37.1%, whereas the silage contained DM 21.9%, CP 9.2%, and NDF 57.9% (DM basis) (Table 3.2). As shown in Table 3.3, the main fatty acids of the concentrate were comprised of 30.72 % C18:2n6c, 20.0% C17:0, 15.34% C12:0, 14.75% C18:1n9c. Concededly, these fatty acids accounted for 1.23%, 0.80%, 0.62%, and 0.59% of the concentrate dry matter respectively. And yet, the main fatty acids of the whole plant corn silage were composed of 39.10% C18:2n6c, 16.60 % C18:1n9c, 14.90% C16:0, and 11.71% C18:3n3, and these fatty acid mad up of 0.70%, 0.30%,0.27%, 0.21% of the corn silage dry matter respectively.

Table 3.2 Chemical compositions of experimental diet (dry matter basis).

Items	**Composition** (%)
Concentrate	
Dry matter	88.8
Organic matter	93.4
Crude protein	13.4
Ether extracts	4.0
Acid insoluble ash	3.8
Acid detergent fiber	28.7
Neutral detergent fiber	37.1
Corn silage	
Dry matter	21.9
Organic matter	88.1
Crude protein	9.2
Ether extract	1.8
Acid insoluble ash	6.1
Acid detergent fiber	46.6
Neutral detergent fiber	57.9

Table 3.3 Fatty acid profiles of concentrate and whole plant core silage (DM basis).

Items	% DM	% Total fatty acid
Concentrate		
C12:0	0.62	15.34
C14:0	0.23	5.83
C16:0	0.25	6.19
C17:0	0.80	20.00
C18:0	0.09	2.28
C18:1n9c	0.59	14.75
C18:2n6c	1.23	30.72
C18:3n3	0.07	1.79
Others	0.12	3.00
Corn silage		
C14:0	0.03	1.60
C16:0	0.27	14.90
C16:1	0.01	0.61
C17:0	0.03	1.60
C18:0	0.07	3.68
C18:1n9c	0.30	16.60
C18:2n6c	0.70	39.10
C18:3n3	0.21	11.71

Others	0.18	10.09

3.5.2 Feed intake and growth performances

No differences existed in whole plant corn silage and concentrate total daily average as well as centesimal body weight dry matter intakes between the treatments. However, as obviously shown in Table 3.4, in terms of g/kg $W^{0.75}$ total dry matter intakes (TDMI) significantly increased with linear, quadratic, also cubic statistical analysis brought on addition of probiotics. Wherein, the increasing levels of supplemented probiotics did not bring out any differences in impacts of probiotics on dry matter intakes. On the contrary, the increasing levels (2.5, 5.0, 7.5 g/h/d) showed similar g/kg $W^{0.75}$ TDMI (52.3, 52.1, 52.5). When judging the growth performance with average daily gain (ADG), the linear, quadratic together with cubic statistical analysis showed that it increased with extremely significant differences. Whereas, the comparisons of the ADG within probiotics supplemented groups were quite close to each other regardless of increasing doses (supplemented probiotics 2.5, 5.0, 7.5 g/h/d, the ADG were 52.7, 54.8, and 51.4 g/d respectively). In the case of checked growth performance with feed efficiency (DMI: ADG), the linear, quadratic as well as cubic significant difference predicatively occurred not only in comparison with control group, but also in comparison within treatment groups (Table 3.4). The DMI: ADG ratios of 2.5 and 5.0 g/h/d probiotics treatment groups were close to each other (7.6 and 7.1). Nevertheless, those of 5.0 and 7.5 g/h/d probiotics treatment groups were significantly differed to each other (7.1 and 8.6). For a holoscopic look of Table 3.4, it showed that the growth performance of 5.0 g/h/d probiotics reached highest ADG and feed efficiency (54.8g/d and 7.1) compared with dose of 2.5 (52.7 g/d and 7.6) and 7.5

g/h/d (51.4 g/d and 8.3).

The growth performance was detected by weekly gain (Figure 3.2). The first weekly gain of control group reduced due to outset of experiment, contrasted with it, probiotics treatment groups showed more steady weekly gain. In the last second week of this experiment (April 9-15, 2007), the hot weather (34±3.8 °C) stressed the animals, and all of them reduced intake owing to the weather change. As a result, the weekly gain promptly decreased, whereas, the probiotics treatment groups turned back to stable gain in the coming week, contrariwise, the control group continued lowering. These evidences testified to the efficiencies of probiotics on adaptation of the animals to feed and heat stress.

Table 3.4 The effect of probiotics on DMI, ADG, and feed conversion of growing goats

	Probiotics (g/h/d)					**Contrast**		
	0	**2.5**	**5.0**	**7.5**	**SEM**	**Linear**	**Quadratic**	**Cubic**
SDMI (g/d)	176.0	173.6	161.9	199.7	10.51	ns	ns	ns
CDMI (g/d)	227.5	227.5	227.5	227.5	1.02	ns	ns	ns
Total (g/d)	403.5	401.1	389.4	427.2	17.53	ns	ns	ns
g/kg $W^{0.75}$	48.4^{b}	52.3^{a}	52.1^{a}	52.5^{a}	1.10	*	*	*
%body weight	2.4	2.7	2.7	2.8	0.12	ns	ns	ns
ADG (g/d)	41.1^{b}	52.7^{a}	54.8^{a}	51.4^{a}	1.30	**	**	**
DMI:ADG	9.8^{a}	7.6^{bc}	7.1^{c}	8.3^{b}	1.32	*	*	*

SDMI=whole plant corn silage dry matter intake; CDMI=concentrate dry matter intake; LWI=%live body weight intake; Means with different superscript letters in the

same row differ significantly (P<0.05); SEM=standard error of the mean; *P<0.05; **P<0.01; ns= not significantly different (P>0.05)

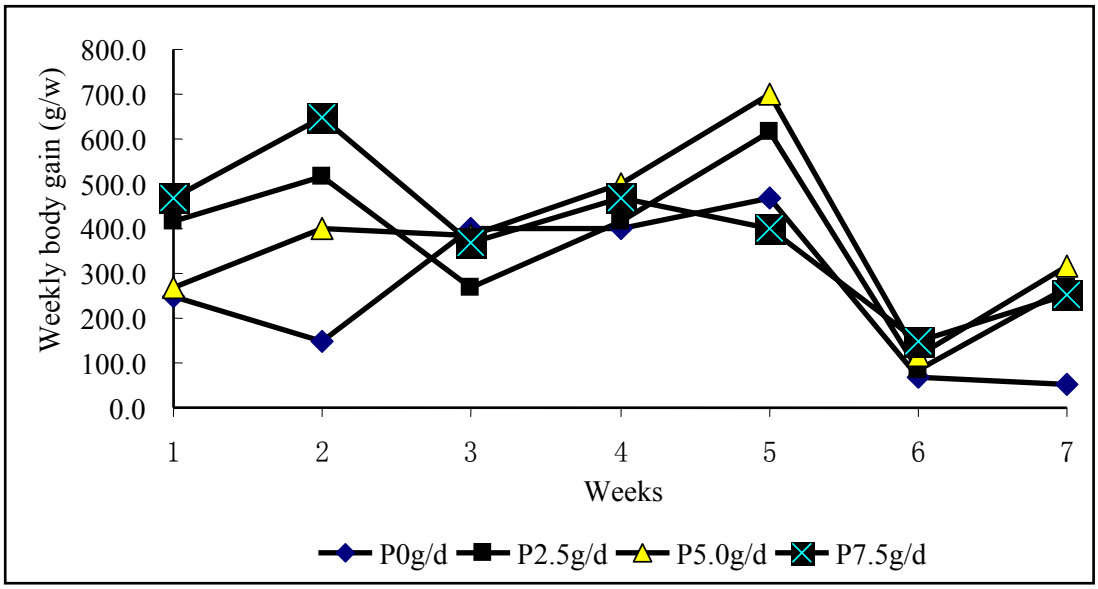

Figure 3.2 The weekly gain of growing goats that supplemented with probiotics (*S.cerevisia* and *L. acidophilus*).

3.5.3 Dietary digestibility

Table 3.5 showed the digestibility of DM, OM, CP, ADF, and EE were not significantly different as a result of additional probiotics. Thereunto, the digestibility of EE, ADF and CP were in the line with expectation to show increasing tendency (P>0.05). At the meantime, the supplementation of probiotics was effectual to elevate the digestibility of NDF with difference in linear, quadratic also cubic significant.

Table 3.5 The effect of probiotics on dietary digestibility of growing goats fed whole plant corn silage (%).

	Probiotics (g/h/d)				SEM	Contrast		
	0	**2.5**	**5**	**7.5**		**Linear**	**Quadratic**	**Cubic**
DDM	69.1	69.9	72.2	69.2	0.88	ns	ns	ns
DOM	73.5	74.6	75.7	74.0	0.85	ns	ns	ns
DCP	61.7	63.3	65.4	64.3	0.95	ns	ns	ns
DADF	39.2	42.7	42.9	39.8	0.87	ns	ns	ns
DNDF	52.8^{b}	56.7^{a}	57.2^{a}	53.2^{b}	0.71	*	*	*
DEE	75.0	76.4	78.0	75.1	1.05	ns	ns	ns

Means with different superscript letters in the same row differ significantly (P<0.05); SEM=standard error of the mean; *P<0.05; **P<0.01; ns= not significantly different (P>0.05).

3.5.4 Ruminal Fluid pH, Ammonia N, PUN, and VFA

Supplementation of probiotics did not conduce to significant changes for the ruminal average pH, howbeit the 5.0 g/h/d group was observed a decreasing tendency comparing to the control (6.42 vs. 6.72) (P>0.05) (Table 3.6). Differed from the case of pH, ammonia nitrogen (NH_3-N) and plasma nitrogen (PUN) significantly increased as a causation of supplementing probiotics (P<0.05). In terms of volatile fatty acid (VFA), the total production of VFA was entailed to a faint increment (P>0.05) and butyric centesimal

proportion in the round way to show a slight decrement (P>0.05) with increasing levels of probiotics. However, the increasing level of probiotics tended to increase the acetic centesimal proportion, and up to a significant amount (P<0.05) in comparison with the control (69.23 vs. 66.28 mM/l) at the level of 7.5 g/h/d. The propionic centesimal proportion showed linear, quadratic, and cubic decrease due to the addition of probiotics (P<0.01), but then it was similar within treatment groups. Regarding to the ratio of C_2 : C_3, addition of probiotics affirmatively brought it on linear, quadratic as well as cubic increase comparing to the control (P<0.05), and yet it was almost the same within the probiotics treatment groups (4.49, 4.42, and 4.42).

Table 3.6 The effect of probiotics on the average pH, ammonia nitrogen (NH_3-N, mg/Dl), plasma nitrogen(PUN, mg/Dl), and VFA (mM/l) of growing goats fed whole plant corn silage.

	Probiotics (g/h/d)					**Contrast**		
	0	**2.5**	**5.0**	**7.5**	**SEM**	**Linear**	**Quadratic**	**Cubic**
pH	6.72	6.63	6.42	6.58	0.06	ns	ns	ns
NH_3-N	10.43^b	12.51^a	12.32^a	12.14^a	0.27	*	*	*
PUN	11.01^b	16.31^a	16.48^a	15.88^a	0.34	*	*	*
TVFA	56.22	56.82	56.93	59.28	0.70	ns	ns	ns
VFA proportion (% TVFA)								
Acetate	66.28^b	67.82^b	68.37^b	69.23^a	1.09	ns	ns	ns
Propionate	21.51^a	19.12^b	19.47^b	19.68^b	0.65	*	*	*
Butyrate	6.83	5.98	6.12	6.23	0.40	ns	ns	ns
C_2:C_3	3.79^b	4.49^a	4.42^a	4.42^a	0.15	*	*	*

Means with different superscript letters in the same row differ significantly ($P<0.05$); SEM=standard error of the mean; *$P<0.05$; ns= not significantly different ($P>0.05$).

3.5.5 Ruminal microbe population

The number of protozoa ranged from 0.68 to 1.18 x 10^4 /ml rumen fluid. And as expected, even though the effectiveness of supplemented probiotics on protozoal population was not significant ($P>0.05$), an overt subtraction was found. Particularly 2.5 and 5.0 g/h/d, 2 levels let the counts of protozoa down by a visible tendency ($P>0.05$) (Table 3.7). As shown in Figure 3.3, in a line chart, the effectiveness grew in number for the counts of protozoa leading by addition of probiotics was more clear, the curved line of the control visibly above those of probiotics treatment groups.

The number of total viable bacteria ranged from 1.17 to 2.02 x 10^{10} /ml rumen fluid. Before morning meal, the addition of probiotics did not open the door for pushing up the counts of total ruminal bacteria by any significance, except for the 2.5 and 5.0 g/h/d, 2 treatments presented a raising tendency ($P>0.05$). Howbeit the effect of additional probiotics on ruminal bacterial number displayed enhancement with significant or highly significant differences after feeding 3 h ($P<0.01$) or went to the length of 6 h ($P<0.05$) with linear, quadratic and also cubic statistical analysis (Table 3.6). The case was congruent with that of protozoa, a line chart could clearly show the elevation of bacterial numbers that were induced by additions of probiotics (Figure 3.4).

Table 3.7 The effect of probiotics on rumen microbe population of growing goats fed whole plant corn silage.

	Probiotics (g/h/d)					Contrast		
	0.0	**2.5**	**5.0**	**7.5**	**SEM**	**Linear**	**Quadratic**	**Cubic**
Protozoal population ($x10^4$)								
0h	0.86	0.68	0.75	0.83	0.09	ns	ns	ns
3h	1.18	1.18	1.10	1.13	0.10	ns	ns	ns
6h	0.88	0.72	0.73	0.86	0.09	ns	ns	ns
Bacterial population ($x10^{10}$)								
0h	1.17	1.27	1.30	1.13	0.08	ns	ns	ns
3h	1.78^b	2.08^a	2.18^a	2.02^a	0.14	**	**	**
6h	1.48^b	1.68^a	1.73^a	1.57^b	0.08	*	*	*

Means with different superscript letters in the same row differ significantly ($P<0.05$); SEM=standard error of the mean; *$P<0.05$; **$P<0.01$; ns= not significantly different ($P>0.05$).

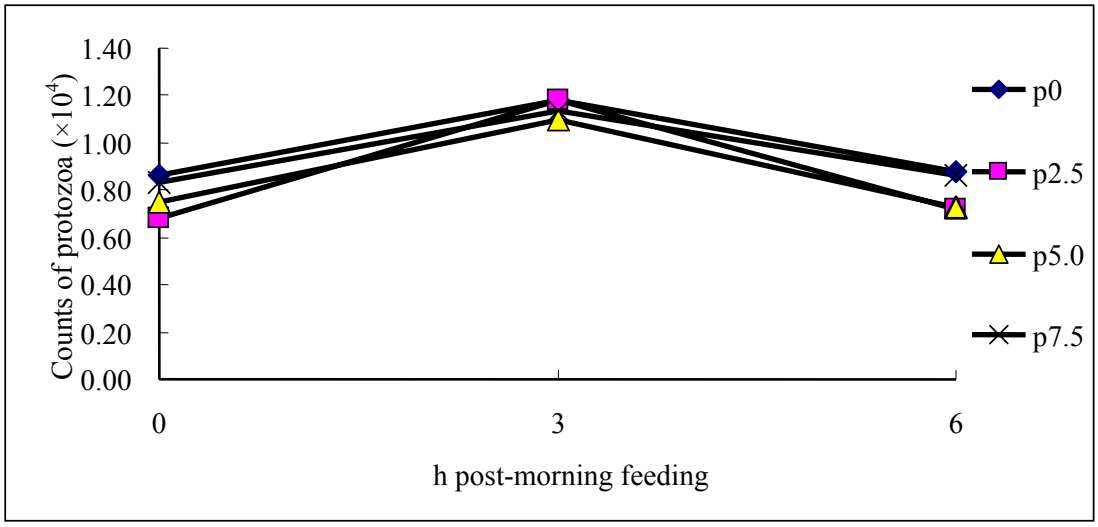

Figure 3.3 Ruminal protozoal population of growing goats supplemented probiotics (*S. cerevisia* and *L. acidophilus*) (x10^6/ml ruminal fluid).

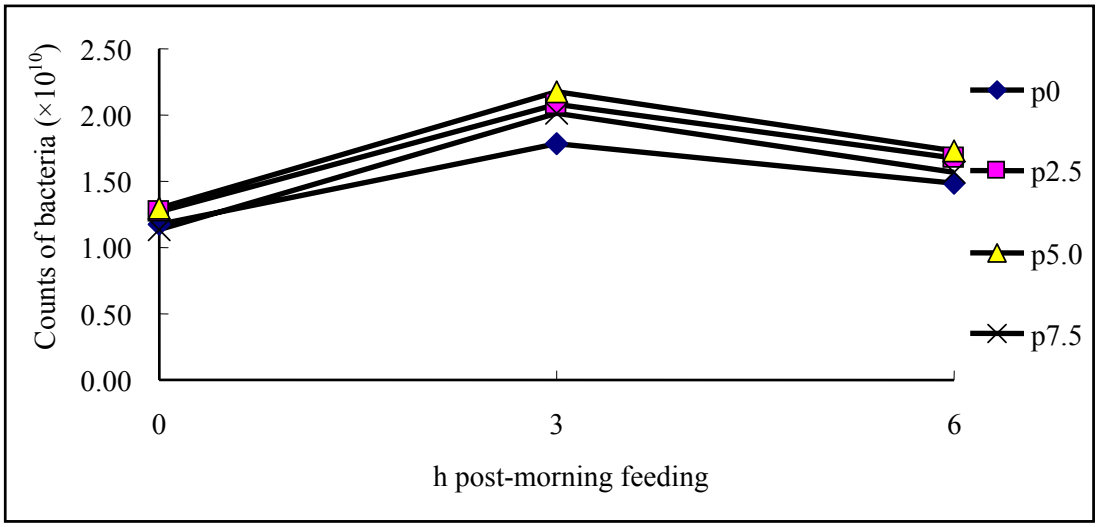

Figure 3.4 Ruminal total viable bacterial population of growing goats supplemented probiotics (*S. cerevisia* and *L. acidophilus*) (x 10^{10}/ml ruminal fluid).

3.5.6 Nitrogen balances

The total dietary N intake and faecal N excretion were not statistically different for all treatments. Yet the urinary and total N excretions were pushed up with the increasing levels of additional probiotics, thereof raised to linear significant difference for the level of 7.5 g/h/d in comparison with the control (1.0 and 3.8 g/d vs. 0.7 and 3.4 g/d) ($P<0.05$) (Table 3.8).

There were not significant effects on the N absorption (g/d), N retention (g/d) as well as N retention (%) because of addition of probiotics compared with the control, but then those of the 7.5 g/h/d treatment were significantly higher ($P<0.05$) than those of 2.5 and 5.0 g/h/d.

To sum up, the effects of supplemented probiotics on the N-balance of growing goats fed whole plant corn silage displayed in enlarging the urinary and accordingly total N excretion. Still, it presented no performances with statistical difference on N absorption also N retention.

Table 3.8 The effect of probiotics on nitrogen balance of growing goats fed whole plant corn silage.

	Probiotic(g/h/d)					Contrast		
	0	**2.5**	**5**	**7.5**	**SEM**	**Linear**	**Quadratic**	**Cubic**
N intake (g/d)	7.5	7.4	7.3	7.8	1.20	ns	ns	ns
N excretion (g/d)								
Faece	3.3	3.3	3.3	3.4	0.43	ns	ns	ns
Urine	1.6^{b}	1.7^{ab}	1.8^{ab}	1.9^{a}	0.04	*	ns	ns
Total	4.9^{b}	5.0^{b}	5.1^{ab}	5.3^{a}	0.31	*	ns	ns
N absorption (g/d)	4.8^{ab}	4.7^{b}	4.6^{b}	5.0^{a}	0.29	ns	ns	ns
N retention (g/d)	2.6^{a}	2.4^{ab}	2.2^{b}	2.5^{a}	0.33	ns	ns	ns
N retention (%)	34.7	32.4	30.2	32.1	1.53	ns	ns	ns

Means with different superscript letters in the same row differ significantly (P<0.05); SEM=standard error of the mean; *P<0.05; **P<0.01; ns= not significantly different (P>0.05).

3.5.7 Fatty acid profiles and conjugated linoleic acid content in plasma

Specifically, supplementation of probiotics was with effect on fatty acids centesimal composition of plasma by: pushing up the C10:0 with linear, quadratic also cubic significance (P<0.01); raising C14:0 with linear and cubic significance (P<0.05); declining C16:0 and C17:0 with tendencies but C15:0 with significant difference (linear: P<0.01; quadratic and cubic: P<0.05).

In point of impacts on C18 fatty acids centesimal composition of plasma resulted from supplementation of probiotics, the highlight existed in the linear, quatrain

likewise cubic enhancements of cis9, trans 11 ($P<0.05$) and trans 10, cis 12 CLA isomers ($P<0.01$). In comparison with the control, cis9, trans 11CLA centesimal compositions of the probiotics treatment groups increased by 27.7%, 40.4%, and 23.4% for the 2.5, 5.0, and 7.5 g/h/d, levels respectively ($P<0.05$). In addition, trans 10, cis 12 CLA was not detected in the control, when they stepped up simultaneously to 0.07, 0.08 and 0.06 % for levels of 2.5, 5.0, and 7.5 g/h/d, respectively ($P<0.01$). About the C18:0, it was increased by tendency ($P>0.05$) in simultaneity with the clear reduction tendency of C18:2n6c ($P>0.05$) and significant subtraction of C18:3n3 by reason of additional probiotics ($P<0.05$).

Concerning with the very long-chain fatty acids (chain length greater than C18), with the exception of C24:1 kept unaffected and C22:6n3 run low by tendency ($P>0.05$), all the centesimal composition of other fatty acids was uplifted with linear, quadratic and also cubic significance (C20:2: $P<0.01$; C20:3n3: $P<0.05$; C20:3n6: $P<0.01$; C20:4n6: $P<0.05$; C20:5n3: $P<0.01$; C24:0: $P<0.01$) (Table 3.9).

About the whole profiles of fatty acids in the plasma, Table 3.9 illustrated that the additional probiotics resulted in an increased tendency for total saturated fatty acid (TSFA) ($P>0.05$). An evident minification for poly-unsaturated fatty acid (pl-USFA) ($P>0.05$) and an overt incensement for desirable fatty acid contrasted with a trivial increment of mono-unsaturated fatty acid (mo-USFA) ($P>0.05$) were observed. The supplementation of probiotics was also the reason for a faint enhancement of total n6 fatty acid (Tn6) ($P>0.05$); a mild subtraction for total n3 fatty acid (Tn3) ($P>0.05$); a small reduction for the pl-USFA: TSFA ratio; but a significant increment for the n−6: n−3 ratio.

Table 3.10 showed that when calculating the centesimal composition of plasma

fatty acids into fatty acid (μg) contained in 1 ml plasma, the effects of probiotics on the fatty acid contents were principally the same in comparison with the centesimal composition that shown in Table 3.9. On the whole， amongst all of the plasma fatty acids that were detected in this experiment, the increment of total saturated fatty acids centesimal composition was observed resulting from addition of probiotics (48.59, 48.58, and 49.04% vs. 47.6%), but kept those of C15:0, C16:0, and C17:0 face-off. At the same time, the addition of probiotics was in force for reducing C18-C22 polyunsaturated fatty acids and heightened the CLA content of plasma as anticipation.

When calculating the centesimal composition of plasma fatty acids into fatty acid (μg) contained in 1 ml plasma, the average contents of total saturated fatty acids (428.6, 441.5, 458.6, and 436.3μg/ ml plasma for control, 2.5, 5.0, and 7.5 g/h/d probiotics treatments, respectively) showed increasing tendency ($P>0.05$). Of the desirable fatty acids, the amounts were 637.3, 660.0, 717.6, and 645.4 μg/ ml plasma for control, 2.5, 5.0, and 7.5 g/h/d probiotics treatments respectively, they showed an increment with linear significance ($P<0.05$). On the ratios of PUFA: SFA and n6: n3 the average values were 0.62, 0.58, 0.59, 0.59 and 2.58, 3.20, 3.33, 3.12 for control, 2.5, 5.0, and 7.5 g/h/d probiotics treatments respectively, the ratio of PUFA: SFA decreased by tendency ($P>0.05$), but that of n6: n3 significantly increased ($P<0.05$). About CLA contents (μg/ ml plasma) of the four group animals, they were 4.2, 5.4, 6.4, 5.1 (μg/ ml plasma) and undetected, 0.6, 0.7, 0.5 (μg/ ml plasma) for cis9, trans11 and trans10, cis12 CLA isomer, respectively, the values of cis9, trans11 CLA presented a significant increment ($P<0.01$), and those of trans10, cis12 CLA showed a growing in number with highly significance ($P<0.05$).

Table 3.9 Plasma fatty acids centesimal profiles of growing goats supplemented probiotics under condition of feeding whole plant corn silage.

	Probiotics (g/h/d)					Contrast		
FA (%TFA)	**0**	**2.5**	**5**	**7.5**	**SEM**	**L**	**Q**	**C**
C8:0	0.72[a]	0.68 [a]	0.50[c]	0.59[b]	0.05	*	*	ns
C10:0	0.15[b]	0.29[a]	0.26 [a]	0.22 [a]	0.03	**	**	**
C12:0	0.38[b]	0.36[bc]	0.50[a]	0.26[c]	0.04	ns	ns	ns
C14:0	3.31[b]	3.89 [a]	3.34[b]	3.89 [a]	0.15	*	ns	*
C15:0	0.45[a]	0.39[a]	0.17[b]	0.23[b]	0.05	**	*	*
C16:0	17.77	16.75	17.59	16.20	0.70	ns	ns	ns
C16:1	0.84	0.88	0.81	0.77	0.08	ns	ns	ns
C17:0	2.92	2.82	2.86	3.10	0.20	ns	ns	ns
C18:0	22.74	23.04	23.15	24.26	1.11	ns	ns	ns
C18:1n9t	1.88	1.87	1.96	1.87	0.06	ns	ns	ns
C18:1n9c	16.60	16.41	17.07	17.58	0.70	ns	ns	ns
C18:2n6c	15.80	15.10	15.44	15.35	0.70	ns	ns	ns
C18:3n3	1.04 [a]	0.96[a]	0.86[b]	0.75 [b]	0.05	*	*	*
C18:c9,t11	**0.47[b]**	**0.60[a]**	**0.66[a]**	**0.58[a]**	**0.03**	*	*	*
C18:t10,c12	**0.00[b]**	**0.07[a]**	**0.08[a]**	**0.06[a]**	**0.01**	**	**	**
C20:2	0.95[a]	0.60[bc]	0.70[b]	0.52[c]	0.02	**	**	**
C20:3n3	2.82[a]	2.21[b]	2.37 [b]	2.57[b]	0.12	*	*	*
C20:3n6	0.30[a]	0.21[b]	0.19 [b]	0.24[b]	0.02	**	**	**
C20:4n6	3.11[c]	3.94[a]	3.51[bc]	3.64[ab]	0.24	*	*	*
C20:5n3	0.41 [a]	0.35[b]	0.35 [b]	0.30[c]	0.01	**	**	**
C24:0	1.16[a]	0.27[b]	0.21[b]	0.29[b]	0.10	**	**	**
C24:1	2.45	2.54	2.37	2.45	0.04	ns	ns	ns
C22:6n3	3.36	3.13	3.34	3.15	0.18	ns	ns	ns

Table 3.9 (Continued)

	Probiotics (g/h/d)					Contrast		
FA (%TFA)	**0**	**2.5**	**5**	**7.5**	**SEM**	**L**	**Q**	**C**
TSFA	47.60	48.59	48.58	49.04	1.52	ns	ns	ns
TMUSFA	21.77	21.70	22.51	22.67	1.07	ns	ns	ns
TPUSFA	28.26	27.10	27.73	27.14	1.00	ns	ns	ns
DFA	70.76	72.94	73.43	73.77	2.37	ns	ns	ns
PUSFA/TSFA	0.59	0.56	0.57	0.55	0.01	ns	ns	ns
Tn6	19.68	21.05	21.04	19.87	0.90	ns	ns	ns
Tn3	7.63	6.65	6.92	6.77	0.09	ns	ns	ns
n−6/n−3	2.58[b]	3.05[a]	2.91[a]	2.94 [a]	0.18	*	*	*

TSFA=total saturated fatty acid; TMUSFA=total mono-unsaturated fatty acid; TPUSFA= total poly-unsaturated fatty acid; DFA=desirable fatty acid; Tn6=total n6 fatty acid; Tn3=total n3 fatty acid; Means with different superscript letters in the same row differ significantly ($P<0.05$); SEM=standard error of the mean; *$P<0.05$; **$P<0.01$; ns= not significantly different ($P>0.05$); L=linear; Q=quadratic; C=cubic.

Table 3.10 Fatty acid and conjugated linoleic acid contents (µg/ml plasma) in plasma of growing goats supplemented probiotics under condition of feeding whole plant corn silage.

	Supplemented probiotics (g/h/d)					**Contrast**		
FA (µg/ml plasma)	**0**	**2.5**	**5.0**	**7.5**	**SEM**	**L**	**Q**	**C**
C8:0	7.0^{a}	6.5^{b}	6.9^{a}	6.8^{ab}	0.31	*	*	*
C10:0	1.3^{c}	2.6^{a}	2.6^{a}	1.9^{b}	0.26	**	**	**
C12:0	3.4^{b}	3.3^{b}	3.8^{a}	3.3^{b}	0.06	*	ns	ns
C14:0	29.8^{b}	35.2^{a}	32.6^{ab}	34.0^{a}	0.64	*	*	*
C15:0	4.1^{a}	3.5^{b}	2.6^{d}	3.0^{c}	0.25	**	*	*
C16:0	160	151.6	151.9	141.7	7.63	*	*	*
C16:1	7.6^{a}	8.0^{a}	7.9^{a}	6.9^{b}	0.47	*	ns	ns
C17:0	26.3^{ab}	25.5^{b}	27.9^{ab}	28.9^{a}	0.39	ns	ns	ns
C18:0	196.8	208.5	226.2	212.2	11.08	ns	ns	ns
C18:1n9t	16.9	16.9	17.1	16.4	1.03	ns	ns	ns
C18:1n9c	149.5	148.5	146.8	153.8	3.14	ns	ns	ns
C18:2n6c	152.3	145.7	150.6	143	3.01	ns	ns	ns
C18:3n3	9.3^{a}	8.7^{a}	8.4^{ab}	6.5^{b}	0.66	*	*	*
C18:c9,t11	**4.2^{c}**	**5.4^{b}**	**6.4^{a}**	**5.1^{b}**	**0.43**	*	*	*
C18:t10,c12	**0.0^{c}**	**0.6^{ab}**	**0.7^{a}**	**0.5^{b}**	**0.40**	**	**	**
C20:2	8.6^{a}	5.4^{c}	6.9^{b}	5.8^{bc}	1.08	**	**	**
C20:3n3	25.4^{a}	20.0^{c}	23.2^{ab}	22.5^{bc}	0.79	*	*	*
C20:3n6	2.7	2.6	2.3	2.1	0.42	*	*	*
C20:4n6	28.1^{b}	35.6^{a}	34.3^{a}	33.1^{a}	0.91	*	*	*
C20:5n3	3.7^{a}	2.3^{c}	2.9^{b}	2.4^{c}	0.20	**	**	**
C24:0	10.4^{a}	4.3^{b}	4.1^{b}	4.5^{b}	1.07	**	**	**

Table 3.10 (Continued)

FA (µg/ml plasma)	Supplemented probiotics (g/h/d)				SEM	Contrast		
	0	2.5	5.0	7.5		L	Q	C
C24:1	22.1	23.0	23.2	21.4	0.90	ns	ns	ns
C22:6n3	30.2^{a}	28.4^{ab}	26.9^{b}	27.6^{b}	1.33	*	ns	ns
TSFA	428.6	441.5	458.6	436.3	5.15	ns	ns	ns
TMUSFA	196.1	196.4	215.0	198.5	2.07	ns	ns	ns
TPUSFA	264.5	254.9	272.6	258.6	1.02	ns	ns	ns
DFA	637.3	660.0	717.6	645.4	10.79	*	ns	ns
PUFA/SFA	0.62	0.58	0.59	0.59	0.01	ns	ns	ns
Tn6	177.3^{b}	190.1^{a}	204.3^{a}	183.8^{b}	5.03	*	*	ns
Tn3	68.6^{a}	59.4^{b}	61.4^{b}	59.0^{b}	0.99	*	ns	ns
n−6/n−3	2.58^{b}	3.20^{a}	3.33^{a}	3.12 ^{a}	0.07	*	*	*

TSFA=total saturated fatty acid; TMUSFA=total mono-unsaturated fatty acid; TPUSFA= total poly-unsaturated fatty acid; DFA=desirable fatty acid; Tn6=total n6 fatty acid; Tn3=total n3 fatty acid; Means with different superscript letters in the same row differ significantly (P<0.05); SEM=standard error of the mean; *P<0.05; ns= not significantly different (P>0.05); L=linear; Q=quadratic; C=cubic.

It caused by difference of fat contained in individual plasma sample, there have been a few disparities on statistical significance. These statistical disparities between Table 3.8 and 3.9 registered as C8:0 ‘C’ (cubic) ‘ns’ (no significance) vs. ‘*’ (significant difference); C12:0 ‘L’ (linear) ‘ns’ vs. ‘*’; C14:0 ‘Q’ (quadratic) ‘ns’ vs. ‘*’; C16:0 ‘L’ ‘ns’, ‘Q’ ‘ns’ and ‘C’ ‘ns’ vs. ‘L’ ‘*’, ‘Q’ ‘*’, ‘C’ ‘*’ respectively; C16:1 ‘L’ ‘ns’ vs. ‘*’; C20:3n6 ‘L’ ‘**’, ‘Q’ ‘**’, ‘C’ ‘**’ vs. ‘L’ ‘*’, ‘Q’ ‘*’, ‘C’ ‘*’ respectively; C22:6n3 ‘L’ ‘ns’ vs. ‘*’; Tn6 ‘L’ ‘ns’ and ‘Q’ ‘ns’ vs. ‘L’ ‘*’ and ‘Q’ ‘*’ ; Tn3 ‘L’ ‘ns’ vs. ‘*’ and desirable fatty acid (DFA) ‘L’ ‘ns’ vs. ‘*’.

3.6 Discussion

3.6.1 Increases of g/kg $W^{0.75}$ ($P<0.05$) and percent of body weight dry matter intake ($P>0.05$), ADG (g/d) ($P<0.01$), and feed conversion (lowered ratio of DMI: ADG) ($P<0.05$) were found for reason of addition of probiotics

The presence of probiotics (*S. cerevisia* and *L. acidophilus*) constituted a healthier and more favorable ruminal setting for digestive and absorption processes. And it is this healthier and more favorable ruminal setting to be responsible for the significant increase of DMI, ADG, and feed efficiency. Chiofalo et al. (2004) completed their studies on twenty growing Maltese goat kids and observed *lactobacilli* significantly increased body weight ($P<0.001$). In the same year, Whitley et al. (2004) reported that in their experiment, the ADG of growing goats was 30 g/d for the probiotics treatment group compared to 10 g/d for the control. In another study, El-Ghani (2004) observed highly significant elevation ($P<0.01$) for feed intake in bucks. In present year, Tripathi et al. (2007) stated that during the digestibility period of their experiment, an increased tendency ($P>0.05$) of DMI was found due to addition of yeast probiotics. More recently, Han et al. (2008) demonstrated that significant increases in DMI and ADG of growing goats resulted from additional probiotics that contained *S. cerevisia* and *L. acidophilus*. The effectiveness of additional probiotics on DMI, ADG, and feed conversion in goats were in the same case as in cattle. For example, Dell'Orto et al. (2000) reported an improvement in the DMI and daily gain of calves for *L. acidophilus* supplementation, Swinney-Floyd et al. (1999) showed improvements in feed efficiency when feedlot steers were supplemented with a combination of *L. acidophilus* 53545 and *P. freudenreichii* P-63 probiotics. In addition, other researches have also shown an improvement in the productive performances of dairy cows (Savoini et al., 2000).

3.6.2 Probiotics was effectual to significantly increase digestibility of NDF (P<0.05), to tend to increase digestibility of EE, ADF and CP (P>0.05), to show a faint enhancement for DM, OM digestibility (P>0.05)

The increase of dietary digestibility for addition of probiotics was the response of increasing colonization of fugal on plant cell; of stimulating growth or/and activity of fibrolytic bacteria; of increasing the activities of xylanase and pectinase and of establishing more favorable ecological conditions for growth and activities of the anaerobic autochtonous microflora. Chaucheyras et al. (1995) stressed that the addition of yeast cells increased the colonization of *Neocallimastix frontalis* fugal on plant cell, and thereby increased cellulose degradation. The effectiveness of some yeast strains to stimulate growth or/and activity of fibrolytic bacteria has been pointed out (Dawson et al., 1990; Harrison et al., 1988). In addition, Chaucheyras-Durand et al. (2001) had proved that the *S. Cerevisiae* I-1077 has effect on establishment of fibrolytic bacteria; on degradation of a lignocellulosic substrate; on the main polysaccharide depolymerase and glycoside hydrolase activities of particle-associated microorganisms and on the development of the rumen digestive function. Recently, Feng et al. (2008) indicated that adding yeast culture increased the activities of xylanase and pectinase. Similar to the present study, Kumagai et al. (2004) had observed that in the condition of both of oat hay and high concentrate feeding, the presence of yeast probiotics tended to increase the digestibility of CP, CF, and organic cell wall. Han et al., (2008) had pointed out that DM ($P<0.01$), organic matter (OM) ($P<0.05$), and NDF ($P<0.05$) digestibility was increased significantly with probiotics, CP digestibility showed an obvious increasing tendency. More over, others (El-Waziry et al., 2000; Kholif et al., 2000; Martins et al., 2000 and Fayed, 2001) have

reported the similar improvements of dietary digestibility. Besides these researches, many of other studies also agreed with the findings (Dawson and Tricarico, 2002; Fadel Elseed et al., 2007; Feng et al., 2008).

3.6.3 Unaffected ruminal average pH, significant raised NH_3-N and PUN ($P<0.05$) were caused be supplementation of probiotics

The findings on pH were in accordance with those from the former studies, Doreau et al. （1998）have suggested that the supplementation of *S. cerevisiae* did not change ruminal pH. More recently, Fonty et al. (2006) demonstrated that *S. cerevisiae* could be efficient to stabilize ruminal pH by stimulating ciliate *Entodiniomorphid* protozoa. Moreover, many findings have emphasized that the yeast probiotics did not affect goats' rumen pH value with any significance (Han et al., 2008; Jiang et al., 2008; Fadel Elseed., 2007; Galp, 2006; Kumagai et al., 2004; Giger-Reverdin et al., 2004; Dawson et al., 1990). On the other hand, supplementation of *L. acidophilus* has shown to decrease ruminal pH (Krehbiel et al., 2003). However, almost all former results showed that addition of probiotics maintained pH in the range that is compatible with the optimal ruminal ecologic dominance.

Supplementation of *S. cerevisiae* alone in the diet of goats has either let the NH_3-N concentration down (Koul et al., 1998; El-Waziry et al., 2000; Nurten. G, 2006), or kept it unaffected ($P>0.05$) (Corona et al., 1999; Tripathi et al., 2007; Jiang et al., 2008). From the results of Nurten Galp (2006), we can get the averages of ruminal fluid NH_3-N and blood urea that calculated from 0, 3, and 6 h post-feeding were 354.0, 308.3 (mmol/l) and 45.50, 43.00 (mg/dl) for control and *S. cerevisiae* treatment group respectively, there were no significant differences. The results of the present study showed that significant raise in NH_3-N was caused by addition of

probiotics, these findings were consistent with those of Fadel- Elseed et al. (2007), which reported that *S. cerevisiae* resulted in a numerical increase in ammonia-N concentration. What is more, the present study also found significant raise in PUN, and it agreed with the results of Galp (2006b), which reported that the means of serum urea were 0.53 (8.9), 0.570 (9.5), and 0.57 (9.4) (g/l and mmol/l) for control, 5, and 10 g/d *S. cerevisiae* treatments, respectively, a significant difference was observed. In point of probiotics effects on ruminal fluid NH_3-N concentration, it can be concluded that this effectiveness is dependent on composition of diet rather than the added doses of probiotics. The study of Kumagai et al. (2004) had provided detail for proving the effectiveness of probiotics on goats' rumen NH_3-N was dependent on the diet.

3.6.4 Addition of probiotics tended to increase TVFA (P>0.05), significantly reduced propionic proportion (P<0.05), tended to raise acetic proportion (P>0.05), and significantly increased C2:C3 ratio (P<0.05).

These results were similar as the previous studies. Thereunto, Fadel Elseed et al. (2007) reported *S. cerevisiae* resulted in a numerical increase in total VFA concentration. El-Waziry et al. (2000) reported that VFA concentration increased with yeast supplementation. El-Ghani, (2004) elucidated in detail that ruminal VFA was significantly heightened for bucks fed *S. cerevisiae* at 6 h. In addition, many other researches on addition of *S. cerevisiae* in goats or lambs had explained the coherence of the results (Jiang et al., 2008; Tripathi et al.,2007; Nurten Galp, 2006; Giger-Reverdin et al., 2004; Chaucheyras-Durand et al., 2001; Enjalbert et al., 1999). The effectiveness of additional yeast probiotics on production of VFA being that it has beneficial effects on growth and H_2-utilisation of acetogenic bacteria (Chaucheyras et

al., 1995b; Chaucheyras-Durand et al., 1997), and since the acetogenic bacteria which produces acetate from CO_2 and H_2, the total VFA and acetic centesimal proportion should appear to be increased. However, in another experiment that was carried out in lambs (Chaucheyras-Durand et al., 2001), even though total VFA was significantly higher in the *S. cerevisiae* group during the 20–50 d period, no any significant effect was observed on the centesimal composition of the major VFA mixture (acetate, propionate, and butyrate) except that of acetate tended to increase. Han et al. (2008) also detected a significant increase of total VFA in probiotics supplemental group, and in the meantime, no significant effect was observed on the centesimal composition of the major VFA mixture as well as the ratio of C_2: C_3 Krehbiel et al. (2003) reported that the supplementation of *L. acidophilus* has shown to increase in ruminal propionate concentrations. This finding was opposite to the present study.

3.6.5 A lowering in number tendency of ruminal protozoa (P>0.05) was simultaneous with a distinct heightening in number of ruminal total viable bacteria (P<0.01) resulted from additional probiotics

The previous findings for effect of *S. cerevisia* on ruminal protozoa were complicated. Thereof, Corona et al. (1999) reported that *S. cerevisia* did not change ruminal protozoa. Recently, Nurten Galp (2006) observed that *S. cerevisiae* treatment decreased *Diplodinium spp.* protozoa significantly but did not affect total protozoal counts. Similarly, Galip (2006b) mentioned the supplementation of *S. cerevisiae* decreased protozoal counts (424.33 vs.383.33) before feeding, but it was not different for the average. Presently, Tripathi et al. (2007) described that ciliate protozoa population did not change due to yeast supplementation. On the contrary, Jouany et al. (1998) found increase of protozoal count by occasion of addition of *S. cerevisiae*.

Krehbiel et al. (2003) stated that supplementation of *L. acidophilus* has been shown to increase ruminal protozoal numbers, to change viable bacterial counts. In the same case, Han et al. (2008) reported the significant increment of protozoal and bacterial counts for the reason of supplementation of blend of *S. cerevisiae* and *L. acidophilus* probiotics. The results of this study were similar to the findings from Krehbiel et al. (2003), Nurten Galp (2006), Han et al. (2008).

3.6.6 Enlarging urinary and total N excretion were in concurrent with unaffected N absorption and N retention due to supplementation of probiotics

Former studies on probiotics were devoid of data for N-balance of goats. More recently, one research on goats showed that N-intake, N-voided in faeces and urine and N-balance did not change due to supplementation of yeast (Tripathi et al., 2007). The results of this study for N-balance had conformity with that of Tripathi et al. (2007). The enlarged urinary N and total N excretion observed in this study were related to the significant increment of ruminal NH_3-N and plasma urea N (PUN) concentration.

3.6.7 An increasing tendency of plasma total saturated fatty acids ($P>0.05$), reduction of C18-C22 polyunsaturated fatty acids ($P<0.05$ or $P<0.01$), a highly significant increase of CLA ($P<0.01$), a raising desirable fatty acids ($P<0.05$), and changing ratios of PUFA: SFA ($P>0.05$) and n6:n3 ($P<0.05$) were observed owing to administering probiotics

Up to now, no other research detailed the effect of probiotics on plasma fatty acid profiles. A similar research in Maltese goat kids found that the *lactobacilli* treatment significantly lowered the levels of blood non-essential fatty acid (NEFA)

(P<0.001) and for triglycerides (P<0.05), but did not mention the fatty acid profiles (Chiofalo et al., 2004). The increasing total plasma saturated fatty acids (P>0.05) centesimal composition, reducing C18-C22 polyunsaturated fatty acids (P<0.05 or P<0.01), and raising desirable fatty acids (P<0.05) resulted from the more effective ruminal biohydrogenation on account of addition of probiotics. The more effective ruminal biohydrogenation resulted in accumulation of saturated fatty acids and subtraction of polyunsaturated fatty acids in the rumen. Consequently, more saturated fatty acids and less polyunsaturated fatty acids went into the blood. The heightening CLA (P<0.01) was caused by the supplemented probiotics (*S. cerevisiae* and *L. acidophilus*) that stimulated the growth and/or activity of ruminal bacteria; accordingly more enzymes accumulated and acted on the substrates of CLA (linolein acid and linoleni acid). As a result, CLA was produced faster and the increasing accumulation appeared in the rumen, subsequently more CLA went into the blood. On the other hand, the *L. acidophilus* itself has been well documented to produce CLA from linolein acid and linoleni acid (Kishino et al., 2002; Julia et al., 2006).

3.7 Conclusions

Additional probiotics (*S. cerevisia* and *L. acidophilus*) increased g/kg W0.75 dry matter intake (P<0.05), ADG (g/d) (P<0.01), and feed conversion (lowered ratio of DMI: ADG) (P<0.05); increased digestibility of NDF (P<0.05), EE, ADF and CP (P>0.05) as well as that of DM and OM (P>0.05).

In the mean time, addition of probiotics unaffected ruminal average pH, but raised the NH_3-N and also PUN (P<0.05), increased TVFA (P>0.05), but reduced propionic proportion (P<0.05) and butyric proportion (P>0.05) in concurrent with

raise of acetic proportion and C2 : C3 ratio (P>0.05).

Depressed ruminal protozoal number (P>0.05) and heightened ruminal total viable bacterial number were entailed by additional probiotics. Enlarged urinary and total N excretions were observed due to supplementation of probiotics.

Supplementation of probiotics increased total saturated fatty acids (P>0.05), contrasted with decrease of C15:0 (P<0.01), C16:0 (P>0.05), and C18-C22 polyunsaturated fatty acids (P<0.05 or P<0.01) centesimal composition in plasma. In addition, supplemented probiotics was in force for heightening CLA (P<0.01); for raising desirable fatty acids (P<0.05); for reducing ratio of PUFA: SFA (P>0.05) and for raising ratio of n6:n3 (P<0.05).

In conclusion, we can claim that supplementation of probiotics was effectual for improvement of stall-feeding growing goats productive performances. Thereunto the levels of 2.5 and 5.0 g/h/d were tested-proof to be appropriated for improvement of growing goat rumen metabolism, growth performance, and plasma CLA concentration. Based on the findings of this experiment, 2.5 and 5.0 g/h/d 2 levels would be chosen for further study.

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CHAPTER IV

EFFECTS OF SUPPLEMENTAL SOYBEAN AND SUNFLOWER OIL ON PERFORMANCES OF GROWING GOATS FED WITH WHOLE PLANT CORN SILAGE

4.1 Abstract

The objectives of this experiment were to check the effects of additional soybean oil and sunflower oil on growth, ruminal metabolism, and plasma fatty acid profiles particularly conjugated linoleic acid (CLA) in growing goats fed corn silage, compared and selected either the soybean oil or the sunflower oil for further study. Thirty growing crossbred (Thai native x Anglo-Nubian) goats that weighed 14.8±2.5 kg, aged about 6 months, were purchased and allocated to 5 treatments according to Randomized Complete Block Design (RCBD) with 6 goats in each treatment. The blocks were made by weight into heavy, medium, and light goats and each of the treatments contained two goats from each of the blocks.

The results presented as significant increase of ADG, significant decrease of DMI: ADG ratio (soybean oil: $P<0.01$; sunflower oil: $P<0.05$) were resulted from both of soybean oil and sunflower oil supplementations. In addition, presence of soybean oil tended to increase digestibility of DM, OM, and NDF ($P>0.05$), but not the sunflower oil. Ruminal average pH were unaffected due to the presences of soybean oil and sunflower oil, but the PUN tended to be decreased ($P>0.05$), and the NH_3-N

were significantly reduced (soybean oil: $P<0.01$; sunflower oil: $P<0.05$). On another hand, TVFA and butyric proportion ($P>0.05$) were not impacted by additions of soybean oil and sunflower oil, but the acetic proportion ($P<0.05$) and C2:C3 ratio ($P<0.05$) significantly increased. Regarding to the N balance, supplementation of sunflower oil resulted in significantly subtraction in dietary N intake, faecal and total N excretion ($P<0.05$); however, both of soybean oil and sunflower oil supplementations increased N absorption and retention. About the plasma fatty acid profiles, the total saturated fatty acids ($P>0.05$) composition tended to be increased, CLA content ($P>0.05$) significantly enhanced, the very long chain fatty acids ($P<0.05$) significantly reduced and DFA also ratio of n6:n3 ($P<0.05$) significantly increased owing to supplementations of soybean oil and sunflower oil.

4.2 Introduction

Since the stall-feeing strategy for goats has been encouraged or pushed to alleviate the degradation of grassland in some countries such as China, India, and so on, supplements that can improve the animals performance and health and particularly to improve the quality and safety of the products attracted much study interests. To fatten or fastened the growth of growing animals, it is necessary to feed the animals with relatively high-concentrate diets that contain fat or oil to enhance dietary energy density.

However, the effects of soybean oil and sunflower oil on the goats were contrasted. Rogério et al. (2005) have verified that the presence of soybean oil in diet of goats decreased the digestibility of NDF contrasted with increase of digestibility of CP, EE, and total digestible nutrients content (TDN). Kucuk et al. (2003) observed

soybean oil fed at approximately 3% of the diet DM did not adversely affect nutrient digestion in sheep limit-fed either high-forage or high-concentrate diets. One year later, Kucuk et al. (2004) further the study and found addition of soybean oil to diets (0, 3.2, 6.3, and 9.4% of dietary DM), the digestibilities of OM, NDF, and N were not affected (P=0.13 to 0.95) by increasing dietary soybean oil level. For the supplementation of sunflower oil in goats, there were no data found by the authors up to now, but recently, Gülşen et al. (2006) who suggested that increasing levels (3, 6, and 9%) of sunflower and soybean oil linear increases pH, did not affect NH_3-N concentration, but depressed ruminal fermentation in cattle.

On the other hand, CLA has been reported for wide range of beneficial effects such as anticarcinogenic, antiatherogenic, antidiabetic and immune stimulatory (Bauman et al., 2000; Lobb and Chow, 2000). It is well known that the ruminal microbial synthesis CLA from the linoleic acid. Soybean oil contains about 52% linoleic acid (Penny, 2006); and the high linoleic acid sunflower oil contains 63%～70% linoleic acid normally (Jasso et al., 2002). Therefore, it is a possible way to enhance chevon CLA contents by added soybean oil and sunflower oil because they were rich in linoleic acid.

4.3 Objectives

The present experiment was carried out to study the effects of additional soybean oil and sunflower oil on growth, ruminal metabolism, and plasma fatty acid profiles particularly conjugated linoleic acid (CLA) in growing goats fed corn silage, and to choose either soybean oil or sunflower oil for further study.

4.4 Materials and methods

4.4.1 Experimental design and treatment

Thirty growing crossbred (Thai native x Anglo-Nubian) goats that weighed 14.8±2.5 kg, aged about 6 months, and were purchased from Pukthongchai district, Nakhon Ratchasima province of Thailand to conduct this experiment. The animals were allocated to 5 treatments according to Randomized Complete Block Design (RCBD) with 6 goats in each treatment. The blocks were made by weight into heavy, medium, and light goats and each of the treatments contained 2 goats from each of the blocks (Table 4.1). Before the outset of experiment, the animals were injected with Ivomic (Merial Ltd., Iselin, NJ) for anti-internal parasite, and housed in individual pens (0.9 x 1.4 m) where the animals could have an easy access to corn silage and fresh water *ad libitum*. What was more, the pens were cleaned and disinfected with Ciber solution prior to the housing of the animals. During the experiment, animals in different treatments received the whole plant corn silage plus concentrate basal diet. The treatments included control, supplementations of 2.5 and 5.0% concentrate basis of soybean oil, and supplementation of 2.5 and 5% concentrate basis of sunflower oil. The additional soybean oil and sunflower oil were mixed evenly with concentrate prior to feeding, and offered to animals by half at 9:00 am and the other at 3:00 pm respectively. The concentrate was supplied with 1.5% *pro rata* body weight for each goat to ensure that the dietary intakes of crude protein, growth net energy, and dry mater in accordance with the Nutrients Requirements of Goats (NUMBER 15, 1989) under the condition of maintenance plus lower activity and 50 g/d weight gain. All animals accessed to the whole plant corn silage and clean water *ad libitum*, and were cared for as described by the Ethics Committee on Animal and Human Experimentation of the

UAB (Reference No. CEEAH 04/481) for the aim of respecting animal welfare and environmental protection. The experiment lasted 8 weeks, excepting 2 weeks for adjustment, 1 week for adaptation, and 1 week post-experiment for urinary and faecal samples collection.

Table 4.1 Lay-out of experimental treatments.

Groups	Animals(n)	BW(kg)	Treatments
I (Control)	6	14.8±2.2	Basal diet
II	6	14.8±2.7	Basal diet + soybean oil 2.5 %
III	6	14.7±1.5	Basal diet +soybean oil 5.0 %
IV	6	14.7±2.5	Basal diet + sunflower oil 2.5 %
Ⅴ	6	14.7±2.1	Basal diet +sunflower oil 5.0 %

Basal diet= whole plant corn silage plus concentrate.

4.4.2 Experimental material

The soybean oil and sunflower oil employed in this study were purchased from Macro supermarket (Muang district, Nakhon Ratchasima province of Thailand). The whole plant corn silage was purchased from Kornburee Cooperatives (Kornburee district, Nakhon Ratchasima province of Thailand). The pelleted concentrate was supplied by farm of Suranaree University of Technology (Nakhon Ratchasima province of Thailand), and it was composed of cassava chip (12.0%), cassava pulp (31.5%), rice bran with germ (10.0%), defatted rice bran (10.0%), molasses (8.0%), palm kernel expeller meal (18.0%), rapeseed meal (4.0%), corn meal (4.0%), urea (1.8%), mineral (1.5%) (Containing Ca 14.5%, P 17%, NaCl 18%, Mg 10%, and carrier), and additional binder (0.2%).

4.4.3 Sampling

The daily offered and left concentrate and whole plant corn silage were weighed (the residues were removed) every morning before offering for the purpose of determination dry matter intake. Body weight of the animals were measured weekly prior to the morning feeding with the aim of evaluating the growing performances. The whole plant corn silage and concentrate were sampled weekly and dried at 60~65 °C hot air oven for determination of dry matter (DM) composition, and followed by grounding through a 1 mm sieve and then kept in tightly covered plastic containers to make a pool respectively for further approximate analysis. During the post-experiment week for urinary and faecal samples total collection, the all-day faece and urine (10% H_2SO_4 was used as a preserving reagent, 30 mL/container) were collected and the total amount was recorded down every morning (measured faece weight and urine volume). Subsequently, 15% of the total amounts was sub-sampled to make a pool respectively for each animal, and then was kept at -20 °C and in the end was dried prior to chemical composition analysis that aimed to determine digestibility and nitrogen balance. For ruminal fluid samples, they were withdrawn on the last day of the experiment through an esophageal stomach tube following 0, 3 and 6 h post-morning meal timing. The samples were strained through three layers of muslin cloth and then were followed by immediately measuring of pH with an OHS-3C pH meter. Thereafter, 1 ml of the samples were measured well and truly with a pipette into the tubes containing 9 ml 10% formalin (V:V=9:1) as a preserving reagent and then were closed tightly with screw caps that with butyl rubber lining for checking the counts of ruminal protozoa and bacteria. At the same time, 20 ml of the samples were measured and then put into small plastic bottles

containing 5 ml 6 N HCl as a preserving reagent, and then the bottles were closed tightly with screw caps that with butyl rubber lining for determination of ruminal ammonia N and volatile fatty acids. With that, all samples were kept at -20□ until further analysis. The blood samples were collected from jugular veins into EDTA-containing vacuum tubes and were centrifuged at 2700 r for 5 min to separate plasma from the cells within 20 minutes after sampling. Subsequently the plasma was collected, and then it was stored at −80 °C for subsequent analyses of blood urea nitrogen and fatty acid profiles.

4.4.4 Chemical analysis and calculation

All the chemical analyses and calculations were done in the same way as described in chapter III.

4.4.5 Preparation of samples for gas chromatography (GC) analysis

The ruminal fluid samples that used to determine total VFA and molar proportion of main volatile fatty acid mix (acetate, propionate, and butyrate) were prepared and analyzed in the same method as described in chapter III.

The preparation of plasma samples for GC analysis was done by using a method as described in chapter III.

4.4.6 Analysis of fatty acids by Gas chromatography (GC)

Total VFA and molar proportion of acetic, propionic, and butyric acids in ruminal fluid and fatty acid profile of plasma samples were determined by HP6890 gas chromatography (GC) (made in USA) that fitted with a Flame Ionization Detector (FID). In addition, a J&W 122~3232 column was applied for determination of VFA, whereas a 100 m x 0.25 mm fused silica capillary column (SP2560, Supelco Inc, Bellefonte, PA, USA) for determination the plasma fatty acid profiles. The column

temperature was fixed at 70°C for 4 min, then it increased at 13 °C /min to 175 °C which lasted for 27 min. Continually it increased at 4 °C /min to 215 °C and kept for 31 min. Nitrogen was adopted as carrier gas with a 60 ml/min flow rate and the oven temperature was 250 °C. FID and injection temperature were fixed at 280°C, and a 1μL injection was done with a 10-μL injector.

4.4.7 Body weight measurement

Body weights of testing animals were measured and calculated in the same way as described in chapter III.

4.4.8 Data analysis

Data were analyzed using the General Linear Models procedure of SAS (SAS, 1985) as a randomized complete block design. Variation due to blocks was extracted in the models employed for the analysis. The protected least significant differences method was used to determine differences among treatment means. Polynomial contrasts (linear, quadratic, and cubic effects) were used to evaluate the all effects. In addition, a non-parametric Mann-Whitney test was used to compare the count means of rumen protozoa also viable bacteria within groups. Differences were considered to be significant at $P<0.05$ (*), highly significant at $P<0.01$ (**), tendencies at $p < P<0.10$, and 'ns' was used to represent no significant difference.

4.4.9 Experimental site

The experiment was conducted on farm of Suranaree University of Technology; whenas chemical analyses were performed in the center of Scientific and Technological Equipments of Suranaree University of Technology.

4.4.10 Duration

The experiment were carried out during June 16, 2007 –September 8, 2007.

4.5 Results

4.5.1 Diet composition

All animals received a diet composing of whole plant silage plus concentrate. The diet was adequate to meet the requirements of crude protein, growth net energy, and dry mater intakes of the goats under the condition of maintenance plus lower activity and 50 g/d weight gain (Nutrients Requirements of Goats, NUMBER 15, 1989). As to the concentrate, it contained DM 90.1%, CP 14.0%, and NDF 34.7%, whereas the silage contained DM 21.2%, CP 9.7%, and NDF 54.9% (DM basis) (Table 4.2). As shown in Table 4.3, the main fatty acids of the concentrate were comprised of 30.37% C18:2n6c, 19.79% C17:0, 15.06% C12:0, 14.47% C18:1n9c. Concededly, these fatty acids accounted for 1.21%, 0.79%, 0.60%, and 0.58% of the concentrate dry matter respectively. And yet, the main fatty acids of the whole plant corn silage were composed of (sorted by size) 38.75% C18:2n6c, 15.98 % C18:1n9c, 14.43% C16:0, and 11.87% C18:3n3, and these fatty acid made up of 0. 81%, 0.34%, 0.30%, 0.25% of the corn silage dry matter, respectively.

The commercial soybean oil and sunflower oil were determined fatty acid profiles by GC, the fatty acids mass were showed in Table 4.4. The main centesimal compositions of the soybean oil were (sorted by size) 48.36% C18:2, 24.67% C18:1, 9.04% C16:0, 5.02% C18:3 and 3.90% C18:0. The main centesimal compositions of the sunflower oil were (sorted by size) 38.33% C18:1, 31.43% C18:2, 7.71% C18:0, 6.07% C16:0, 3.82% C22:0, 3.53% C20:5n3, and 2.34% C18:3.

Table 4.2 Chemical compositions of experimental diet (dry matter basis).

Items	**Composition (%)**
Concentrate	
Dry matter	90.1
Organic matter	94.0
Crude protein	14.0
Ether extracts	4.0
Acid insoluble ash	3.1
Acid detergent fiber	26.5
Neutral detergent fiber	34.7
Corn silage	
Dry matter	21.2
Organic matter	89.3
Crude protein	9.7
Ether extract	2.1
Acid insoluble ash	5.1
Acid detergent fiber	42.4
Neutral detergent fiber	54.9

Table 4.3 Fatty acid profiles of concentrate and whole plant core silage (DM basis).

Items	% DM	% Total fatty acid
Concentrate		
C12:0	0.60	15.06
C14:0	0.24	5.92
C16:0	0.25	6.28
C17:0	0.79	19.79
C18:0	0.09	2.31
C18:1n9c	0.58	14.47
C18:2n6c	1.21	30.37
C18:3n3	0.07	1.82
Others	0.12	3.04
Corn silage		
C14:0	0.04	1.77
C16:0	0.30	14.43
C16:1	0.01	0.71
C17:0	0.04	1.67
C18:0	0.07	3.54
C18:1n9c	0.34	15.98
C18:2n6c	0.81	38.75
C18:3n3	0.25	11.87
Others	0.22	10.56

Table 4.4 Fatty acid profiles of the soybean oil and sunflower oil that used in this experiment.

	%Total fatty acid	
Fatty acids	**Soybean oil**	**Sunflower oil**
C14:0	0.37	0.48
C15:0	0.18	-
C16:0	9.04	6.07
C17:0	0.27	1.36
C18:0	3.90	7.71
C18:1	24.67	38.33
C18:2	48.36	31.43
C18:3	5.02	2.34
C20:0	1.79	1.74
C20:2	0.38	0.62
C22:0	1.77	3.82
C20:3n6	2.04	0.60
C23:0	0.23	0.69
C22:2	0.21	0.62
C20:5n3	1.33	3.53
C24:1	0.12	0.29

4.5.2 Feed intake and growth performances

No differences existed in whole plant corn silage and concentrate total daily average as well as % body weight dry matter intakes between the treatments. However, as obviously shown in Table 4.5, in comparison with the addition of sunflower oil, the silage dry mater intake (SDMI), total dry mater intake (TDMI), and *pro rata* body weight intake ($P>0.05$) tended to be brought up by the supplementation of soybean oil. Thereof, dry matter intakes were not significantly affected with the increasing levels of supplemented oil. The average daily gain (ADG) increased with significantly with supplementation of soybean oil ($P<0.05$), and it was tended to pushed up by the presence of sunflower oil. Compared with additional sunflower oil, ADG of the goats supplemented soybean oil was elevated significantly ($P<0.05$). Whereof the levels of oil, the increasing levels of soybean oil (2.5 and 5.0) showed a faint depressing effect on ADG (71.6 vs. 67.6). Whereas, the increasing levels of sunflower oil (2.5 and 5.0%) contrasted with those of soybean oil to display increasing ADG (43.1 VS. 53.1g/d). In reference to feed conversion, it was illustrated with extremely significant depression of DMI: ADG ratio due to supplementation of soybean oil ($P<0.01$), and was illustrated with significant depression of the DMI: ADG ratio owing to presence of sunflower oil ($P<0.05$). In accordance with the case of DMI, the difference of supplemented soybean oil levels (2.5 and 5.0%) showed a mild increment of DMI: ADG ratio (5.8 vs. 6.1). On the contrary, the difference of supplemented sunflower oil levels (2.5 and 5.0%) reduced the DMI: ADG ratio (8.2 vs. 6.1) significantly ($P<0.05$).

Table 4.5 The effects of linoleic acid enriched soybean oil and sunflower oil on DMI, ADG, and feed conversion of growing goats (% concentrate basis).

	Control	SB oil (%)		SF oil (%)		SEM	Effect	
		2.5	5.0	2.5	5.0		SB	SF
S DMI(g/d)	273.5	307.5	302.1	237.0	269.6	11.5	ns	ns
C DMI(g/d)	210.4	217.6	215.0	208.2	212.0	3.8	ns	ns
Total (g/d)	483.9	525.1	517.1	445.2	481.6	12.0	ns	ns
$W^{0.75}_{(g/kg/d)}$	48.9	50.7	49.6	44.7	49.6	2.2	ns	ns
LWB (%)	2.7	2.7	2.7	2.5	2.6	0.1	ns	ns
ADG (g/d)	42.4^{c}	71.6^{a}	67.6^{a}	43.1^{c}	53.1^{b}	4.3	*	ns
DMI:ADG	11.4^{a}	7.33^{b}	7.65^{b}	10.33^{a}	9.07^{b}	0.4	**	*

S DMI=whole plant corn silage dry mater intake; C DMI=concentrate dry mater intake; LWB=%live body weight intake; SB=soybean oil; SF=sunflower oil; SEM=standard error of the mean; *P<0.05; **P<0.01; ns= not significantly different (P>0.05); Means with different superscript letters in the same row differ significantly (P<0.05).

4.5.3 Dietary digestibility

Table 4.6 showed the digestibility of DM, OM, CP, NDF, and EE were not significantly different because of additional soybean oil and sunflower oil. Of which, the digestibility of DM, OM and NDF presented an increasing tendency (P>0.05) going with a small stressing of CP and EE digestibility due to the supplementation of soybean oil. In the meantime, the supplementation of sunflower oil was not effectual to display statistical difference in affecting on dietary digestibility (P>0.05).

Considering the effects of supplemented levels of soybean oil and sunflower oil on dietary digestibility, the increasing levels of soybean oil (2.5 and 5.0%) revealed a mild reduction in dietary digestibility (P>0.05), in contrast, the increasing levels of sunflower oil (2.5 and 5.0%) appeared to uplift the dietary digestibility (P>0.05). Compared the effects of supplemental soybean oil and sunflower oil on dietary digestibility, the better results were observed for the addition of soybean oil rather than sunflower oil (Table 4.6).

Table 4.6 The effects of linoleic acid enriched soybean oil and sunflower oil on dietary digestibility of growing goats fed whole plant corn silage (%).

	Control	SB oil (%)		SF oil (%)		SEM	Effect	
		2.5	5.0	2.5	5.0		SB	SF
DDM	62.2	71.5	68.2	62.9	63.7	4.9	ns	ns
DOM	66.2	75.8	71.2	66.1	71.4	4.6	ns	ns
DCP	58.3	56.7	56.3	55.5	57.9	3.3	ns	ns
DNDF	56.2	59.8	57.4	55.0	56.6	3.2	ns	ns
DEE	76.2	74.6	74.5	73.5	75.3	4.0	ns	ns

SB=soybean oil; SF=sunflower oil; SEM=standard error of the mean.

4.5.4 Ruminal Fluid pH, Ammonia N, PUN, and VFA

Supplementation of soybean oil and sunflower oil did not conduce to significant changes for the ruminal average pH (Table 4.7), the pH ranged form 6.23 to 6.42. Howbeit, as shown in Figure 4.1, before morning meal, the pH of soybean oil and sunflower oil treatment groups tended to lower down (P>0.05), and particularly

the supplementation of 5.0% sunflower oil resulted in obvious decrease of pH in comparison with the control (6.4 vs. 6.8) ($P<0.05$). Post-morning feeding 3 and 6 h, the soybean oil and sunflower oil treatments were not responsible for changes of pH. Moreover, the value of pH changed according to the sampling time. It presented highest value before morning feeding, and then decreased in concurrence with the morning meal and reached the lowest value post-morning feeding 3 h, after that turned to continual increase, and after meal 6 h the pH values were similar to that were measured before morning meal.

Differed from the case of pH, supplementation of soybean oil resulted in an extremely significant decrease in average ammonia nitrogen (NH_3-N) concentration contrasting with the control ($P<0.01$); the average NH_3-N concentration also revealed an significant decrease resulting from the supplementation of sunflower oil in contrast to the control ($P<0.05$). Still, the dosages of the supplemented soybean oil and sunflower oil did not bring significant changes in the average NH_3-N (Table 4.7). In details (Figure 4.2), the effects of additional linoleic acid enriched soybean oil and sunflower oil on ruminal NH_3-N were related to the sampling time; the changes of the NH_3-N occurred before morning meal and post-morning feeding 6 h, but left it unaffected at post-morning feeding 3 h.

The linear equation and r^2 of the plasma urea nitrogen (PUN) standard were r^2 =0.9837 and y=0.0274x+0.0023. Where: y is the amount of PUN, and x is the concentration of PUN standard. PUN concentration presented a slight subtraction due to presence of sunflower oil ($P>0.05$), but displayed a decreasing tendency owing to the addition of soybean oil ($P>0.05$) (Table 4.7). As shown in Figure 4.3, the effects of supplemented soybean oil and sunflower oil on PUN appeared before morning meal

rather than post-morning feeding 3 and 6 h.

In terms of volatile fatty acid (VFA), the total production of VFA was not affected by the supplementation of soybean oil and sunflower oil (Table 4.7). In addition, as shown in Figure 4.4, the VFA changed according to the sampling time, its peak presented at post-morning feeding 3 h and then lowered gradually. Concurrently, the additions of soybean oil and sunflower oil did not significantly affected the main VFA mixture molar composition as well as the ratio of C_2:C_3 (Table 4.7).

Table 4.7 The effects of soybean oil and sunflower oil on the average pH, ammonia nitrogen (NH_3-N, mg/Dl), plasma nitrogen (PUN, mg/Dl), and VFA (mM/l) of growing goats fed whole plant corn silage.

	Control	SB oil (%)		SF oil (%)		SEM	Effect	
		2.5	5.0	2.5	5.0		SB	SF
pH	6.42	6.38	6.32	6.35	6.23	0.04	ns	ns
NH_3-N	17.82^{a}	15.29^{c}	16.43^{b}	16.91^{b}	15.91^{bc}	0.83	**	*
PUN	17.03	16.06	16.49	16.30	16.71	1.28	ns	ns
TVFA	84.4	82.8	83.4	79.6	81.1	3.59	ns	ns
The main VFA mixture centesimal proportion (% TVFA)								
Acetate	67.0	67.1	66.6	67.1	65.6	0.67	ns	ns
Propionate	22.1	23.7	23.8	23.2	24.4	0.55	ns	ns
Butyrate	6.01	5.23	5.56	5.74	5.94	0.45	ns	ns
C2:C3	3.03	2.83	2.80	2.89	2.70	0.21	ns	ns

SB=soybean oil; SF=sunflower oil; SEM=standard error of the mean; *P<0.05; **P<0.01; ns= not significantly different (P>0.05); Means with different superscript letters in the same row differ significantly (P<0.05).

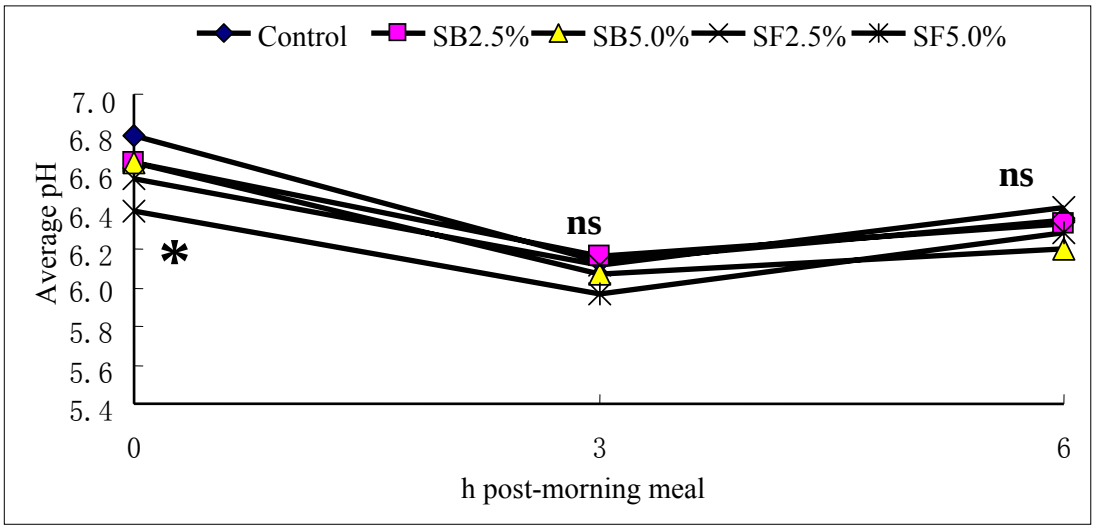

Figure 4.1 Ruminal pH of growing goats supplemented soybean oil (SB) and sunflower oil (SF).

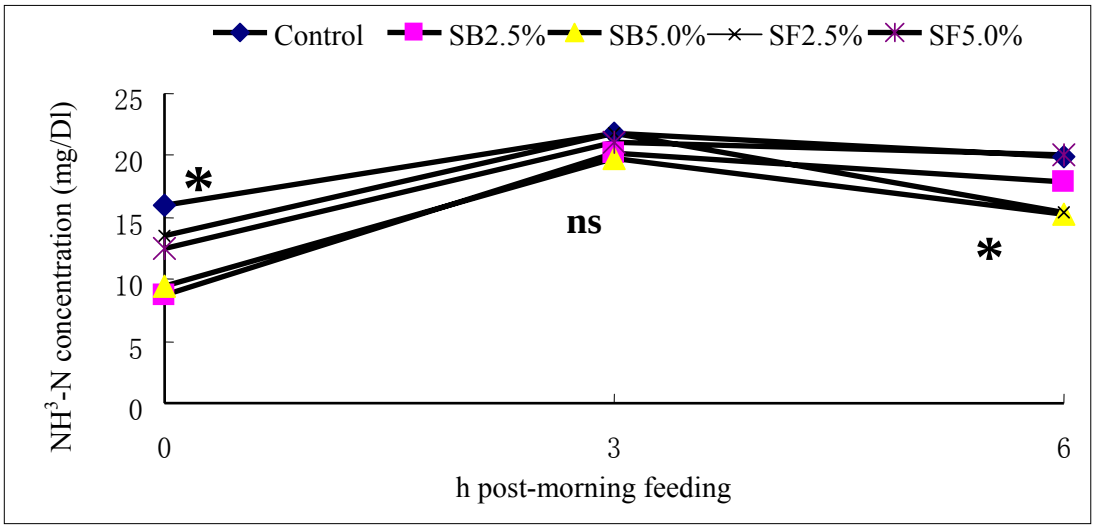

Figure 4.2 Ruminal NH_3-N of growing goats supplemented soybean oil (SB) and sunflower oil (SF).

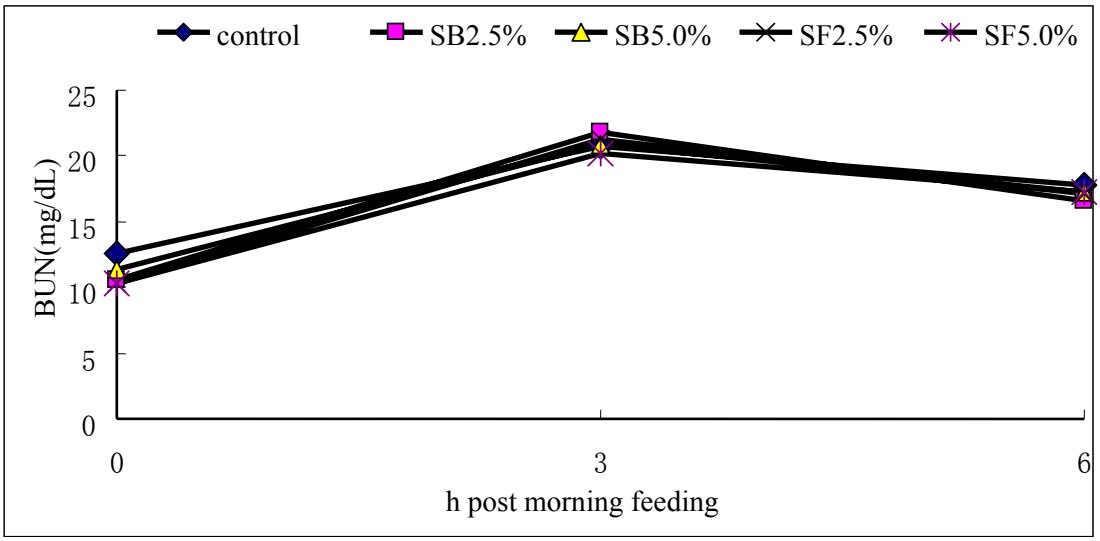

Figure 4.3 Plasma urea nitrogen of growing goats supplemented soybean oil (SB) and sunflower oil (SF).

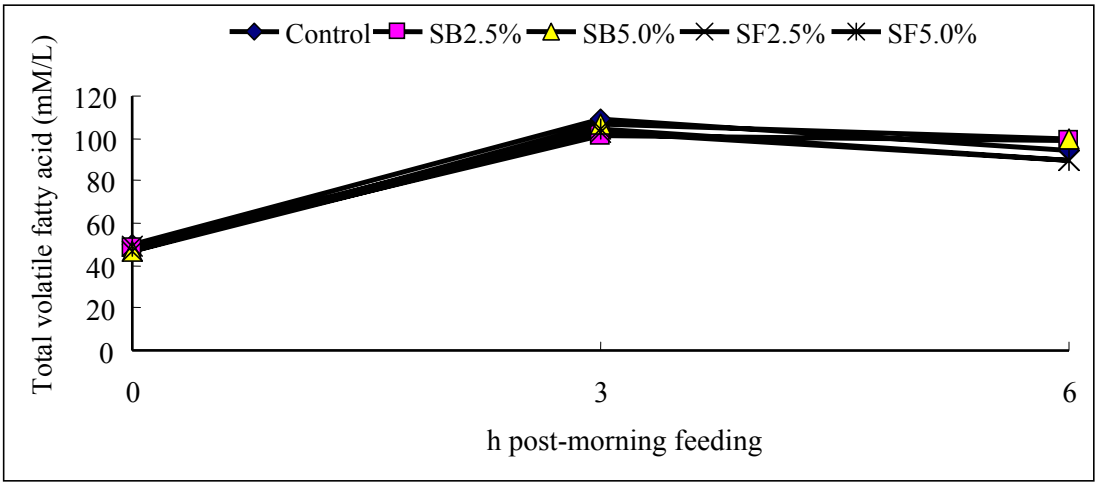

Figure 4.4 Plasma urea nitrogen of growing goats supplemented soybean oil (SB) and sunflower oil (SF).

4.5.4 Ruminal microbe population

The number of protozoa ranged from 1.53 to 2.35 x10^4 /ml rumen fluid

(Table 4.8). The supplementations of linoleic acid enriched soybean oil and sunflower oil caused highly significant increase in protozoal counts ($P<0.01$). In addition, as revealed in Figure 4.5, the significant increase of protozoal counts presented at all the sampling time (0, 3, and 6 h). However, the difference of dosages did not result in significant changes in protozoal counts ($P>0.05$) (Table 4.8).

The number of total viable bacteria ranged from 0.40 to 1.77 x 10^{10} /ml rumen fluid; and in the same case as protozoa, the supplementations of soybean oil and sunflower oil caused highly significant increase in protozoal counts ($P<0.01$) (Table 4.8). Also the significant increase of bacterial counts presented at all the sampling time (0, 3, and 6 h) and the difference of dosages did not result in significant changes ($P>0.05$) (Table 4.8) (Figure 4.6).

Table 4.8 The effects of soybean oil and sunflower oil on rumen microbe population of growing goats fed whole plant corn silage.

	Control	SB oil (%)		SF oil (%)		SEM	Effect	
		2.5	5.0	2.5	5.0		SB	SF
Protozoal population ($x10^4$)								
0h	1.73^{b}	2.12^{a}	2.25^{a}	2.11^{a}	2.18^{a}	0.13	**	**
3h	1.53^{b}	1.82^{a}	1.84^{a}	1.81^{a}	1.86^{a}	0.15	**	**
6h	1.98^{b}	2.64^{a}	2.29^{a}	2.35^{a}	2.11^{a}	0.22	**	**
Bacterial population ($x10^{10}$)								
0h	1.01^{c}	1.49^{b}	1.45^{b}	1.60^{a}	1.54^{ab}	0.14	**	**
3h	0.40^{b}	0.63^{a}	0.51^{a}	0.63^{a}	0.47^{ab}	0.05	**	**
6h	1.00^{c}	1.61^{a}	1.41^{b}	1.77^{a}	1.43^{b}	0.10	**	**

SB=soybean oil; SF=sunflower oil; SEM=standard error of the mean; *P<0.05; **P<0.01; ns= not significantly different (P>0.05); Means with different superscript letters in the same row differ significantly (P<0.05).

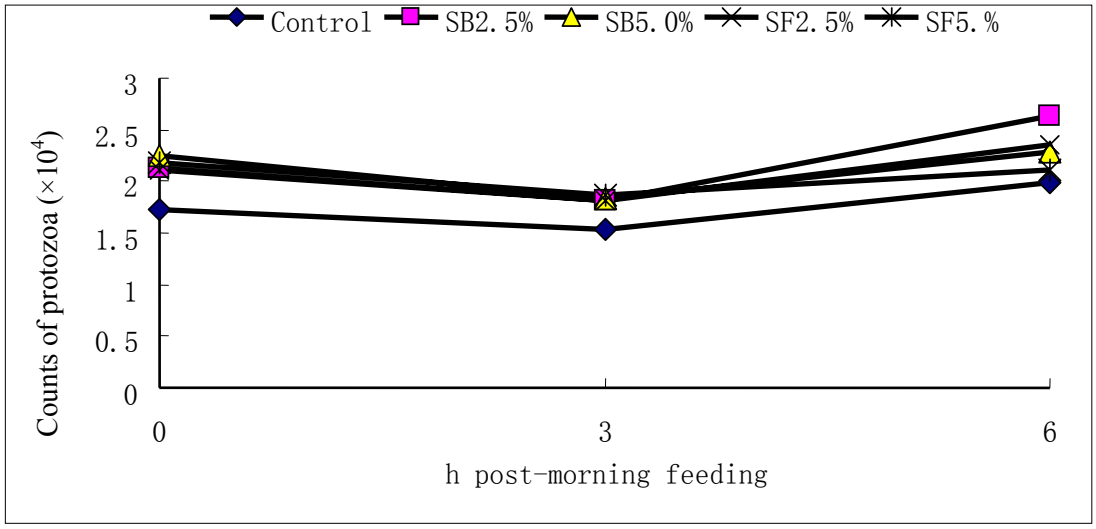

Figure 4.5 Counts of ruminal protozoa for growing goats supplemented soybean oil and sunflower oil.

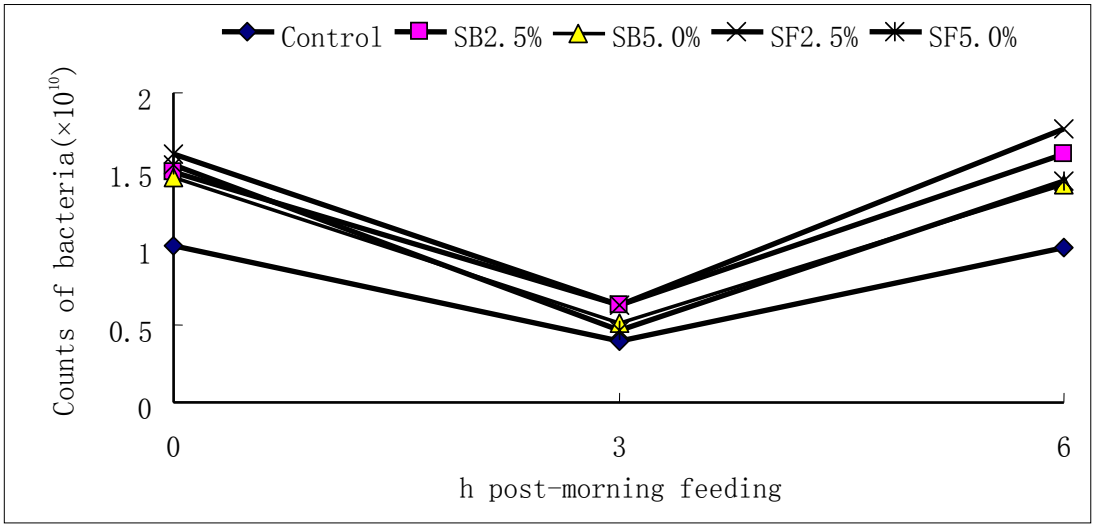

Figure 4.6 Counts of ruminal bacteria for growing goats supplemented linoleic acid enriched soybean oil and sunflower oil.

4.5.5 Nitrogen balances

Additions of soybean oil did not significantly affect total dietary N intake and excretion, but remarkably put the nitrogen absorption (NA), nitrogen retention up (NR) ($P<0.05$). The supplementation of sunflower oil leaded to event reduction in total dietary N intake and excretion except for urinary nitrogen excretion ($P<0.05$), whereas, the supplementation of sunflower oil substantially increased the NA and NR ($P<0.05$) (Table 4. 9).

Table 4.9 The effects of soybean oil and sunflower oil on nitrogen balance of growing goats (% of concentrate basis)

	Control	SB%		SF%		SEM	Effect	
		2.5	5.0	2.5	5.0		SB	SF
N intake(g/d)	7.1^{a}	7.5^{a}	7.2^{a}	6.4^{b}	6.5^{b}	0.27	ns	*
N excretion (g/d)								
Faece	3.9^{a}	3.6^{a}	3.6^{a}	3.4^{ab}	2.9^{b}	0.17	ns	*
Urine	1.4	1.3	1.2	1.2	1.2	0.10	ns	*
Total	5.3^{a}	4.9^{a}	4.8^{a}	4.6^{ab}	4.1^{b}	0.13	ns	*
N A (g/d)	3.2^{b}	4.0^{a}	4.0^{a}	3.0^{b}	3.7^{a}	0.26	*	*
N R (g/d)	1.8^{b}	2.7^{a}	2.4^{a}	1.8^{b}	2.4^{a}	0.11	*	*
N R (%)	25.0^{c}	35.2^{ab}	33.0^{b}	27.9^{c}	36.9^{a}	1.33	**	*

NR=N retention; NA=N absorption; SB=soybean oil; SF=sunflower oil; SEM=standard error of the mean; *P<0.05; **P<0.01; ns= not significantly different (P>0.05); Means with different superscript letters in the same row differ significantly (P<0.05).

4.5.6 Fatty acid profiles and conjugated linoleic acid content in plasma

As the plasma C12-C17 long chain fatty acids centesimal composition as concerned, the C15:0 ($P<0.01$), C16:1 ($P<0.05$), and C17:0 ($P<0.05$) markedly increased for the reason of additional soybean oil. At the same time, the supplementation of sunflower oil also was clear to raise C15:0 ($P<0.01$) and C16:1 ($P<0.05$) but keeping C17:0 unaffecting (Table 4.10).

The plasma C18:0 content tended to increase ($P>0.05$) contrasting with C18:1 tended to decreased ($P>0.05$) owing to the additions of the soybean oil and sunflower oil. Concurrently, the C18:2n6 and C18:3n3 noticeably increased due to the supplementation of soybean ($P<0.05$) and sunflower oil ($P<0.01$). In the mean time, the detected C18:c9,t11 CLA isomer composition made up 0.42-1.16% of total plasma fatty acids, it increased with highly significance ($P<0.01$); the detected C18:t10,c12 ranged from undetectable (the control) to 0.20 (2.5% soybean oil), they also presented extremely significance in comparison with the control ($P<0.01$) (Table 4.10).

Except for no significant impacts on the detected C24:1 and C22:6n3, the presences of soybean oil and sunflower oil resulted in distinct enhancements in very long-chain fatty acids (length of chain larger than 18 C). Both of the soybean oil and sunflower oil increased the contents of C20:3n3 significantly ($P<0.05$), in concomitancy to increase the C20:4n6 and C20:5n3 with highly significance ($P<0.01$), and on the contrary to evidently decrease the C24:0 ($P<0.05$) (Table 4.10).

The plasma total CLA centesimal proportion ranged form 0.41-1.22%, it increased 197.6% due to presences of soybean oil, increased 129.3% (2.5% sunflower oil) and 141.5% (5.0% sunflower oil). The total saturated fatty acids (TAFA) tended

to increase owing to the presence of soybean oil (P>0.05), however, total poly-unsaturated fatty acids (TPUSFA) and desirable fatty acids (DFA=C18:0+TUSFA) tended to increase owing to both of supplementations of soybean oil and sunflower oil. The total n6 fatty acids (Tn6) significantly increased (P<0.05) contrasted with significant decrease of the total n3 fatty acids (T36) (P<0.05), and accordingly, a highly significant increase in n6:n3 ratios were led by supplementations of soybean oil and sunflower oil (Table 4.10).

On the other hand, the calculations of centesimal composition of plasma fatty acids into fatty acid (μg) contained in 1 ml plasma showed some statistical disparities (Table 4.11).

On the whole, the C18:c9, t11 isomer (μg) contained in 1 ml plasma ranged from 3.6-12.0μg/ml plasma. It increased 166.7 and 233.3% for additional dosages of 2.5 and 5.0% soybean oil. At the same time, it increased 130.6 and 161.1% due to additional dosages of 2.5 and 5.0% sunflower oil respectively. The C18:t10, c12 CLA isomer (μg) contained in 1 ml plasma ranged from undetected-0.2 μg/ml plasma. It contained 0.9 and 0.6 μg/ml plasma for additional dosages of 2.5 and 5.0% soybean oil respectively, at the meantime it increased 0.4 and 0.6 μg/ml plasma for additional dosages of 2.5 and 5.0% sunflower oil respectively. The total detected CLA contained 3.6, 10.5, 12.6, 8.7, and 10.0 μg/ml plasma for the control, 2.5% soybean oil, 5.0% soybean oil, 2.5% sunflower oil and 5.0% sunflower oil respectively. In comparison with the control, the soybean oil and sunflower oil treatments increased 191.7%, 250.0%, 141.7% and 177.8% respectively. Obviously, the soybean oil was more effectual on improvement of plasma CLA content than sunflower oil.

Table 4.10 Plasma fatty acids centesimal composition profiles of growing goats supplemented linoleic acid enriched soybean oil and sunflower oil under condition of feeding whole plant corn silage.

FA (%)	Control	SB (%)		SF (%)		SEM	Effect	
		2.5	5.0	2.5	5.0		SB	SF
C12:0	1.14	1.28	1.24	1.22	0.91	0.11	ns	ns
C14:0	3.57	3.16	2.93	3.92	3.46	0.23	ns	ns
C15:0	0.72^{a}	0.50^{b}	0.25^{c}	0.28^{c}	0.41^{b}	0.01	**	**
C16:0	18.32	19.73	19.90	18.22	17.93	1.33	ns	ns
C16:1	1.68^{a}	1.34^{b}	1.35^{b}	1.14^{b}	1.62^{a}	0.02	*	*
C17:0	2.48^{a}	2.15^{b}	2.09^{b}	2.64^{a}	2.38^{a}	0.01	*	ns
C18:0	20.36	21.71	21.83	20.38	21.14	1.40	ns	ns
C18:1nc	18.19	17.09	17.94	17.51	18.35	1.06	ns	ns
C18:2n6c	14.72^{b}	17.55^{ab}	19.68^{a}	19.45^{a}	20.88^{a}	1.43	*	**
C18:3n3	1.76^{a}	1.89^{a}	1.25^{b}	1.17^{b}	1.13^{b}	0.09	*	**
C18:c9,t11	**0.41^{b}**	**1.02^{a}**	**1.16^{a}**	**0.92^{a}**	**0.93^{a}**	0.09	**	**
C18:t10,c12	**0.00^{d}**	**0.20^{a}**	**0.06^{b}**	**0.02^{c}**	**0.06^{b}**	0.09	**	**
C20:2	1.05^{a}	0.60^{b}	0.70^{b}	0.32^{c}	0.34^{c}	0.09	*	**
C20:3n3	3.15^{a}	2.21^{b}	1.37^{c}	1.76^{bc}	2.39^{b}	0.21	*	*
C20:4n6	1.68^{a}	1.23^{b}	1.13^{b}	1.06^{b}	1.09^{b}	0.06	**	**
C20:5n3	0.16^{b}	0.22^{ab}	0.40^{a}	0.00^{c}	0.00^{c}	0.09	**	**
C24:0	1.21^{a}	0.47^{c}	0.21^{d}	0.29^{d}	0.81^{ab}	0.07	**	**
C24:1	2.69	2.54	2.37	2.45	2.27	0.05	ns	ns
C22:6n3	3.55	3.05	2.58	3.46	2.57	0.12	ns	ns

Table 4.10 (Continued)

FA (%)	Control	SB (%)		SF (%)		SEM	Effect	
		2.5	5.0	2.5	5.0		SB	SF
TCLA	**0.41^{b}**	**1.22^{a}**	**1.22^{a}**	**0.94^{a}**	**0.99^{a}**	**0.08**	**	**
TSFA	47.80	49.20	48.45	46.95	47.04	2.10	ns	ns
TMUSFA	22.56	20.97	21.67	21.11	22.24	0.53	ns	ns
TPUSFA	26.48	27.96	28.33	28.14	29.39	0.96	ns	ns
DFA	69.40	70.65	71.82	69.63	72.77	1.37	ns	ns
PUFA/SFA	0.55	0.57	0.58	0.60	0.62	0.01	ns	ns
Tn6	16.81^{b}	20.00^{a}	22.02^{a}	21.44^{a}	22.96^{a}	1.76	*	*
Tn3	8.62^{a}	7.36^{ab}	5.60^{b}	6.39^{b}	6.09^{b}	0.22	*	*
n−6/n−3	1.95^{c}	2.72^{b}	3.93^{a}	3.36^{a}	3.77^{a}	0.23	**	**

SB=soybean oil; SF=sunflower oil; TSFA=total saturated fatty acid; TMUSFA=total mono-unsaturated fatty acid; TPUSFA= total poly-unsaturated fatty acid; DFA=desirable fatty acid; Tn6=total n6 fatty acid; Tn3=total n3 fatty acid; SEM=standard error of the mean; *P<0.05; **P<0.01; ns= not significantly different (P>0.05); Means with different superscript letters in the same row differ significantly (P<0.05).

Table 4.11 Fatty acid and conjugated linoleic acid contents (µg/ml plasma) in plasma of growing goats supplemented linoleic acid enriched soybean oil and sunflower oil under condition of feeding whole plant corn silage.

FA (µg/ml plasma)	Control	SB (%)		SF (%)			Effect	
		2.5	5.0	2.5	5.0	SEM	SB	SF
C12:0	9.9^{b}	12.0^{a}	12.8^{a}	11^{ab}	9.2^{b}	0.64	*	ns
C14:0	31.3^{ab}	29.7^{b}	30.3^{b}	35.4^{a}	35^{a}	1.09	ns	ns
C15:0	6.3^{a}	6.6^{a}	2.6^{c}	2.5^{c}	4.1^{b}	0.29	*	**
C16:0	160.8	185.7	185.9	174.3	181.1	1.35	ns	ns
C16:1	14.8^{a}	12.6^{ab}	14.0^{a}	10.3^{b}	11.4^{b}	0.61	ns	*
C17:0	21.8	20.2	21.6	23.8	24.0	0.37	ns	ns
C18:0	178.8	204.3	205.8	183.8	213.5	4.33	ns	ns
C18:1nc	159.7^{b}	160.9^{b}	185.6^{a}	158.0^{b}	165.4^{b}	2.17	*	ns
C18:2n6c	129.2^{c}	165.2^{b}	203.6^{s}	175.5^{ab}	210.9^{s}	10.19	**	**
C18:3n3	15.5^{a}	14.8^{a}	12.9^{ab}	10.6^{b}	11.4^{b}	0.73	*	**
C18:c9,t11	**3.6^{c}**	**9.6^{a}**	**12.0^{a}**	**8.3^{b}**	**9.4^{ab}**	**0.24**	**	**
C18:t10,c12	**0.0^{c}**	**0.9^{a}**	**0.6^{ab}**	**0.4^{b}**	**0.6^{ab}**	**0.09**	**	**
C20:2	9.2^{a}	5.6^{b}	7.3^{ab}	2.8^{c}	3.4^{c}	0.31	*	**
C20:3n3	27.6^{a}	20.8^{b}	14.2^{c}	15.8^{c}	24.1^{ab}	1.08	*	*
C20:4n6	14.8^{a}	11.6^{b}	11.6^{b}	9.5^{c}	11.0^{bc}	0.44	*	**
C20:5n3	1.4^{b}	2.1^{b}	4.1^{a}	0.0^{c}	0.0^{c}	0.07	**	**
C24:0	10.6^{a}	4.4^{b}	2.2^{c}	2.6^{c}	5.2^{b}	0.2	**	**
C24:1	23.6	23.9	24.5	22.1	23.0	0.14	ns	ns
C22:6n3	31.2	28.7	26.7	31.2	25.9	1.21	ns	ns
T CLA	**3.6^{c}**	**10.5^{ab}**	**12.6^{a}**	**8.7^{b}**	**10.0^{ab}**	**0.78**	**	**

Table 4.11 (Continued)

FA (µg/ml plasma)	Control	SB (%)		SF (%)		SEM	Effect	
		2.5	5.0	2.5	5.0		SB	SF
TSFA	419.5^{b}	462.9^{a}	461.2^{a}	433.4^{b}	472.1^{a}	3.12	*	*
TMUSFA	198.1	197.4	224.1	190.4	199.8	1.00	ns	ns
TPUSFA	232.5	259.3	293.0	254.1	296.7	2.03	ns	ns
DFA	609.4^{b}	661.0^{ab}	722.9^{a}	628.3^{b}	710.0^{a}	13.47	**	**
PUFA/SFA	0.55	0.56	0.64	0.59	0.63	0.03	ns	ns
Tn6	147.6^{c}	187.3^{b}	227.8^{a}	193.7^{b}	231.9^{a}	6.98	*	*
Tn3	75.7	66.4	57.9	57.6	61.4	1.27	ns	ns
n−6/n−3	1.95^{c}	2.82^{b}	3.93^{a}	3.36^{a}	3.78^{a}	0.13	**	**

SB=soybean oil; SF=sunflower oil; TSFA=total saturated fatty acid; TMUSFA=total mono-unsaturated fatty acid; TPUSFA= total poly-unsaturated fatty acid; DFA=desirable fatty acid; Tn6=total n6 fatty acid; Tn3=total n3 fatty acid; SEM=standard error of the mean; *P<0.05; **P<0.01; ns= not significantly different (P>0.05); Means with different superscript letters in the same row differ significantly (P<0.05).

The statistical disparities between Table 4.10 and 4.11 registered as C12:0 ‘SB’ ‘ns’ (no significance) vs. ‘*’ (significant difference); C15:0 ‘SB’ ‘**’ vs. ‘*’; C16:1 ‘SB’ ‘*’vs. ‘ns’; C17:0‘SB’ ‘*’ vs. ‘ns’; TSFA ‘SB’ and ‘SF’ ‘ns’ vs. ‘*’; DFA ‘SB’ and ‘SF’ ‘ns’ vs. ‘**’; and finally Tn3 ‘SB’ and ‘SF’ ‘ns’ vs. ‘*’.

4.6 Discussion

4.6.1 Supplementation of soybean oil and sunflower oil significantly increased ADG and feed conversion (soybean oil: P<0.01; sunflower oil: P<0.05), but not affect DMI of growing goats fed with whole plant corn silage.

The former findings about the effects of soybean oil and sunflower oil on ADG, DMI, and feed efficiency of ruminants were inconsistent, dietary sunflower oil added at 6% of the diet tended to increase (P = 0.07) ADG by 8% in cattle (Mir et al., 2002). This result concurred with observations in cattle fed sunflower seed (Gibb et al., 2001). On the contrary, feeding sunflower seed oil in sheep not affected the ADG, DMI, and feed efficiency (Ivan et al., 2001). However, Rogério et al. (2005) have verified that presence of soybean oil in goats' diet decreased the intakes of dry matter (%BW and g/kg BW0.75), neutral detergent fiber (NDF) and non-fibrous carbohydrates. Contrasted with this, Bouattour et al. (2008) stressed that feeding soybean oil (2.5% dietary DM basis) did not change the DIM in dairy goats. The results in the present study agreed with Bouattour et al. (2008).

In general, DMI is usually affected when added fat or oil levels more than 5% diet DM basis and this impact was related to the dietary NDF content (Rick et al., 1996). In the present study, 2.5 and 5.0% concentrate basis 2 levels were used in both of soybean oil and sunflower oil, limited the concentrate feeding as 1.5% of body weight for each goat, and the animals accessed to whole plant corn silage ad libitum. This ensured that the added soybean oil and sunflower oil levels were lowere than the optimum that can affect DMI of the animals. At the same time, free choice feeding of whole plant corn silage kept high NDF content of the diets. It is the dietary

management in the present study can be responsible for the significantly increased ADG and significantly decreased ratio of DMI: ADG in the tested goats.

4.6.2 Addition of soybean oil tended to increase digestibility of DM, OM, and NDF (P>0.05); sunflower oil was not effectual to increase dietary digestibility; increasing dosages of soybean oil showed a slight decrease in dietary digestibility; increasing levels of sunflower oil did not affect the dietary digestibility.

Eweedah et al. (1997) stated that fullfat soybean and sunflower seed did not impact the apparent digestibility of DM, OM, NFE and CP as well as nutritive value in Holstein bulls (179-203 kg) fed corn silage. However, the increasing fat level tended to decrease digestibility of CF, ADF and NDF. Rogério et al. (2005) have verified that the presence of soybean oil in diet of goats decreased the digestibility of NDF contrasted with increase of digestibility of CP, EE, and total digestible nutrients content (TDN). However, in the present study, the additions of soybean oil tended to increase NDF digestibility, and the supplementation of sunflower oil did not affect the NDF digestibility. The results were similar to Kucuk et al. (2004) who added soybean oil to diets at 0, 3.2, 6.3, and 9.4% of dietary DM, and found digestibilities of OM, NDF, and N were not affected (P = 0.13 to 0.95) by increasing dietary soybean oil level. It was found that the added levels of the soybean oil and sunflower oil have no significant effects on dietary digestibility. This was because of the highly significant increase of the protozoal and bacterial counts that resulted from additional soybean oil and sunflower oil (Table 4.8). Generally, fiber digestibility was adversely affected by dietary fat but the magnitude of this response was affected by source and amount of dietary fat and fiber contained in the dietary (Jenkins, 1993). Again, it is

the supplemented dosages of the soybean oil and sunflower, and the fatty acid profiles of them together with the amount and types of the roughage in the present study can be responsible for the different results on dietary digestibility from those of some previous study.

4.6.3 Presences of soybean oil and sunflower oil unaffected on ruminal average pH, but tended to decrease PUN (P>0.05) and significantly reduced NH_3-N (soybean oil: P<0.01; sunflower oil: P<0.05)

Ivan et al. (2001) explained that a decrease existed in ammonia N concentration, but pH significantly increased in rumen fluid for sunflower oil fed sheep. In contrast, other found ruminal pH and ammonia were not changed (P = 0.31) with increasing dietary soybean oil level in sheep (Kukcu et al., 2004). In addition, Beaulieu et al. (2002) described that supplementation of soybean oil did not alter ruminal pH in beef. However, Gülşen et al. (2006) suggested that increasing levels (3, 6, and 9%) of sunflower and soybean oil linearly increased pH, did not affect NH_3-N concentration, however depressed ruminal fermentation in cattle. On the contrary, Brokaw et al. (2001) observed the ruminal ammonia was decreased in cattle for receiving supplemental soybean oil. Furthermore, Rogério et al. (2005) observed an increase in ruminal pH of goats on account of presence of soybean oil. The result of present study in ruminal ammonia was in consistent with Brokaw et al. (2001). The presence of ciliate protozoa in the rumen ecosystem is associated with increased recycling of microbial nitrogen in the rumen (Jounany, 1996). Protozoa exert a stabilizing effect on ruminal pH because they rapidly ingest the starch and prevent fermentation of lactate producing bacteria on it (Williams and Dinusson, 1973). The results of the present study showed pH unchanged, PUN and NH_3-N were

significantly reduced, it were attributed to increased demand for NH_3-N uptake to support microbial growth and production that registered as significant increased of ruminal protozoal and bacterial counts (Table 4.8), and consequently less NH_3-N went into the blood which caused decrease of PUN.

4.6.4 Additions of soybean oil and sunflower oil were of no effect on increases of TVFA , VFA molar proportions and C2:C3 ratio

The proportion of acetate tended to increase quadratically ($P \geq 0.06$) as the levels of soybean oil in the diet increased from 2.5 to 7.5%, but the molar proportions of the other VFA, total VFA concentration were not affected in beef (Beaulieu et al., 2002). Whereas, total VFA decreased as the levels of soybean oil in the diet increased from 0 to 3.2%, and molar proportion of butyrate was not affected in sheep (Kukcu et al., 2004).Total VFA decreased ($P<0.05$), molar propoinic proportion increased, ratio of acetate: propionate decreased when feed the sheep with sunflower seeds oil (Ivan et al., 2001). In addition, Rogério et al. (2005) have verified that the presence of soybean oil in diet of goats decreased the acetate: propionate ratio in the ruminal fluid. When supplementing dietary oil or fat in ruminants, propionate molar proportions were expected to increase from conversion of glycerol to propionate, with the glycerol supplied from hydrolysis of dietary triacylglycerol (Chalupa et al., 1986). The findings of the present study showed that the VFA molar proportions did not be significantly changed, in part, these were put down to the triacylglycerol hydrolysis may not have been completed. At the meantime, the medium added dosages of soybean oil and sunflower oil and the abundant roughage caused no significant change of VFA molar proportion, and accordingly no significant change of C2:C3 ratio.

4.6.5 Supplementation of soybean oil did not significantly change the dietary N intake and N excretion; supplementation of sunflower oil resulted in significantly subtraction in dietary N intake, faecal and total N excretion (P<0.05); both of soybean oil and sunflower oil supplementations increased N absorption and retention

Up to now, it has not found the references concerned with the effects of supplementations of soybean oil and sunflower oil on N balance of goats. However, the findings of the present study were in accordance with the case of lamb, for example, Kucuk et al. (2004) observed that the intake of N in lamb changed very little owing to the presence of soybean oil. The subtraction in dietary N intake in present study due to addition of sunflower oil was caused by the concurred faint decrease of whole plant corn silage, and the increased N absorption and retention for soybean oil and sunflower oil supplementations were because decrements of N excretion related with the dietary N intake.

4.6.6 Supplementations of soybean oil and sunflower oil tended to increase plasma total saturated fatty acids (P>0.05) centesimal composition, but significantly decreased C15:0 (P<0.01), significantly elevated CLA content (P>0.05), significantly reduced the very long chain fatty acids (P<0.05), significantly increased DFA and ratio of n6:n3 (P<0.05)

The above findings agreed with that of Yeom et al. (2003) who demonstrated the supplementation of soybean oil in goat diet, significantly (P<0.05) elevated the linoleic acid (C18:2n-6) content by 9.3% on the contrary to decrease C14:0, C17:0 , C18:1n-9, C18:3n-3, and C20:5n-3 by highly significance. As shown in Table 4.4.

The FA profile of the soybean oil and sunflower oil used were characterized by a high concentration of C18:2, a relatively high content of C18:1, and lower levels of C18:3 and C18:0. Consequently, intakes of all C18 FA were higher in the oil added diets than in the control diet. These differences in the FA composition of the diets may explain the changes in plasma from the soybean oil and sunflower oil treatments. What is more, the high concentration of C18:2 together with significant increase of ruminal microbe (Table 4.8) in the soybean oil and sunflower oil treatments, can be the main reason for increase of plasma CLA concentration.

4.7 Conclusions

ADG and ratio of DMI: ADG of growing goats fed with whole plant corn silage increased significantly on account of additional soybean oil ($P<0.01$) and sunflower oil ($P<0.05$), but the DMI was not changed with significances.

DM, OM, and NDF digestibility of growing goats fed with whole plant corn silage tended to increase ($P>0.05$) for addition of soybean oil, but the supplementation of sunflower oil was not effectual to significantly change the dietary digestibility.

Supplementation of soybean oil and sunflower oil decreased NH_3-N (soybean oil: $P<0.01$; sunflower oil: $P<0.05$) as well as tended to decrease PUN ($P>0.05$), however, the ruminal average pH was not significantly changed for the reason of presences of soybean oil and sunflower oil.

Supplementations of soybean oil and sunflower oil unaffected TVFA and butyric proportion, but significantly increased acetic proportion ($P<0.05$) and C2:C3 ratio ($P<0.05$).

The ruminal total viable bacteria and protozoa ($P<0.01$) number increased significantly ($P<0.01$) due to additions of soybean oil and sunflower oil.

Significant reductions in dietary intake N, faecal N excretion and total N excretion were caused by supplementations of sunflower oil ($P<0.05$), whereas, supplementations of sunflower oil increased N absorption and N retention ($P<0.05$). At the same time, addition of soybean oil did not affect the total dietary N intake and N excretion, but significantly increased N absorption ($P<0.05$) and N retention ($P<0.01$).

Supplementations of soybean oil and sunflower oil significantly decreased centesimal composition of plasma C15:0 fatty acid ($P<0.01$), however, tended to increase total saturated fatty acids ($P>0.05$), significantly enhanced plasma CLA content ($P>0.05$), but significant reduced very long chain fatty acids ($P<0.05$). Significant increases also were found in DFA and Tn6 fatty acids and ratio of n6:n3 for the reasons of additional soybean oil and sunflower oil ($P<0.05$).

In summary, the supplementation of the soybean oil was more efficient than the sunflower oil in improvement of ADG and ruminal metabolism of growing goats fed with whole plant corn silage, the soybean oil was more effectual to enhance the plasma CLA and DFA contents. Based on the findings of this experiment, the soybean oil was chosen for further study.

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CHAPTER V

EFFECTS OF SUPPLEMENTAL SOYBEAN OIL AND PROBIOTICS ON PERFORMANCES OF GROWING GOATS FED WITH WHOLE PLANT CORN SILAGE

5.1 Abstract

The objectives of this experiment were to study the effects of additional soybean oil together with probiotics on growth, ruminal metabolism, plasma fatty acid profiles particularly CLA, on carcass quality, meat quality, meat fatty acid profiles particularly CLA in growing goats fed with whole plant corn silage. The thirty growing crossbred (Thai native x Anglo-Nubian) goats that used to perform the second experiment were prepared for the present study after 5 weeks adjustment. They were allocated to 5 treatments according to factorial in RCBD with 6 goats in each treatment.

The results showed that ADG and feed efficiency increased significantly ($P<0.05$). There were distinct interactions between soybean oil and probiotics on ADG ($P=0.07$) and feed conversion ($P=0.04$). Digestibility of DM and OM significantly increased ($P=0.02$), there were significant interaction on DM ($P=0.05$) and OM ($P=0.05$) digestibility for soybean oil and probiotics. Sampling time affected ruminal NH_3-N and PUN ($P<0.05$). There were a significant synergistic effect on the total VFA for soybean oil and probiotics ($P=0.05$). The C18:c9,t11 and C18:t10,c12CLA increased with highly significance ($P<0.01$). There were significant synergistic impact between soybean oil and

probiotics on increase of CLA isomers. The ratios of PUFA/SFA and n−6/n−3 increased (PUFA/SFA: $P>0.05$; n−6/n−3: $P<0.05$). The kidney, pelvic, and heart (KPH) fat significantly increased ($P<0.05$), but others slaughter attributes were not significantly affect. The ether extracts of the meat significantly increased ($P<0.05$), but the OM, DM, and CP were unaffected. The C14:0 ($P<0.05$), C15:0 ($P<0.05$), C16:0 ($P>0.05$), C16:1 ($P>0.05$) and C17:1 ($P<0.05$) fatty acid composition decreased.

All C18 fatty acids of the meat increased, particularly the C18:c9,t11 CLA increased 100 to 139.6% ($P<0.01$), the C18:t10,c12 CLA increased 100 to 300%($P<0.01$). There were significant synergistic effect of soybean oil and probiotics on CLA isomers was found ($P<0.05$).

The total CLA isomers ($P<0.01$), total n-6 ($P<0.05$), and total poly-unsaturated fatty acids ($P<0.05$) significantly increased; total saturated (TSFA), total n-3, total mono-unsaturated, and desirable fatty acids tended to increased ($P>0.05$). Supplementation of 5.0% soybean oil significantly increased the ratios of poly-unsaturated fatty acids to total saturated fatty acids ($P<0.05$), whereas, significantly decreased the ratios of total n-6 fatty acids to n-3 fatty acids ($P<0.05$). A remarkable interaction between soybean oil and probiotics existed in total CLA isomers ($P=0.04$), total n-6 fatty acids (=0.03), total saturated fatty acids ($P=0.09$), and total n-3 fatty acids.

5.2 Introduction

Nowadays, probiotics are widely used in animal nutrition with purpose of inducing favorable changes in the activity of the digestive microflora (Chiofalo et al., 2004). Lots of research had demonstrated that *Lactobacillus acidophilus* in combination with fungal cultures were more efficacious for increasing milk

production in lactating dairy cows (Komari et al., 1999; Block et al., 2000). In chapter 3 the effects of supplementation of additional *saccharomyces cerevisiae* and *L. acidophilus* probities on growth, ruminal metabolism, and plasma fatty acid profiles particularly CLA in growing goats fed with corn silage had been studied. The results showed that probiotics was effectual on increasing ADG and on establishing healthier and more favorable gastro-enteric setting for digestion and absorption. The probiotics also was effectual on reducing plasma myristic (C14:0) and palmitic (C16:0) that are hypercholesteremic and associated with the increased incidence of arteriosclerosis and coronary heart disease, was effectual on increasing plasma CLA. Based on the findings of the experiment, two 2.5 and 5.0 g/h/d 2 levels were chosen for the present study.

In addition, as the food safety and origin become the concern of the public, the chevon was preferred to many people due to it was looked as natural and low in fat and cholesterol. Furthermore, there is an interest in value-added goat meat that enriched with CLA, which could offer potential benefits in terms of human health, since CLA have been reported for wide range of beneficial effects such as anticarcinogenic, antiatherogenic, antidiabetic and immune stimulatory. In fact, biosynthesis of CLA happen in 2 ways (Bauman et al., 2001): the first is the partial biohydrogenation of linoleic acid and linolenic acid in the rumen, and the second is the desaturation of trans-11 C18:1 (TVA; trans-vaccenic acid) by the action of Δ9-desaturase in gland and tissue (Griinari et al., 2000). Soybean oil contains about 52% linoleic acid (Penny, 2006), and sunflower oil contains 63%-70% linoleic acid normally (Jasso et al., 2002). Chapter 4 had testified that the supplementation of soybean oil was more effectual on improvement of growing goats' growth, rumen

metabolism, and plasma CLA content than sunflower oil. In addition, an important criterion for the selection of lactobacilli for probiotic purposes is their adherence properties (Nemcova et al., 1997). Ringo et al. (1998) and Kaste et al. (2007) had asserted that PUFA increases the colonisation of fish and piglets intestine with lactobacilli. Accordingly, the selected levels of probiotics (2.5 and 5.0 g/h/d) and soybean oil (2.5 and 5.0% concentrate basis) were used in the present study to testify their synergistic effects on on growth, ruminal metabolism, and plasma fatty acid profiles particularly CLA, on carcass quality, meat quality, meat fatty acid profiles particularly CLA in growing goats fed with corn silage.

5.3 Objectives

This experiment was conducted to study the effects of additional soybean oil together with probiotics on growth, ruminal metabolism, and plasma fatty acid profiles particularly CLA, on carcass quality, meat quality, meat fatty acid profiles particularly CLA in growing goats fed with corn silage.

5.4 Materials and Methods

5.4.1 Experimental design and treatment

The thirty growing crossbred (Thai native x Anglo-Nubian) goats that used to perform the second experiment were prepared for the present study. After the second experiment was finished, the animals was fed the concentrate 100 g/d/h and accessed to the whole plant corn silage *ad libitum* for 5 weeks to scavenge the possible difference that caused by the experiment. Subsequently, the animals were weighed and allocated to the present experiment. The weights of animals were (18.29

± 2.7) kg, ages were about 9 months. They were allocated to 5 treatments according to factorial in Randomized Complete Block Design (RCBD) with 6 goats in each treatment. The blocks were mad by weight into heavy, medium, and light goats and each of the treatments contained 2 goats from each of the blocks (Table 5.1). Before the experiment, the animals were injected with Ivomic (Merial Ltd., Iselin, NJ) for anti-internal parasite, and housed in individual pens (0.9x1.4 m) where the animals could have an easy access to corn silage and fresh water *ad libitum*. And also, the pens were cleaned and disinfected with Ciber solution prior to the housing of the animals. During the experiment, animals in different treatments received the whole plant corn silage plus concentrate basal diet. The treatments included control, supplementations of 2.5 and 5.0% concentrate basis of soybean oil together with 2.5 and 5.0 g/h/d probiotics. The additional soybean oil and probiotics were mixed evenly with concentrate prior to feeding, and offered to animals by half at 9:00 am and the other at 3:00 pm, respectively. The concentrate was supplied with 1.5% *pro rata* body weight for each goat to ensure that the dietary intakes of crude protein, growth net energy, and dry matter in accordance with the Nutrients Requirements of Goats No.15 (NRC, 1989) under the condition of maintenance plus lower activity and 50 g/d weight gain. All animals accessed to the whole plant corn silage and clean water *ad libitum*, and were cared for as described by the Ethics Committee on Animal and Human Experimentation of the UAB (Reference No. CEEAH 04/481) for the aim of respecting animal welfare and environmental protection. The experiment lasted 8 weeks, excepting 2 weeks for adjustment, 1 week for adaptation, and 1 week post-experiment for urinary and faecal samples collection.

Table 5.1 Lay-out of experimental treatments.

Groups	Animals(n)	BW(kg)	Treatments
I (Control)	6	18.30±2.0	Basal diet
II	6	18.35±1.7	Basal diet + SB 2.5 % + P 2.5 g/h/d
III	6	18.25±1.9	Basal diet +SB 2.5 % + P5.0 g/h/d
IV	6	18.20±2.3	Basal diet +SB 5.0 % + P 2.5 g/h/d
V	6	18.35±2.0	Basal diet+SB 5.0 % + P 5.0 g/h/d

Basal diet= whole plant corn silage plus concentrate; SB=soybean oil; andP= probiotics.

5.4.2 Experimental material

The soybean oil and probiotics employed in this study were prepared at the same time as that used in the first and second experiment and with the same batch number. The soybean oil was purchased from Macro supermarket (Muang district, Nakhon Ratchasima province of Thailand). The probiotics was purchased from L. P. Feeds Tech Co., Ltd (Bangkok, Thailand), containing *Lactobacillus acidophilus* 2.0 x 10^{12} cfu/g and *Saccharomyces cerevisia* 5.0x10^{11} cfu/g. The whole plant corn silage was purchased from Kornburee Cooperatives (Kornburee district, Nakhon Ratchasima province of Thailand) at the same time as the second experiment. The pelleted concentrate that was the same as the second experiment was supplied by farm of Suranaree University of Technology (Nakhon Ratchasima province of Thailand), and it was composed of cassava chip (12.0%), cassava pulp (31.5%), rice bran with germ (10.0%), defatted rice bran (10.0%), molasses (8.0%), palm kernel expeller meal (18.0%), rapeseed meal (4.0%), corn meal (4.0%), urea (1.8%), mineral (1.5%) (Containing Ca 14.5%, P 17%, NaCl 18%, Mg 10%, and carrier), and additional binder (0.2%).

5.4.3 Sampling

The daily offered and left concentrate and whole plant corn silage were weighed (the residues were removed) every morning before offering for the purpose of determination of dry matter intake. Body weight of the animals were measured weekly prior to the morning feeding with the aim of evaluating the growing performances. The whole plant corn silage and concentrate were sampled weekly and dried at 60~65 °C hot air oven for determination of dry matter (DM) composition, and followed by grounding through a 1 mm sieve and then kept in tightly covered plastic containers to make a pool respectively for further approximate analysis. During the post-experiment week for urinary and faecal samples total collection, the all-day faece and urine (10% H_2SO_4 was used as a preserving reagent, 30 mL/container) were collected and the total amount was recorded down every morning (measured faece weight and urine volume). Subsequently, 15% of the total amounts was sub-sampled to make a pool respectively for each animal, and then was kept at -20°C and in the end was dried prior to chemical composition analysis that aimed to determine digestibility and nitrogen balance. For ruminal fluid samples, they were withdrawn on the last day of the experiment through an esophageal stomach tube following 0, 3 and 6 h post-morning meal timing. The samples were strained through three layers of muslin cloth and then were followed by immediately measuring of pH with an OHS-3C pH meter. Thereafter, 1 ml of the samples were measured well and truly with a pipette into the tubes containing 9 ml 10% formalin (V:V=9:1) as a preserving reagent and then were closed tightly with screw caps that with butyl rubber lining for checking the counts of ruminal protozoa and bacteria. At the same time, 20 ml of the samples were measured and then put into small plastic bottles containing 5 ml 6 N HCl as a preserving reagent, and then the bottles were closed tightly

with screw caps that with butyl rubber lining for determination of ruminal ammonia N and volatile fatty acids. With that, all samples were kept at -20 °C until further analysis. The blood samples were collected from jugular veins into EDTA-containing vacuum tubes and were centrifuged at 2700 x r for 5 min to separate plasma from the cells within 20 minutes after sampling. Subsequently the plasma was collected, and then it was stored at −80 °C for subsequent analyses of blood urea nitrogen and fatty acid profiles.

At the end of the experiment, 3 goats were randomly chosen from each group and were euthanatized with a captive bolt stun gun followed by exsanguinating at Pukthongchai slaughter plant (Pukthongchai district, Nakhon Ratchasima province of Thailand). The carcass scores, hot carcass weights, Kidney, pelvic and heart (KPH) fat weights, empty free fat tissue alimentary tract and internal organ weights were obtained at the time of slaughter. The carcass were scored by three persons individually and recorded as the means. The criterions for evaluating the carcass were as described by USDA (1992). After chilling at 5 °C for 24 h, the carcasses were split along the vertebrae and the left side was separated between the 12th and13th ribs and used for all measurements and analyses. In each carcass, the following measurements were taken: longissimus dorsal muscle area between the 12th and 13th rib; body wall thickness between the 12th and 13th rib and 5 cm from the midline of the carcass. The longissimus dorsal muscle area was traced adopting the method that described by Y´a˜nez et al. (2006), measured using a LI-COR portable area meter (LI-3000A). Then the *semimembranosus* muscle, *Triceps humeralis* muscle, and *longissimus* dorsal muscle samples were taken from hindquarter, forequarter, and loin (from 12th rib counted backwards to 8th rib) of the left side of the carcasses. All samples were placed in plastic bags that air was expelled, and were frozen at -20 °C.

5.4.4 Chemical analysis and calculation

All the chemical analysis and calculation were done in the same way as described in the former chapter.

Shear force determination of meat samples were done with a TAXT2 texture analyzer with crosshead speed at 3.5 mm/s (Chilled for 24 h at 4 °C). Chroma of the meat samples were measured with a MINLTA electronic chroma meter.

5.4.5 Preparation of samples for gas chromatography (GC) analysis

The ruminal fluid and plasma samples were prepared in the way as the former chapter described for GC analysis.

The *semitendinosis* muscle, *Triceps humeralis* muscle, and *longissimus muscle* samples come from each animal were made a pool respectively for fatty acid profiles and CLA analysis, and the analyzing was done by GC. The preparation of meat samples for GC analysis was done by using a modified method explained by Cordain et al. (2002).

5.4.6 Analysis of fatty acids

Total VFA and molar proportion of acetic, propionic, and butyric acids in ruminal fluid and fatty acid profile of plasma samples were determined by HP6890 gas chromatography (GC) (made in USA) that fitted with a Flame Ionization Detector (FID). In addition, a J&W 122~3232 column was applied for determination of VFA, whereas a 100 m x 0.25 mm fused silica capillary column (SP2560, Supelco Inc, Bellefonte, PA, USA) for determination the plasma fatty acid profiles. The column temperature was fixed at 70 °C for 4 min, then it increased at 13 °C /min to 175 °C which lasted for 27 min. Continually it increased at 4 °C /min to 215 °C and kept for 31 min. Nitrogen was adopted as carrier gas with a 60 ml/min flow rate and the oven

temperature was 250 °C. FID and injection temperature were fixed at 280 °C, and a 1μL injection was done with a 10-μL injector.

5.4.7 Body weight measurement

Body weights of testing animals were measured every Saturday morning before morning meal. The average daily gain (ADG) was calculated as:

$$ADG\,(g/d) = \frac{Total\ \ weekly\ gain\,(g)}{Number of\ weeks \times 7}$$

5.4.8 Data analysis

The effects that compared with the control were analyzed with the General Linear Models procedure of the Statistical Analysis System Institute (SAS Inst. Inc., Cary, NC 1988) as a randomized complete block design. The effects between soybean oil and probiotics were analyzed with a 2x2 factorial arrangement using the General Linear Models procedure of the Statistical Analysis System Institute (SAS Inst. Inc., Cary, NC 1988). Variation due to blocks was extracted in the models employed for the analysis. Duncan's New Multiple Range Test and Orthogonal Contrast Analysis (Steel and Torrie, 1980) were used to compare treatment means both of the above analyses. In addition, a non-parametric Mann-Whitney test was used to compare the count means of rumen protozoa also viable bacteria within groups. Differences were considered to be significant at $P<0.05$ (*), highly significant at $P<0.01$ (**), tendencies at $0.05< P<0.1$, and 'ns' was used to represent no significant difference.

5.4.9 Experimental site

The experiment was conducted on the farm of Suranaree University of Technology; whenas chemical analyses were performed in the center of Scientific and Technological Equipments of Suranaree University of Technology.

5.4.10 Duration

The experiment was carried out during October 13, 2007–January12, 2008.

5.5 Results

5.5.1 Diet compositions

All animals received a diet composing of whole plant silage plus concentrate. The diet was adequate to meet the requirements of crude protein, growth net energy, and dry mater intakes of the goats under the condition of maintenance plus lower activity and 50 g/d weight gain (Nutrients Requirements of Goats, NUMBER 15, 1989). The concentrate and whole plant corn silage were prepared in the same time as those using in the second experiment, so the chemical composition and fatty acid profiles were the same. As to the concentrate, it contained DM 90.1%, CP 14.0%, and NDF 34.7%, whereas the silage contained DM 21.2%, CP 9.7%, and NDF 54.9% (DM basis) (Table 5.2). As shown in Table 5.3, the main fatty acids of the concentrate were comprised of 30.37% C18:2n6c, 19.79% C17:0, 15.06% C12:0, 14.47% C18:1n9c. Concededly, these fatty acids accounted for 1.21%, 0.79%, 0.60%, and 0.58% of the concentrate dry matter respectively. And yet, the main fatty acids of the whole plant corn silage were composed of (sorted by size) 38.75% C18:2n6c, 15.98 % C18:1n9c, 14.43% C16:0, and 11.87% C18:3n3, and these fatty acid mad up of 0. 81%, 0.34%, 0.30%, 0.25% of the corn silage dry matter respectively.

The fatty acids mass of the soybean oil were showed in Table 5.4. The main centesimal compositions were (sorted by size) 48.36% C18:2, 24.67% C18:1, 9.04% C16:0, 5.02% C18:3 and 3.90% C18:0.

Table 5.2 Chemical compositions of experimental diet (dry matter basis).

Items	**Composition (%)**
Concentrate	
Dry matter	90.1
Organic matter	94.0
Crude protein	14.0
Ether extracts	4.0
Acid insoluble ash	3.1
Acid detergent fiber	26.5
Neutral detergent fiber	34.7
Corn silage	
Dry matter	21.2
Organic matter	89.3
Crude protein	9.7
Ether extract	2.1
Acid insoluble ash	5.1
Acid detergent fiber	42.4
Neutral detergent fiber	54.9

Table 5.3 Fatty acid profiles of concentrate and whole plant core silage (DM basis).

Items	% DM	% Total fatty acid
Concentrate		
C12:0	0.60	15.06
C14:0	0.24	5.92
C16:0	0.25	6.28
C17:0	0.79	19.79
C18:0	0.09	2.31
C18:1n9c	0.58	14.47
C18:2n6c	1.21	30.37
C18:3n3	0.07	1.82
Others	0.12	3.04
Corn silage		
C14:0	0.04	1.77
C16:0	0.30	14.43
C16:1	0.01	0.71
C17:0	0.04	1.67
C18:0	0.07	3.54
C18:1n9c	0.34	15.98
C18:2n6c	0.81	38.75
C18:3n3	0.25	11.87
Others	0.22	10.56

Table 5.4 Fatty acid profiles of the soybean oil that used in this experiment.

Fatty acids	Soybean oil (%total fatty acids)
C14:0	0.37
C15:0	0.18
C16:0	9.04
C17:0	0.27
C18:0	3.90
C18:1	24.67
C18:2	48.36
C18:3	5.02
C20:0	1.79
C20:2	0.38
C22:0	1.77
C20:3n6	2.04
C23:0	0.23
C22:2	0.21
C20:5n3	1.33
C24:1	0.12

5.5.2 Feed intake and growth performances

No differences existed in whole plant corn silage and concentrate total daily average as well as the percentage of body weight dry matter intakes between the treatments. On the other side, as shown in Table 5.5, the supplementation of soybean oil tended to decrease the whole plant corn silage DMI (P=0.09), $W^{0.75}$ DMI (P=0.06),

and percentage on body weight DMI (P=0.06) contrasted with the additions of probiotics and combination of soybean oil plus probiotics. 2.5% soybean oil plus probiotics (2.5 and 5.0 g/h/d) treatments tended to increase DMI in contrast to the control and 5.0% soybean oil plus probiotics (2.5 and 5.0 g/h/d) treatments. Thereof, the increasing levels of supplemental soybean oil showed a faint decrease on DMI, but it was not affected with the increasing levels of supplemental probiotics.

In comparison with the control, ADG was significantly increased with supplementations of soybean oil and probiotics (P=0.05) except for 2.5% soybean oil plus 2.5 g/h/d probiotics treatment (P=0.52). Comparing within the soybean oil and probiotics supplementations, the ADG tended to be increased due to additions of soybean oil (P=0.09), probiotics (P=0.07) also soybean oil plus probiotics (P=0.07).

In reference to feed conversion, it was illustrated with significant depression of DMI: ADG ratios due to supplementations of probiotics (P=0.05) and soybean oil plus probiotics (P=0.04). In addition, the DMI: ADG ratio was obviously decreased owing to presence of soybean oil (P=0.08).

Table 5.5 The effect of soybean oil and probiotics on DMI, ADG, and feed conversion of growing goats (% concentrate).

	Control	SB (%)	2.5		5.0		SEM	P-value		
		P (g/d)	2.5	5.0	2.5	5.0		SB	P	SBxP
SDMI(g/d)	249.1	272.9	267.8	245.0	224.5	18.72	0.09	0.88	0.91	
CDMI(g/d)	235.0	235.0	233.1	233.7	230.9	14.91	0.99	0.99	0.93	
Total (g/d)	484.1	507.9	500.9	478.7	455.4	19.50	0.25	0.67	0.84	
$W^{0.75}$(g/kg)	51.3	55.0	53.3	51.0	49.0	1.13	0.06	0.45	0.51	
LWB (%)	2.4	2.6	2.5	2.4	2.3	0.11	0.06	0.38	0.71	
ADG (g/d)	44.4^{c}	49.5^{c}	63.1^{a}	57.4^{b}	56.7^{b}	3.32	0.09	0.07	0.07	
DMI:ADG	10.0^{a}	8.7^{b}	8.4^{b}	8.02^{b}	8.6^{b}	0.41	0.08	0.05	0.04	

SDMI=whole plant corn silage dry mater intake; CDMI=concentrate dry mater intake; LWB=percentage on live body weight DMI; SB=soybean oil; P=probiotics; SEM=standard error of the mean of the SB and P treatments (except for the control); P-value is for the SB and P treatments (except for the control); Means with different superscript letters in the same row differ significantly ($P<0.05$).

5.5.3 Dietary digestibility

As shown in Table 5.6, the digestibility of DM and OM were significantly increased by probiotics (P=0.02) and soybean oil plus probiotics treatments (P=0.05). In contrasted to the control, 2.5% soybean oil plus 5.0 g/h/d probiotics increased DM and OM digestibility significantly (P=0.04). The CP and NDF digestibility were not affected by supplementations of soybean oil and probiotics in comparison with the control. EE digestibility of 5% soybean oil plus probiotics treatments (2.5 and 5.0

g/h/d) was significantly lower than those of 2.5% soybean oil plus probiotics treatments (2.5 and 5.0 g/h/d) (P<0.05). Comparing with the control, the soybean oil treatment tended to reduce EE digestibility (P=0.08), and there was no synergistic impact on EE digestibility for probiotics and soybean oil.

In conclusion, the dietary digestion was a slight greater for goats fed with 2.5% soybean oil plus probiotics (2.5 and 5.0 g/h/d) than goats fed with 2.5% soybean oil plus probiotics (2.5 and 5.0 g/h/d), there were significant interactions on DM (P=0.05) and OM (P=0.05) digestibility for soybean oil and probiotics.

Table 5.6 The effect of soybean oil and probiotics on dietary digestibility of growing goats fed whole plant corn silage (%).

	Control	SB (%)	2.5		5		SEM	P-value		
		P (g/d)	2.5	5	2.5	5		SB	P	SBxP
DDM	64.9 [b]		67.2[ab]	71.8[a]	65.5[b]	66.8[ab]	3.16	0.53	0.02	0.05
DOM	67.9[b]		71.4[a]	74.5[a]	70.1[ab]	70.0[ab]	3.18	0.52	0.02	0.05
DCP	67.3		69.0	73.8	68.7	66.8	3.64	0.33	0.69	0.37
DNDF	48.2		50.2	51.4	53.7	52.5	6.51	0.78	0.34	0.27
DEE	78.5[a]		76.8[a]	77.6[a]	69.2[b]	68.1[b]	4.82	0.08	0.50	0.69

SB=soybean oil; P=probiotics; DDM=digestibility of DM; DOM=digestibility of OM; DCP=digestibility of CP; DNDF=digestibility of NDF; DEE=digestibility; SEM=standard error of the mean of the SB and P treatments (except for the control); P value is for the SB and P treatments (except for the control); Means with different superscript letters in the same row differ significantly (P<0.05).

5.5.4 Ruminal Fluid pH, Ammonia N, PUN, and VFA

The pH ranged form 6.21 to 6.53, they were little higher for 5.0% soybean oil plus probiotics (2.5 and 5.0 g/h/d) treatments than 2.5% soybean oil plus probiotics treatments (P>0.05) (Table 5.7). As shown in Figure 5.1, the lowest pH appeared 3 h post-morning feeding and existed significant difference between treatments. Howbeit, before morning meal and post-morning meal 6 h, the pH values were similar and no much difference between the treatments.

The linear equation and r^2 of plasma urea nitrogen standard were r^2=0.9837 and y=0.0274x+0.0023. Where: y is the amount of PUN, and x is the concentration of PUN standard. The plasma urea nitrogen and NH_3-N were slightly greater for goats supplemented with 2.5% soybean oil plus probiotics (2.5 and 5.0 g/h/d) than goats supplemented with 5.0% soybean oil plus probiotics (2.5 and 5.0 g/h/d). At the same time, no significant synergistic effects on both of NH_3-N (P=0.57) and PUN (P=0.84) for soybean oil and probiotics (Table 5.7). Furthermore, as shown in Figure 5.2 and 5.4, the effects of supplementations of soybean oil and probiotics on the NH_3-N and PUN were related to the sampling time, before morning feeding the NH_3-N and PUN were similar between treatments, nevertheless, some significance differences existed 3 and 6 h post-morning meal (P<0.05).

The total VFA was faint greater for goats supplemented with 5.0% soybean oil plus probiotics (2.5 and 5.0 g/h/d) than 2.5% soybean oil plus probiotics (2.5 and 5.0 g/h/d) (P>0.05), and there were a significant synergistic effect on the total VFA for soybean oil and probiotics (P=0.05). In addition, the sampling time affected the total VFA evidently, as shown in Figure 5.4, the highest total VFA value appeared at 3h post-morning feeding and there were significant difference between the treatments

(P<0.05), however, total VFA of before and 6h post-morning feeding were similar between treatments and presented no differences.

On the other hand, the 5% soybean oil plus probiotics displayed slight increase in propoinic proportion, in contrast, 2.5% soybean oil plus probiotics showed slight increase in acetic proportion. There were not any significant interaction on the main VFA mixture molar compositions (C_2: P=0.63; C_3: P= 0.79 and C_4: P=0.55) and C_2 to C_3 ratio (P=0.99) for additions of soybean oil and probiotics (Table 5.7).

Table 5.7 The effects of soybean oil and probiotics on the average pH, ammonia nitrogen (NH_3-N, mg/dL), plasma nitrogen (PUN, mg/dL), and VFA (mM/l) of growing goats fed with whole plant corn silage.

	Control	SB (%)	2.5		5.0		SEM	P value		
		P (g/d)	2.5	5.0	2.5	5.0		SB	P	SBxP
pH	6.53		6.34	6.21	6.52	6.50	0.11	0.05	0.46	0.06
NH_3-N	11.2		11.6	11.7	10.9	11.3	0.64	0.34	0.64	0.57
PUN	19.3		19.7	17.0	17.9	20.6	1.75	0.55	0.98	0.84
TVFA	72.0		68.5	66.9	69.3	70.3	4.12	0.40	0.18	0.05
The main VFA mixture centesimal proportion (% TVFA)										
Acetate	67.6		65.1	63.0	64.1	62.7	1.51	0.47	0.44	0.63
Propionate	20.7		21.0	22.3	23.5	22.7	1.06	0.49	0.68	0.79
Butyrate	4.8		4.7	5.7	5.4	5.6	0.75	0.65	0.34	0.55
C2:C3	3.2		3.1	2.9	2.7	2.8	0.70	0.60	0.47	0.99

SB=soybean oil; P=probiotics; SEM=standard error of the mean of the SB and P treatments (except for the control); P value is for the SB and P treatments (except for the control); Means with different superscript letters in the same row differ significantly (P<0.05)

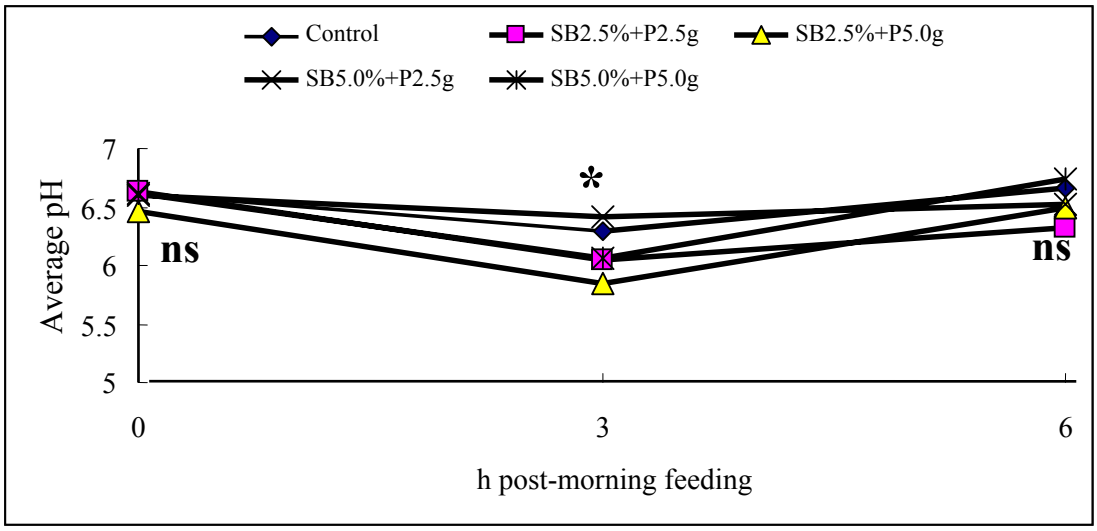

Figure 5.1 Ruminal pH of growing goats supplemented soybean oil (SB) and probiotics (P).

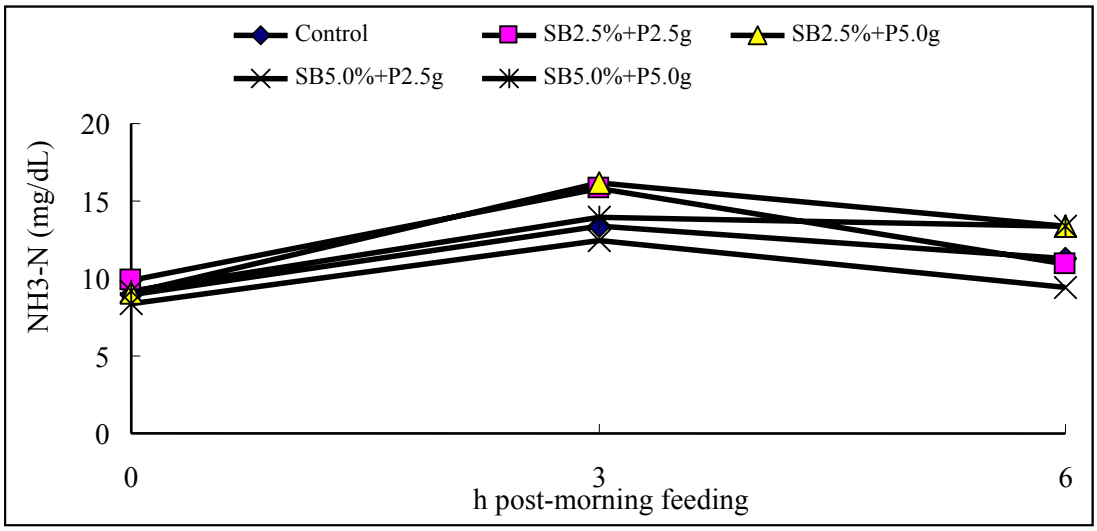

Figure 5.2 Ruminal NH_3-N of growing goats supplemented soybean oil (SB) and probiotics (P).

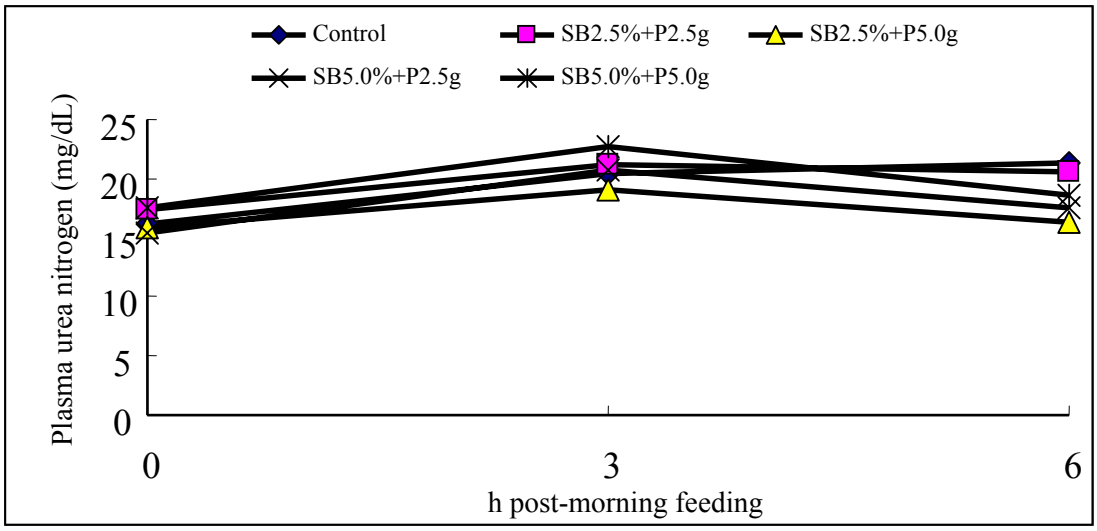

Figure 5.3 Plasma urea nitrogen of growing goats supplemented soybean oil (SB) and probiotics (P).

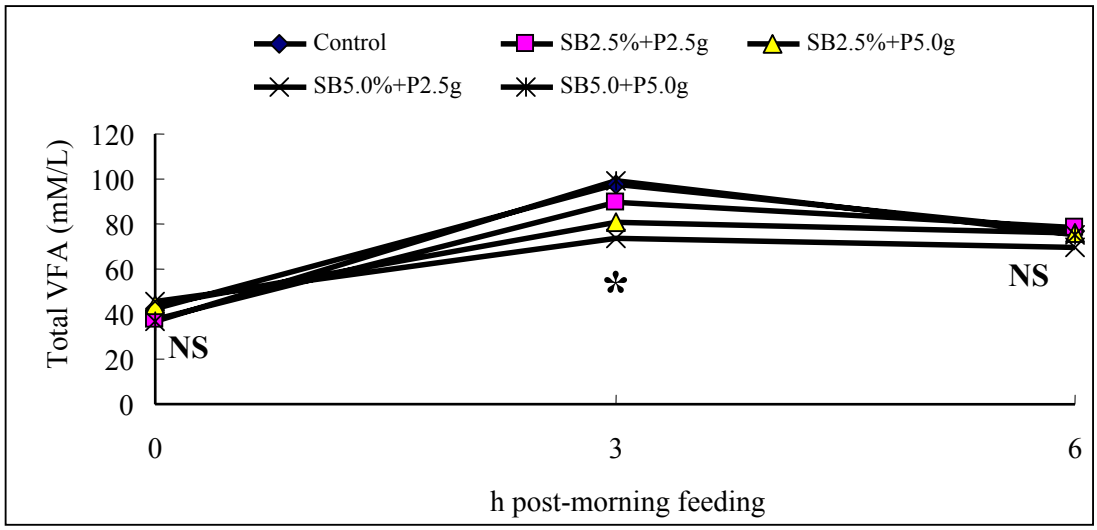

Figure 5.4 Total ruminal VFA of growing goats supplemented soybean oil (SB) and probiotics (P).

5.5.4 Ruminal microbe population

The number of protozoa ranged from 1.04 to 1.95 $x10^4$ per ml rumen fluid (Table 5.8). The protozoal counts were significantly greater for 5.0 g/h/d probiotics plus soybean oil treatments than for 2.5 g/h/d probiotics plus soybean oil treatments and the control ($P<0.05$) through the 3 sampling times. In addition, as revealed in Figure 5.5, the significant difference of protozoal counts presented before, 3h, and 6 h post morning feeding ($P<0.05$). However, as Table 5.8 presented, there were not any significant interaction in protozoal number between soybean oil and probiotics.

The number of total viable bacteria ranged from 1.35 to 2.57 x 10^{10} per ml rumen fluid. And in similar case to the protozoa, the supplementations of 5 g/h/d probiotics plus 2.5% soybean oil obviously greater than 2.5 g/h/d probiotics plus 5.0% soybean oil treatment ($P=0.04$). The supplementations of 5 g/h/d probiotics plus 5.0% soybean oil also greater than 2.5 g/h/d probiotics plus 5.0% soybean oil treatment, but no significance ($P=0.14$). In addition, the significant differences of bacterial counts presented through all the sampling time (0, 3, and 6 h) and no significant synergistic effects on the number of bacteria resulted from additions of soybean oil and probiotics (Figure 5.6).

Table 5.8 The effect of soybean oil and probiotics on rumen microbe population of growing goats fed whole plant corn silage.

	Control	SB (%)	2.5		5.0		SEM	P-value		
		P (g/d)	2.5	5.0	2.5	5.0		SB	P	SBxP
Protozoal population ($x10^4$)										
0h	1.25^{c}		1.53^{b}	1.83^{a}	1.46^{b}	1.89^{a}	0.29	0.53	0.09	0.41
3h	1.04^{c}		1.11^{c}	1.63^{a}	1.21^{bc}	1.48^{ab}	0.25	0.91	0.08	0.55
6h	1.43^{b}		1.87^{a}	1.95^{a}	1.71^{a}	1.91^{a}	0.11	0.80	0.70	0.89
Bacterial population ($x10^{10}$)										
0h	1.35^{b}		1.49^{b}	1.57^{ab}	1.73^{a}	1.73^{a}	0.09	0.02	0.57	0.57
3h	2.02^{c}		2.25^{bc}	2.57^{a}	2.37^{ab}	2.62^{a}	0.15	0.46	0.74	0.03
6h	1.61^{c}		1.68^{c}	2.39^{a}	2.05^{b}	2.30^{a}	0.11	0.13	0.03	0.001

SB=soybean oil; P=probiotics; SEM=standard error of the mean of the SB and P treatments (except for the control); P-value is for the SB and P treatments(except for the control); Means with different superscript letters in the same row differ significantly ($P<0.05$).

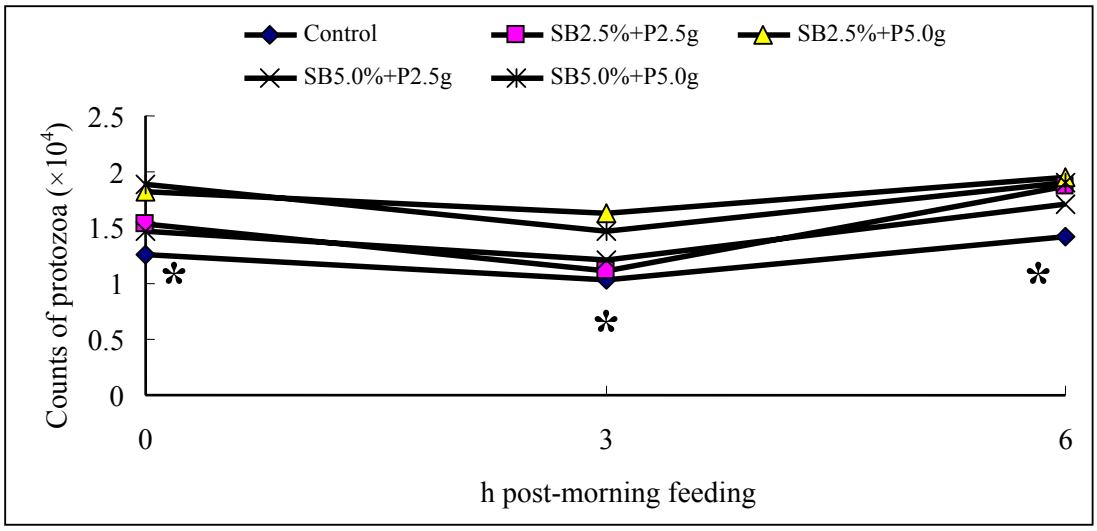

Figure 5.5 Counts of ruminal protozoa for growing goats supplemented with soybean oil (SB) and probiotics (P).

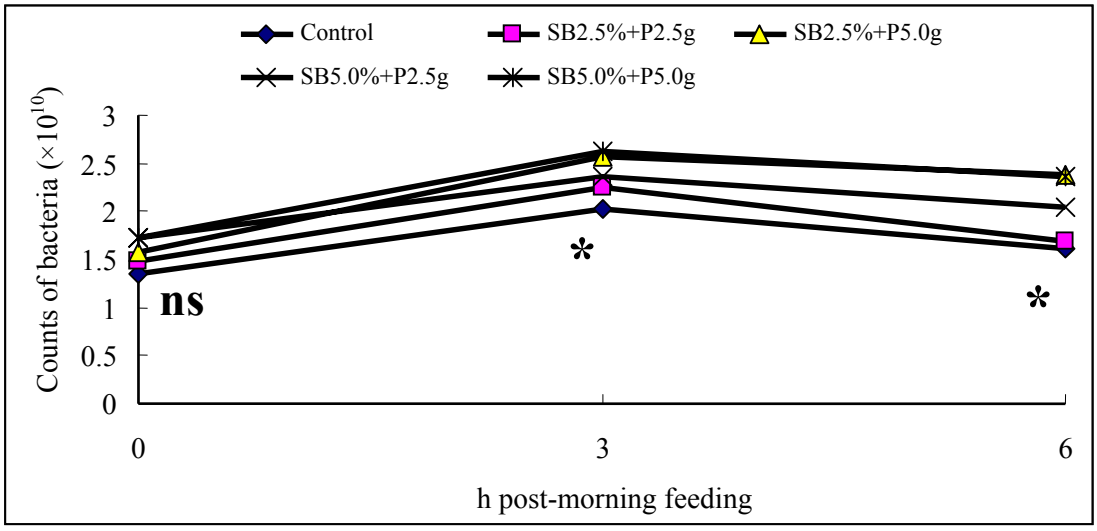

Figure 5.6 Counts of ruminal bacteria for growing goats supplemented soybean oil (SB) and probiotics (P).

5.5.5 Nitrogen balance

The dietary nitrogen intake for goats supplemented with 2.5% soybean oil plus probiotics were slightly higher than those supplemented with 5.0% soybean oil plus probiotics ($P>0.05$) (Table 5.9). The nitrogen excretion were not significantly affected with supplementations of soybean oil and probiotics. However, the nitrogen absorption was substantially greater for the goats supplemented with 2.5% soybean oil plus probiotics than those supplemented with 5.0% soybean oil plus probiotics ($P<0.05$), the nitrogen retention also in the similar case but no significant difference ($P>0.05$). Moreover, nitrogen retention in terms of percentage for the soybean oil and probiotics treatments were significantly greater than the control ($P<0.05$), and the average daily nitrogen retention (g/d) for goats supplemented with 2.5% soybean oil plus probiotics remarkably higher the control.

To sum up, the supplementation of soybean oil and probiotics had not synergistic effects on dietary nitrogen intake ($P=0.82$), on faecal nitrogen excretion ($P=0.36$), and on urinary nitrogen excretion ($P=0.19$). Nevertheless, it tended to display interaction for nitrogen absorption ($P=0.07$), for average daily nitrogen retention (g/d) ($P=0.08$), and for percentage of nitrogen retention ($P=0.1$) (Table 5. 9).

Table 5.9 The effects of soybean oil and probiotics on nitrogen balance of growing goats (% concentrate)

	Control	SB (%)	2.5		5.0		SEM	P-value		
		P (g/d)	2.5	5.0	2.5	5.0		SB	P	SBxP
N intake(g/d)	9.2	10.2	10.1		9.3	9.1	0.58	0.98	0.06	0.82
N excretion (g/d)										
Faece	4.1	4.3	3.6		3.8	3.7	0.36	0.32	0.57	0.36
Urine	2.4	2.2	2.8		2.4	2.2	0.22	0.84	0.84	0.19
Total	6.5	6.5	6.4		6.2	5.9	0.12	0.33	0.69	0.94
N A (g/d)	5.1^{b}	5.9^{ab}	6.5^{a}		5.5^{b}	5.4^{b}	0.06	0.58	0.04	0.07
N R (g/d)	2.7^{b}	3.7^{a}	3.7^{a}		3.1^{ab}	3.2^{ab}	0.07	0.42	0.05	0.08
N R (%)	29.3^{b}	36.3^{a}	36.6^{a}		33.3^{a}	35.2^{a}	1.73	0.37	0.57	0.90

NR= N retention; NA=N absorption; SB=soybean oil; P=probiotics; SEM=standard error of the mean of the SB and P treatments (except for the control); P-value is for the SB and P treatments (except for the control); Means with different superscript letters in the same row differ significantly ($P<0.05$).

5.5.6 Fattyacid profiles and conjugated linoleic acid content in plasma

Plasma C8:0 and C10:0 for goats supplemented with 2.5% soybean oil plus probiotics were higher than those supplemented with 5.0% soybean oil plus probiotics (C8:0: $P>0.05$; C10:0: $P<0.05$). Moreover, the soybean oil and probiotics treatments significantly decreased plasma C8:0 in comparison with the control, and there were significant interaction between soybean oil and probiotics on the plasma C8:0 concentration ($P=0.04$). Plasma C10:0 for goats supplemented with 2.5% soybean oil plus probiotics were significantly higher than the control ($P<0.05$), and there were not

any significant interaction between soybean oil and probiotics on the plasma C10:0 concentration (P=0.31) (Table 5.10).

For the plasma C12-C17 long chain fatty acids, C12:0 and C14:0 were higher for the goats supplemented with 2.5% soybean oil plus probiotics than those supplemented with 5.0% soybean oil plus probiotics (C12:0: P<0.05; C14:0: P>0.05). In addition, a significant synergistic effect on C12:0 existed between soybean oil and probiotics (P=0.03). The C15:0 (P>0.05), C16:0 (P>0.05), C16:1 (P>0.05), and C17:0 (P>0.05) were greater for the goats supplemented with 5.0 g/h/d probiotics plus soybean oil than those supplemented with 2.5 g/h/d probiotics plus soybean oil. Furthermore, the C15:0 and C16:0 for goats supplemented with soybean oil and probiotics were significantly decreased contrasting to the control (P<0.05), and the obvious interactions between soybean oil and probiotics on the plasma C15:0 (P=0.06) and C17:0 (P=0.05) were found (Table 5.10).

The plasma C18:0 for goats supplemented with soybean oil and probiotics was numerically greater than the control (P>0.05). It was faintly higher for the goats supplemented with 5.0% soybean oil plus probiotics than those supplemented with 2.5% soybean oil plus probiotics(P>0.05), and it was slightly higher or the goats supplemented with 5.0 g/h/d probiotics plus soybean oil than those supplemented with 2.5 g/h/d probiotics plus soybean oil. C18:1n9t, C18:1n9c, C18:2n6c, and C18:3n3 for the soybean oil and probiotics treatments were significantly greater that the control (P>0.05). Thereof, the 5.0% soybean oil plus probiotics treatments greater than those supplemented with 2.5% soybean oil plus probiotics, and the 5.0 g/h/d probiotics plus soybean oil treatments greater than those supplemented with 2.5 g/h/d probiotics plus soybean oil (C18:1n9t: P=0.05; C18:1n9c: P=0.03; C18:2n6c: P=0.37; C18:3n3:

P=0.05). There were remarkable interaction between soybean oil and probiotics on C18:1n9c (P=0.09) and C18:3n3 (P=0.05) (Table 5.10).

The plasma C18:c9,t11 and C18:t10,c12 CLA isomers significantly increased for the soybean oil and probiotics treatments in contrast to the control (P<0.01). The increments of C18:c9,t11 CLA isomer were 134.0% for 2.5% soybean oil plus 2.5 g/h/d probiotics treatment (1.24 vs.0.53), 145.3% for 2.5% soybean oil plus 5.0 g/h/d probiotics treatment (1.30 vs.0.53), 152.8% for 5.0% soybean oil plus 2.5 g/h/d probiotics treatment (1.34 vs.0.53) and 156.6% for 5.0% soybean oil plus 5.0 g/h/d probiotics treatment (1.36 vs.0.53) respectively. The C18:t10,c12 CLA isomer was undetectable in the control and 2.5% soybean plus 2.5 g/h/d probiotics treatment, they were 0.09, 0.11, and 0.21% of total plasma fatty acids for 2.5% soybean plus 5.0 g/h/d probiotics, 5.0% soybean plus 2.5 g/h/d probiotics, and 5.0% soybean plus 5.0 g/h/d probiotics treatments respectively. Thereinto, the 5.0% soybean oil plus probiotics and 5.0 g/h/d probiotics plus soybean treatments were greater than others (P>0.05), and there were significant interaction between soybean oil and probiotics on the plasma C18:c9,t11 (P=0.05) and C18:t10,c12 (P=0.01) CLA isomers (Table 5.10).

In contrast to the control, the supplementations of soybean oil and probiotics significantly decreased the plasma very long-chain fatty acids (chain length large than C18) (P<0.05) except for the C20:4n6. In addition, there were evident synergistic impacts between soybean oil and probiotics on C20:3n6 (P=0.05), C20:4n6 (P=0.09), and C20:5n3 (P=0.04) (Table 5.10).

To sum up, the plasma total CLA centesimal proportion ranged form 0.53-1.50%. It increased 134.0 (1.24 vs. 0.53), 173.6 (1.45 vs. 0.53), 173.6 (1.45 vs. 0.53), and 183.0% (1.50 vs. 0.53) for 2.5% soybean oil plus 2.5 g/h/d probiotics, 2.5%

soybean oil plus 5.0 g/h/d probiotics, 5.0% soybean oil plus 2.5 g/h/d probiotics, and 5.0% soybean oil plus 5.0 g/h/d probiotics treatment respectively. Meanwhile, there were significant synergistic impact between soybean oil and probiotics on the total plasma CLA centesimal composition (P=0.04). The ratios of PUFA/SFA and n−6/n−3 ranged from 0.57 to 0.69 and 5.87 to 8.64, thereof, the control was lower than the soybean oil and probiotics treatments (PUFA/SFA: P>0.05; n−6/n−3: P<0.05). There were not any significant interactions between soybean oil and probiotics in total saturated fatty acids (TSFA) (P=0.46), in total mono- unsaturated fatty acids (Tmo-USFA) (P=0.19), in total poly-unsaturated fatty acids (TPUSFA) (P=0.56), in total n6 fatty acids (Tn6) (P=0.67), in total n3 fatty acids (Tn3) (P=0.45), and in desirable fatty acids (DFA=C18:0+ TUSFA) (P=0.75).

On the other hand, the calculations of centesimal composition of plasma fatty acids into fatty acid (μg) contained in 1 ml plasma showed that the statistical analyses were similar to those of no calculations (Table 5.11). On the whole， the total plasma CLA isomers were 4.8 μg/ml for the control, and those of soybean oil and probiotics treatments ranged from 10.4 to 14.3 μg/ml. The total plasma saturated fatty acids was 435.9 μg/ml for the control, and those of soybean oil and probiotics treatments ranged from 382.4 to 470.1 μg/ml. The desirable fatty acids was 655.0 μg/ml for the control, and those of soybean oil and probiotics treatments ranged from 631.1 to 708.4 μg/ml.

Table 5.10 Plasma fatty acids centesimal composition profiles of growing goats supplemented soybean oil and probiotics under condition of feeding whole plant corn silage.

%TFA	Control	SB (%)	2.5		5.0		SEM	P-value		
		P (g/d)	2.5	5.0	2.5	5.0		SB	P	SBxP
C8:0	0.41[a]		0.21[b]	0.20[b]	0.17 [b]	0.20[b]	0.05	0.01	0.01	0.04
C10:0	0.16[b]		0.29[a]	0.26[a]	0.22[ab]	0.20[b]	0.02	0.01	0.42	0.31
C12:0	0.66[a]		0.59[a]	0.55 [a]	0.31[b]	0.24[b]	0.09	0.05	0.47	0.03
C14:0	3.94		3.72	3.75	3.85	3.61	0.30	0.72	0.64	0.67
C15:0	0.19[a]		0.05[c]	0.07[bc]	0.07[bc]	0.09[b]	0.02	0.05	0.17	0.06
C16:0	17.32[a]		13.78[b]	15.28[b]	14.17[b]	13.76[b]	0.98	0.46	0.82	0.55
C16:1	0.00[c]		0.22[b]	0.26[b]	0.21[b]	0.37[a]	0.14	0.43	0.05	0.19
C17:0	3.36		3.59	3.63	3.96	4.39	0.42	0.05	0.08	0.05
C18:0	23.11		24.36	24.47	27.14	24.84	0.78	0.64	0.29	0.15
C18:1n9c	16.34[b]		18.9[ab]	19.11[a]	19.58[a]	19.61[a]	1.99	0.04	0.97	0.55
C18:1n9t	0.42[c]		0.57 [b]	0.62[b]	0.81[a]	0.98[a]	0.07	0.03	0.05	0.09
C18:2n6c	18.87		20.65	19.16	19.25	20.69	1.76	0.17	0.76	0.38
C18:3n3	0.31[b]		0.43[a]	0.47[b]	0.53[a]	0.61[a]	0.02	0.02	0.25	0.05
C18:c9,t11	0.53[b]		1.24[a]	1.30[a]	1.34[a]	1.36 [a]	0.16	0.01	0.05	0.05
C18:t10,c12	0.00[c]		0.00[c]	0.09[b]	0.11[b]	0.21[a]	0.07	0.01	0.05	0.01
C20:3n6	0.36[a]		0.31[a]	0.13 [b]	0.00[c]	0.00[c]	0.11	0.01	0.03	0.05
C20:4n6	4.55		6.65	5.90	4.22	4.37	0.29	0.04	0.81	0.09
C20:5n3	0.67[a]		0.29[b]	0.31[b]	0.00[c]	0.00[c]	0.12	0.01	0.01	0.04
C22:6n3	3.17 [a]		2.22[b]	2.30[b]	2.85[a]	3.12[a]	0.23	0.04	0.57	0.62

Table 5.10 (Continued)

%TFA	Control	SB (%)	2.5		5.0		SEM	P-value		
		P (g/d)	2.5	5.0	2.5	5.0		SB	P	SBxP
TCLA	0.53[b]		1.24[a]	1.45[a]	1.45[a]	1.50[a]	0.05	0.02	0.06	0.04
TSFA	49.15		46.42	48.23	49.89	48.28	1.46	0.05	0.73	0.46
TMUSFA	20.76		19.69	20.97	20.60	18.96	1.03	0.64	0.53	0.19
TPUSFA	28.45		31.99	29.72	28.29	31.10	2.11	0.46	0.37	0.56
PUFA/SFA	0.58		0.69	0.62	0.57	0.64	0.04	0.55	0.84	0.38
Tn6	24.31		28.86	26.64	22.92	26.37	2.01	0.52	0.32	0.67
Tn3	4.14		4.13	3.08	3.18	4.12	0.37	0.26	0.17	0.45
n−6/n−3	5.87[b]		6.99[ab]	8.64[a]	7.22[a]	6.40[b]	0.54	0.24	0.34	0.55
DFA	72.32		76.04	75.16	76.03	74.90	3.46	0.86	0.92	0.75

SB=soybean oil; P=probiotics; TSFA=total saturated fatty acid; TMUSFA=total mono-unsaturated fatty acid; TPUSFA=total poly-unsaturated fatty acid; DFA=desirable fatty acid; Tn6=total n6 fatty acid; Tn3=total n3 fatty acid; SEM=standard error of the mean of the SB and P treatments (except for the control); P value is for the SB and P treatments (except for the control); Means with different superscript letters in the same row differ significantly ($P<0.05$).

Table 5.11 Plasma fatty acid contents in one ml plasma of growing goats supplemented soybean oil and probiotics under condition of feeding whole plant corn silage.

µg/ml plasma	Control	SB (%) 2.5		5.0		SEM	P value		
		P (g/d) 2.5	5.0	2.5	5.0		SB	P	SBxP
C8:0	3.5[a]	2.4[b]	2.3[b]	2.0[b]	2.0[b]	0.17	0.01	0.04	0.10
C10:0	1.5[b]	3.2[a]	3.1[a]	3.1[a]	2.0[b]	0.24	0.02	0.50	0.52
C12:0	6.0[a]	5.0[a]	5.5[a]	2.7[b]	2.3[b]	0.26	0.08	0.82	0.07
C14:0	35.7	34.8	32.0	34.0	34.4	1.88	0.89	0.78	0.93
C15:0	1.7[a]	0.6[bc]	0.8[b]	0.5[c]	0.5[c]	0.14	0.65	0.70	0.63
C16:0	148.2[a]	115.6[b]	130.4[a]	125.1[b]	140.8[a]	2.42	0.58	0.71	0.61
C16:1	0.0[b]	3.5[a]	3.1[a]	2.8[a]	3.5[a]	0.38	0.31	0.03	0.10
C17:0	30.4	29.2	31.0	34.9	39.4	1.87	0.03	0.05	0.08
C18:0	187.6[b]	199.3[b]	208.8[b]	236.8[a]	248.4[a]	9.90	0.14	0.87	0.09
C18:1n9c	150.2[b]	158.6[ab]	168.0[a]	181.7[a]	171.5[a]	3.21	0.98	0.98	0.55
C18:1n9t	3.8[c]	4.8[c]	6.0[b]	7.2[ab]	9.3[a]	0.76	0.04	0.05	0.12
C18:2n6c	170.9	173.2	173.5	182.3	195.4	3.62	0.26	0.89	0.29
C18:3n3	2.8[c]	5.3[b]	4.0[b]	5.9 [b]	9.5[a]	1.07	0.01	0.15	0.04
C18:c9,t11	4.8[b]	10.4[a]	11.6[a]	11.8[a]	12.4[a]	0.41	0.01	0.08	0.17
C18:t10,c12	0.0[c]	0.0[c]	0.8[b]	1.0[b]	2.0[a]	0.16	0.04	012	0.09
C20:3n6	3.3[a]	2.6 [a]	1.1[b]	0.0[c]	0.0[c]	0.22	0.01	0.87	0.05
C20:4n6	41.2[b]	55.8[a]	50.4[a]	37.3[b]	41.7[b]	2.7	0.05	0.90	0.07
C20:5n3	6.0[a]	2.4[b]	2.7[b]	0.0[c]	0.0[c]	0.99	0.00	0.01	0.04
C22:6n3	28.7[a]	27.0[a]	19.7[b]	25.2[a]	27.8[a]	1.53	0.01	0.99	0.49

Table 5.11 (Continued)

%TFA	Control	SB (%)	2.5		5.0		SEM	P-value		
		P (g/d)	2.5	5.0	2.5	5.0		SB	P	SBxP
TCLA	4.8[c]		10.4[b]	12.3[ab]	12.8[ab]	14.3[a]	1.12	0.03	0.05	0.05
TSFA	435.9		382.4	413.9	450.7	470.1	7.98	0.05	0.85	0.38
TMUSFA	188.0		166.8	180.7	191.7	180.8	4.04	0.84	0.65	0.21
TPUSFA	257.6		276.7	253.7	232.4	290.8	10.02	0.60	0.32	0.56
PUFA/SFA	0.58		0.69	0.62	0.57	0.64	0.07	0.27	0.14	0.27
Tn6	220.1		242.1	228.3	202.3	251.4	3.92	0.59	0.20	0.58
Tn3	37.5[a]		34.6[a]	26.3[b]	28.0[b]	39.3[a]	1.77	0.20	0.11	0.34
n−6/n−3	5.9[b]		7.0[ab]	8.7[a]	7.2[a]	6.4[b]	0.51	0.37	0.21	0.52
DFA	655.0		631.1	641.2	669.4	708.4	15.84	0.34	0.55	0.41

SB=soybean oil; P=probiotics; TSFA=total saturated fatty acid; TMUSFA=total mono-unsaturated fatty acid; TPUSFA=total poly-unsaturated fatty acid; DFA=desirable fatty acid; Tn6=total n6 fatty acid; Tn3=total n3 fatty acid; SEM=standard error of the mean of the SB and P treatments (except for the control); P value is for the SB and P treatments (except for the control); Means with different superscript letters in the same row differ significantly ($P<0.05$).

5.5.7 Slaughter performances

As presented in Table 5.12, the living weight, carcass weight, and the weights of free fat alimentary tract and organs were similar between the control and treatments. However, the weights of kidney, pelvic, and heart fat (KPHfat) and percentage of KPHfat on carcass of the soybean oil and probiotics treatments were significantly greater than the control ($P<0.05$). Thereof, the leg scores, body wall

thickness, and LD muscle area for the goats supplemented with soybean oil and probiotics were mildly higher than the control. In addition, the leg scores ($P>0.05$), body wall thickness ($P>0.05$), and LD muscle area ($P>0.05$), dressing rate ($P>0.05$), percentage of KPH fat on carcass ($P<0.05$) as well as the weights of KPH fat ($P<0.05$) were greater for goats supplemented with 5.0% soybean oil plus probiotics (2.5 and 5.0 g/h/d) than those of supplemented with 2.5% soybean oil plus probiotics (2.5 and 5.0 g/h/d). And they were greater for goats supplemented with 5.0 g/h/d probiotics plus soybean oil (2.5 and 5.0%) than those of supplemented with 2.5 g/h/d probiotics plus soybean oil (2.5 and 5.0%). There were no significant interactions between soybean oil and probiotics in the slaughter performances of growing goats fed with whole plant corn silage.

Table 5.12 Slaughter performances of growing goats supplemented soybean oil and probiotics under condition of feeding whole plant corn silage.

	Control	SB (%) 2.5		5.0		SEM	P-value		
		P (g/d) 2.5	5.0	2.5	5		SB	P	SBxP
LW(kg)	20.0	20.8	18.0	20.7	21.0	1.04	0.46	0.51	0.41
CW(kg)	9.1	9.0	8.0	9.7	9.8	0.58	0.25	0.66	0.63
AW(kg)	1.5	1.5	1.5	1.4	1.3	0.14	0.10	0.22	0.22
KPHfat(g)	123.3^{b}	133.3^{b}	133.3^{b}	203.3^{a}	194.0^{a}	17.44	0.06	0.88	0.88
spleen(g)	18.3	26.7	20.0	21.7	25.0	2.59	1.00	0.72	0.30
liver(g)	238.3	261.7	215.0	241.7	261.7	10.41	0.48	0.48	0.10
heart(g)	73.3	75.0	66.7	85.0	86.7	4.38	0.08	0.67	0.53
lung(g)	118.3	150.0	115	133.3	128.3	9.27	0.92	0.25	0.38
Kidney(g)	42.7	56.7	45.0	51.7	50.0	2.15	1.00	0.11	0.22
Leg scores	12.2	11.5	12.3	11.8	12.7	0.46	0.68	0.32	1.00
BWT(cm)	0.73	0.65	0.73	0.80	1.10	0.09	0.16	0.29	0.54
LD area(cm^2)	11.3	13.3	12.1	12.1	11.3	0.81	0.72	0.30	0.65
Dressing (%)	45.2	43.0	44.1	47.2	46.3	0.93	0.04	0.93	0.48
KPHfat(%)	1.3^{b}	1.5^{b}	1.9^{a}	2.1^{a}	2.0^{a}	0.18	0.22	0.75	0.37

SB=soybean oil; P=probiotics;CW=carcass weight; AW=alimentary tract weight; KPHfat=kidney, pelvic, and heart fat; BWT=body wall thickness; LD area=*longissimus dorsal* muscle area; SEM=standard error of the mean of the SB and P treatments (except for the control); P value is for the SB and P treatments (except for the control); Means with different superscript letters in the same row differ significantly ($P<0.05$).

5.5.8 Meat quality traits

As displayed in Table 5.13, the L* color for M. *semimembranosus*, M. *Longissimus dorsal* muscle, and M. *Triceps humeralis* ranged from 41.0-48.0, 36.8-41.9, and 40.1-45.4 respectively. In addition, the B* color ranged from 14.5-16.1, 12.7-18.1, and 14.2-16.4 respectively. What was more, the a* ranged from 6.7-11.7, 7.4-9.1, and 6.7-11.7 respectively.

The a* color the administered soybean oil and probiotics in feed significantly decreased the *Semitendinosis*, and *Triceps humeralismuscle* a* color (less redder) contrasting with the control, but kept those of *Longissimus dorsal* muscle were untouched. The l* and b* color for the *Semitendinosis*, and *Triceps humeralismuscle* did not changed significantly due to the administering of soybean oil and probiotics. On the contrary, the l* and b* color of *Longissimus dorsal* muscle for the goats supplemented with 2.5% soybean oil plus probiotics (2.5 and 5.0 g/h/d) were greater than those administered 5.0% soybean oil plus probiotics (2.5 and 5.0 g/h/d) and the control ($P<0.05$). There were no significant interactions between soybean oil and probiotics in the goat meat chroma.

Table 5.13 Meat chroma of growing goats supplemented soybean oil and probiotics under condition of feeding whole plant corn silage.

	Control	SB (%)	2.5		5.0		SEM	P -value		
		P (g/d)	2.5	5.0	2.5	5.0		SB	P	SBxP
***Semimembranosus* muscle**										
l*	41.0		42.6	48.0	43.6	42.9	2.34	0.63	0.58	0.46
b*	16.1		15.1	14.4	15.1	14.5	0.79	0.97	0.65	0.96
a*	10.3^{a}		7.3^{b}	7.2^{b}	8.2^{ab}	7.1^{b}	0.19	0.57	0.44	0.43
***Longissimus dorsal* muscle**										
l*	39.5^{b}		41.9^{ab}	45.2^{a}	39.3^{b}	36.8^{b}	1.06	0.02	0.83	0.15
b*	14.9^{b}		18.1^{a}	14.1^{b}	15.1^{ab}	12.7^{b}	0.57	0.03	0.01	0.30
a*	8.1		9.1	8.1	9.3	7.4	0.65	0.81	0.24	0.71
***Triceps humeralis* muscle**										
l*	41.6		40.1	45.4	43.4	42.3	1.61	0.98	0.47	0.29
b*	16.4		14.7	14.0	15.4	14.2	0.55	0.65	0.31	0.80
a*	11.7^{a}		8.2^{b}	7.0^{b}	7.7^{b}	6.7^{b}	0.67	0.70	0.37	0.95

SB=soybean oil; P=probiotics; l=dark to light; b=blue to yellow; a=green to red; SEM=standard error of the mean of the SB and P treatments (except for the control); P value is for the SB and P treatments (except for the control); Means with different superscript letters in the same row differ significantly ($P<0.05$).

The shear force values of M. *semimembranosus*, M. *longissimus dorsi* and M. *Triceps humeralis* ranged from 5387.1-6697.1 g/cm^2, 4269.5-5360.9 g/cm^2 and 5534.1-6665.2 g/cm^2. Administered soybean oil and probiotics in diet of goats did not

significantly change the M. *semimembranosus*, M. *longissimus dorsi* as well as M. Triceps humeralis shear force, and there were not any significant synergistic impact on meat shear force for supplementations of soybean oil and probiotics. The OM, DM, and CP of M. *semimembranosus*, M. *longissimus dorsi*, and M. *Triceps humeralis* sample blend (W:W:W=1:1:1) for the goats administering soybean oil and probiotics were slightly higher than the control, particularly, the composition of ether extracts was significantly higher than the control ($P<0.05$). There were no significant interactions between soybean oil and probiotics in meat shear force and M. *semimembranosus*, M. *longissimus dorsi*, and M. *Triceps humeralis* sample blend chemical compositions (Table 5.14).

Table 5.14 Mixed meat quality traits of growing goats supplemented soybean oil and probiotics under condition of feeding whole plant corn silage.

	Control	SB (%)	2.5		5.0		SEM	P -value		
		P (g/d)	2.5	5.0	2.5	5.0		SB	P	SBxP
Share force (g/cm2)										
SM	6697.1		5786.4	5863.5	6399.6	5387.1	238.93	0.87	0.29	0.22
LD	4624.9		4964.3	4269.5	5360.9	4653.8	207.31	0.32	0.10	0.98
TM	6665.2		5794.5	5948.7	6475.8	5534.1	244.35	0.74	0.32	0.27
Mixed meat composition (SM:LD:TM=1:1:1)(% DM basis)										
DM	25.5		26.1	26.0	26.6	26.0	0.42	0.71	0.59	0.77
OM	96.0		96.2	95.9	96.3	96.0	0.09	0.75	0.12	0.84
CP	75.1		77.8	77.9	75.9	77.6	1.66	0.70	0.75	0.79
EE	8.6^{b}		11.4^{ab}	11.90^{a}	12.1^{a}	13.8^{a}	0.59	0.25	0.32	0.58
AIA	1.8		1.7	1.8	1.7	1.8	0.05	0.93	0.24	0.83

SB=soybean oil; P=probiotics; SM= M. *semimembranosus*; LD= M. *longissimus dorsi*; TM= M. *triceps humeralis*; SEM=standard error of the mean of the SB and P treatments (except for the control); P value is for the SB and P treatments (except for the control); Means with different superscript letters in the same row differ significantly ($P<0.05$).

5.5.9 Fatty Acid Profiles and conjugated linoleic acid content in goat meat

Centesimal fatty acid profiles of M. *semimembranosus* (SM), M. *longissimus dorsi* (LD), and M. *Triceps humeralis* (TM) mixed sample were presented in Table 5.15 (SM: LC: TM=1:1:1). Administering 5% soybean oil plus probiotics

(2.5 and 5.0g/h/d) in feed of goats got higher C12:0 than those of adding 2.5% soybean oil plus probiotics (2.5 and 5.0g/h/d). At the same time, supplementations of soybean oil and probiotics significantly increased percentage of C12:0 in contrast to the control ($P<0.05$), and there was substantial interaction between soybean oil and probiotics in composition of C12:0. On the other hand, supplementations of soybean oil and probiotics contrasted the control to decrease C14:0 ($P<0.05$), C15:0 ($P<0.05$), C16:0 ($P>0.05$), C16:1 ($P>0.05$) and C17:1($P<0.05$), but left C17:0 to be untouched. Amongst them, treatments of 5.0% soybean oil plus probiotics (2.5 and 5.0g/h/d) were mild greater than those of 2.5% soybean oil plus probiotics (2.5 and 5.0g/h/d), the soybean oil and probiotics displayed synergistic impact on reduction of C15:0 ($P=0.05$) but not for others.

C18:0 and C18:1fatty acids were greater for soybean oil and probiotics treatments in comparison with the control with no significance. Whereas, the supplementations of soybean oil and probiotics remarkably increased C18:2n6c and C18:3n3 ($P<0.05$). There was not any obvious interaction between soybean oil and probiotics in C18 fatty acids except for CLA isomers. Administering soybean oil and probiotics in feed of goats increased the meat C18:c9,t11 CLA isomer with highly significance in contrast to the control ($P<0.01$). In contrast to the control, the increments were 100% (0.96 vs. 0.48), 95.8% (0.94 vs. 0.48) for 2.5% soybean oil plus 2.5 and 5.0 g/h/d probiotics treatments respectively. The increments were 120.8% (1.06 vs. 0.48), and 139.6% (1.15 vs. 0.48) for 5.0% soybean oil plus 2.5 and 5.0 g/h/d probiotics treatments respectively. What was more, the C18:c9,t11 CLA isomer for the 5.% soybean oil plus probiotics (2.5 and 5.0 g/h/d) treatments tended to be higher than those of 2.5% soybean oil plus 2.5 and 5.0 g/h/d probiotics treatments ($P>0.05$).

In addition, a significant synergistic effect of soybean oil and probiotics on C18:c9,t11 CLA isomer was found (P=0.03). C18:t10,c12 CLA isomer also was distinctly increased with supplementations of soybean oil and probiotics (P<0.01). The increments were 100% (0.04 vs. 0.02) and 150% (0.05 vs. 0.02) for 2.5% soybean oil plus 2.5 and 5.0 g/h/d probiotics treatments in comparison to the control respectively; and were 250% (0.07 vs. 0.02) and 300% (0.08vs. 0.02) 5.0% soybean oil plus 2.5 and 5.0 g/h/d probiotics treatments respectively. Comparison between the treatments showed that C18:t10,c12 CLA isomer for 5% soybean oil plus probiotics (2.5 and 5.0 g/h/d) treatments were significantly greater than those of 2.5% soybean oil plus probiotics (2.5 and 5.0 g/h/d) treatments, and there was a distinct interaction between soybean oil and probiotics on C18:t10,c12 CLA (P=0.04) (Table 5.15).

The very long chain saturated fatty acids C20:0 and C22:0 decreased significantly due to supplementations of soybean oil and probiotics in contrast to the control (P<0.05). On the contrary, presences of soybean oil and probiotics increased the very long chain unsaturated fatty acids C20:2 (P<0.05) and C20:3n (P>0.05). There were obvious interactions between soybean and probiotics on C20:0 (P=0.08), C22:0 (P=0.05), C20:2 (P=0.09) and C20:3n (P=0.07).

To sum up, administration of soybean oil and probiotics in goat feed significantly increased total CLA isomers (P<0.01), total n-6 (P<0.05), and total poly-unsaturated fatty acids (P<0.05); tended to increased total saturated (TSFA), total n-3, total mono-unsaturated, and desirable fatty acids (P>0.05). Supplementation of 5.0% soybean oil plus probiotics significantly increased the ratios of poly-unsaturated fatty acids to total saturated fatty acids (PUFA/SFA) (P<0.05), whereas, significantly decreased the ratios of total n-6 fatty acids to n-3 fatty acids (n6/n3) (P<0.05). A

remarkable interaction between soybean oil and probiotics existed in total CLA isomers (P=0.04), total n-6 fatty acids (P=0.03), total saturated fatty acids (P=0.09), and total n-3 fatty acids.

Table 5.15 Meat fatty acids centesimal composition profiles of growing goats supplemented soybean oil and probiotics under condition of feeding whole plant corn silage.

%TFA	Control	SB (%)	2.5		5.0		SEM	P value		
		P (g/d)	2.5	5.0	2.5	5.0		SB	P	SBxP
C12:0	0.27^{c}		0.29^{bc}	0.31^{abc}	0.43^{a}	0.40^{a}	0.03	0.02	0.07	0.03
C14:0	5.36^{a}		3.83^{b}	2.97^{b}	4.22^{a}	4.13^{ab}	0.39	0.02	0.06	0.18
C15:0	0.76^{a}		0.48^{c}	0.66^{ab}	0.68^{ab}	0.60^{bc}	0.04	0.02	0.99	0.05
C16:0	23.92		19.74	20.00	20.55	19.22	1.88	0.99	0.62	0.46
C16:1	0.65		0.40	0.51	0.44	0.54	0.12	0.82	0.47	0.99
C17:0	4.87		4.84	5.36	4.81	4.68	0.59	0.61	0.78	0.64
C17:1	0.79^{a}		0.50^{b}	0.45^{b}	0.62^{ab}	0.46^{b}	0.08	0.50	0.18	0.04
C18:0	19.93		23.82	24.71	24.47	22.53	1.71	0.71	0.81	0.49
C18:1	30.62		33.48	32.32	34.64	35.01	2.12	0.07	0.66	0.22
C18:2n6c	4.64^{c}		5.90^{b}	7.96^{a}	5.23^{b}	5.47^{b}	0.56	0.05	0.38	0.56
C18:3n3	1.16^{b}		1.45^{ab}	2.24^{a}	2.25^{a}	2.18^{a}	0.31	0.03	0.96	0.53
C18:c9,t11	0.48^{b}		0.96^{a}	0.94^{a}	1.06^{a}	1.15^{a}	0.19	0.001	0.01	0.03
C18:t10,c12	0.02^{c}		0.04^{b}	0.05^{b}	0.07^{a}	0.08^{a}	0.01	0.001	0.01	0.04
C20:0	0.31^{a}		0.15^{b}	0.18^{b}	0.24^{ab}	0.19^{b}	0.03	0.21	0.09	0.08
C20:2	0.79^{b}		1.05^{a}	0.99^{a}	1.08^{a}	1.05^{a}	0.16	0.04	0.92	0.09
C22:0	0.18^{a}		0.13a^{b}	0.11^{b}	0.10^{b}	0.10^{b}	0.02	0.01	0.87	0.05
C20:3n	0.62		0.62	0.59	0.60	0.52	0.17	0.05	0.09	0.07

Table 5.15 (Continued)

%TFA	Control	SB (%)	2.5		5.0		SEM	P value		
		P (g/d)	2.5	5.0	2.5	5.0		SB	P	SBxP
TCLA	0.51^{c}	1.01^{b}	0.99^{b}		1.13^{ab}	1.23^{a}	0.19	0.001	0.01	0.04
TSFA	54.24	53.28	54.32		56.61	51.85	1.53	0.01	0.69	0.09
Tn6	5.30^{b}	6.91^{b}	8.96^{a}		6.33^{b}	6.72^{ab}	0.32	0.03	0.12	0.03
tn3	1.78^{b}	2.06^{b}	2.82^{a}		2.85^{a}	2.71^{a}	0.30	0.05	0.85	0.08
TMUSFA	32.07	34.39	33.29		35.68	36.01	2.04	0.84	0.65	0.21
TPUSFA	8.35^{c}	10.23^{b}	12.42^{a}		10.31^{b}	10.90^{ab}	0.72	0.16	0.32	0.26
DFA	63.34	68.44	69.42		68.45	69.44	2.73	0.27	0.04	0.17
PUFA/SFA	0.16^{b}	0.19^{b}	0.24^{a}		0.20^{ab}	0.22^{a}	0.02	0.59	0.20	0.58
n−6/*n*−3	3.03^{ab}	3.37^{a}	3.14^{a}		2.25^{c}	2.37^{c}	0.27	0.20	0.71	0.34

SB=soybean oil; P=probiotics; TSFA=total saturated fatty acid;TMUSFA=total mono-unsaturated fatty acid; TPUSFA= total poly-unsaturated fatty acid; DFA=desirable fatty acid; Tn6=total n6 fatty acid; Tn3=total n3 fatty acid; SEM=standard error of the mean of the SB and P treatments (except for the control); P value is for the SB and P treatments(except for the control); Means with different superscript letters in the same row differ significantly ($P<0.05$).

As shown in Table 5.16, when calculating the centesimal composition of fatty acids into fatty acid contained in per gram meat lipid (mg/g lipid), the statistical analyses were similar to those in percentage on total detected fatty acids (Table 5.15). Collectively, total CLA isomers was 2.34 mg/g lipid for the control, and ranged from 5.36-8.17 mg/g lipid for administration of soybean oil and probiotics in feed, 5.0% soybean oil plus probiotics treatments were significantly higher than the control

($P<0.01$) and 2.5% soybean oil plus probiotics ($P<0.05$). The desirable fatty acids was 566.6 mg/g lipid for the control, and ranged form 597.6 to 665.5 for administration of soybean oil and probiotics in feed, in the same case as total CLA, 5.0% soybean oil plus probiotics treatments were higher than the control ($P<0.05$) and 2.5% soybean oil plus probiotics ($P>0.05$). The ratio of PUFA/SFA was 0.15 for the control, and ranged from 0.18 to 0.20 for the soybean oil and probiotics treatments. The n6/n3 ratio of the control was 0.15, and ranged from 2.09 to 2.78 for the soybean oil and probiotics treatments.

Table 5.16 Fatty acid and conjugated linoleic acid contents (mg/g lipid) in chevon of growing goats supplemented soybean oil and probiotics under condition of feeding whole plant corn silage.

mg/g lipid	Control	SB (%)	2.5		5.0		SEM	P-value		
		P (g/d)	2.5	5.0	2.5	5.0		SB	P	SBxP
C12:0	1.4^{c}		1.6^{c}	1.8^{bc}	2.5^{a}	2.4^{ab}	0.08	0.003	0.06	0.02
C14:0	28.3^{a}		19.1^{bc}	17.5^{bc}	20.3^{b}	21.4^{b}	1.84	0.01	0.72	0.21
C15:0	3.6^{a}		2.7^{b}	3.0^{b}	3.2^{ab}	3.4^{a}	0.17	0.08	0.51	0.37
C16:0	121.2		114.2	117.2	117.9	119.6	4.65	0.73	0.53	0.73
C16:1	3.4^{a}		2.5^{c}	2.5^{c}	2.6^{bc}	3.1^{ab}	0.79	0.70	0.13	0.04
C17:0	25.8		24.7	25.5	27.9	28.7	1.82	0.15	0.83	0.22
C17:1	5.7^{a}		2.4^{b}	2.6^{b}	2.6^{b}	2.4^{b}	0.14	0.61	0.57	0.35
C18:0	98.9^{b}		112.8^{b}	140.8^{a}	144.4^{a}	145.6^{a}	8.46	0.15	0.33	0.10
C18:1	183.9^{c}		198.2^{bc}	195.0^{bc}	204.0^{ab}	225.2^{a}	11.26	0.04	0.56	0.17
C18:2n6c	24.5^{b}		31.0^{a}	33.9^{a}	30.9^{a}	34.8^{a}	1.51	0.08	0.91	0.82
C18:3n3	5.6^{c}		7.9^{b}	8.7^{b}	12.4^{a}	14.4^{a}	0.84	0.01	0.48	0.08
C18:c9,t11	2.22^{d}		5.12^{c}	5.54^{c}	6.58^{a}	7.40^{a}	0.72	0.001	0.03	0.05
C18:t10,c12	0.11^{e}		0.22^{d}	0.30^{c}	0.43^{b}	0.52^{a}	0.09	0.001	0.05	0.03
C20:0	1.6^{a}		0.9^{b}	1.1^{b}	1.3^{ab}	1.3^{ab}	0.03	0.10	0.16	0.10
C20:2	3.7^{c}		5.2^{b}	5.8^{b}	6.4^{a}	6.7^{a}	0.34	0.07	0.65	0.87
C22:0	0.8		0.7	0.6	0.6	0.6	0.07	0.66	0.07	0.54
C20:3n	4.7^{b}		3.4^{c}	5. 7^{a}	5.1^{ab}	5.2^{ab}	0.37	0.10	0.72	0.99

Table 5.16 (Continued)

mg/g lipid	Control	SB (%)	2.5		5.0		SEM	P value		
		P (g/d)	2.5	5.0	2.5	5.0		SB	P	SBxP
TCLA	2.34^{c}		5.36^{b}	5.86^{b}	7.04^{a}	8.17^{a}	0.94	0.01	0.03	0.02
TSFA	274.9		278.8	304.2	315.8	320.7	12.65	0.12	0.10	0.03
Tn6	27.2^{b}		33.7^{ab}	38.2^{a}	36.7^{a}	35.6^{a}	1.54	0.14	0.87	0.13
tn3	10.1^{c}		11.7^{c}	14.5^{b}	16.9^{ab}	17.5^{a}	1.14	0.14	0.07	0.04
TMUSFA	190.3		202.0	194.4	208.1	190.7	10.16	0.67	0.25	0.77
TPUSFA	41.4^{b}		49.3^{b}	59.6^{a}	62.8^{a}	61.0^{a}	4.54	0.12	0.80	0.83
DFA	566.6^{b}		597.6^{ab}	637.3^{a}	665.5^{a}	658.2^{a}	16.18	0.04	0.01	0.23
PUFA/SFA	0.15^{b}		0.18^{a}	0.19^{a}	0.19^{a}	0.20^{a}	0.04	0.18	0.45	0.33
n−6/*n*−3	2.62		2.78	2.69	2.17	2.09	0.61	0.63	0.83	0.61

SB=soybean oil; P=probiotics; TSFA=total saturated fatty acid;TMUSFA=total mono-unsaturated fatty acid; TPUSFA= total poly-unsaturated fatty acid; DFA=desirable fatty acid; Tn6=total n6 fatty acid; Tn3=total n3 fatty acid; SEM=standard error of the mean of the SB and P treatments (except for the control); P value is for the SB and P treatments(except for the control); Means with different superscript letters in the same row differ significantly ($P<0.05$).

5.6 Discussion

5.6.1 The administration of soybean oil (2.5 and 5.0 % of concentrate) and probiotics (2.5 and 5.0 g/h/d) in diet of growing goats did not significantly affect DMI, but significantly increased ADG and feed efficiency (P<0.05); there were distinct interaction between soybean oil and probiotics on increases of ADG (P=0.07) and feed conversion (P=0.04)

Rogério et al. (2005) have verified that supplemented soybean oil singly the goats, the intakes of dry matter (%BW and g/kg BW0.75), NDF and non-fibrous carbohydrates decreased. Contrasted with this, Bouattour et al. (2008) stressed that feeding soybean oil (2.5% dietary DM basis) did not change the DIM in dairy goats. There were abundant studies had test-proof that the supplementation of probiotics positively affects the DMI, ADG, and feed conversion. E.g., Chiofalo et al. (2004), Whitley et al. (2004), El-Ghani (2004) and Tripathi et al. (2007) had testified the increases of body weight, DME also feed efficiency. The presence of probiotics (*S. cerevisia* and *L. acidophilus*) constituted a healthier and more favorable ruminal setting for digestive and absorption processes. This healthier and more favorable ruminal setting was responsible for the significant increase of DMI, ADG, and feed efficiency. So even the administration of soybean oil showed no effect or negative effect on DMI of the animals, the combining supplementations of soybean oil and probiotics showed synergistic effects on DMI, ADG, and feed efficiency in the animals. The findings of increased ADG and feed efficiency in present study were in accordance with our previous study (Han et al., 2008).

5.6.2 Supplementation of probiotics significantly increased digestibility of DM and OM (P=0.02); significant synergistic effects of soybean oil and probiotics on DM and OM digestibility were observed (P=0.05)

Dietary digestibility particularly fiber digestibility is adversely affected by dietary fat (Jenkins, 1993). Nevertheless, Kucuk et al. (2004) who added soybean oil to diets at 0, 3.2, 6.3, and 9.4% of dietary DM, and found digestibility of OM, NDF, and N were not affected by increasing dietary soybean oil level. However, dietary digestibility for addition of probiotics always be reported as increase. It was the increasing colonization of fugal on plant cell; the stimulating growth and activity of fibrolytic bacteria; the increasing activities of xylanase and pectinase and the establishing more favorable ecological conditions for growth and activities of the anaerobic autochtonous microflora responsible for the increase of dietary digestibility due to addition of probiotics. Many studies had approved that the presence of probiotics positively affected dietary digestibility (Dawson and Tricarico, 2002; Fadel Elseed et al., 2007; Feng et al., 2008). Our former study on supplementation of probiotics together with palm oil by-pass fat (Han et al., 2008) already demonstrated the increase of dietary DM and OM. Thereupon, the findings in increase and interaction of dietary DM and OM for administration of soybean oil and probiotics were reasonable.

5.6.3 The pH were higher for 5.0% than 2.5% soybean oil treatments, ranging form 6.21 to 6.53; There were not any significant synergistic effects on NH_3-N (P=0.57) and PUN (P=0.84) for soybean oil and probiotics; sampling time affected ruminal NH_3-N and PUN significantly (P<0.05)

The above results in consisted with findings of other, for example, Gülşen et al. (2006) who suggested that increasing levels (3, 6, and 9%) of sunflower and soybean oil linear increases pH, did not affect NH_3-N concentration, and depressed ruminal fermentation in cattles. Rogério et al. (2005) observed increase in ruminal pH of goats on account of presence of soybean oil. Kukcu et al. (2004) observed that ruminal ammonia were not affected by increased dietary soybean oil in sheep. Many findings have emphasized that the probiotics did not affect goats' rumen pH value with any significance (Han et al., 2008; Jiang et al., 2008; Fadel Elseed., 2007; Galp, 2006; Kumagai et al., 2004; Giger-Reverdin et al., 2004; Dawson et al., 1990), but maintained pH in the range that is compatible with the optimal ruminal ecologic dominance. Because of the changes of rumen pH, microbial population, and metabolism in concurrent with feeding, the sampling time significantly changed the ruminal NH_3-N and PUN.

5.6.4 Total VFA and propoinic proportion were greater for goats supplemented with 5.0% than 2.5% soybean oil; there were a significant synergistic effect on the total VFA for soybean oil and probiotics (P=0.05)

Propionate molar proportions also the total VFA were expected to increase from conversion of glycerol to propionate, with the glycerol supplied from

hydrolysis of dietary triacylglycerol (Chalupa et al., 1986). Rogério et al. (2005) have verified that the presence of soybean oil in diet of goats decreased the acetate: propionate ratio in the ruminal fluid for the increase of propionate. The effectiveness of additional yeast probiotics on production of VFA being that it has beneficial effects on growth and H_2-utilisation of acetogenic bacteria (Chaucheyras et al., 1995b; Chaucheyras-Durand et al., 1997), and since the acetogenic bacteria which produces acetate from CO_2 and H_2, the total VFA and acetic centesimal proportion should appear to be increased. Fadel Elseed et al. (2007) reported S. cerevisiae resulted in a numerical increase in total VFA concentration. El-Waziry et al. (2000) reported that VFA concentration increased with yeast supplementation. El-Ghani, (2004) elucidated in detail that ruminal VFA was significantly heightened for bucks fed S. cerevisiae at 6 h.

The supplementation of 5% soybean oil showed higher propionate owing to the glycerol from 5% soybean oil higher than that from 2.5% soybean oil. What was more, both of soybean oil and probiotics supplementations were of to increase VFA, and the linoleic acid that contained in the soybean oil was the substrate for ruminal microbe to biosynthesis CLA, this caused synergistic effect on total VFA between soybean oil and probiotics.

5.6.5 The number of protozoa and total viable bacteria were significantly greater for supplementation of 5.0 than 2.5 g/h/d probiotics ($P<0.05$); there was not significant interaction on ruminal microbe population for soybean oil and probiotics

Jouany et al. (1998) found increase of protozoal count due to addition of S. *cerevisiae*. Krehbiel et al. (2003) stated that supplementation of L. *acidophilus* has been shown to increase ruminal protozoal numbers, to change viable bacterial counts.

In the same case, Han et al. (2008) reported the significant increment of protozoal and bacterial counts for the reason of supplementation of blend of S. *cerevisiae* and L. *acidophilus* probiotics. Kucuk et al. (2004) observed increases for ruminal protozoa and bacteria when administering soybean oil in sheep. The results of the first experiment in chapter III showed that supplementation of 5.0 g/h/d probiotics got the highest bacterial and protozoa counts. In addition, the second experiment in chapter IV showed increasing levels of soybean oil did not affect the ruminal microbial population significantly. These may be responsible for no interaction was found for soybean oil and probiotics on ruminal microbial population.

5.6.6 Dietary nitrogen intake (P>0.05), absorption (P<0.05), and retention were higher for goats supplemented with 2.5% than 5.0% soybean oil; there were not synergistic effects on dietary nitrogen intake and excretion for soybean oil and probiotics, but the obvious synergistic effects on nitrogen absorption (P=0.07) were found

The nitrogen absorption and retention were related to ruminal nitrogen metabolism, flow of ruminal microbial protein, and efficiency of ruminal urea recycle. There were not any reference addressed the effect of soybean oil and probiotics were found. The whole plant corn silage dry matter intake was slightly decreased for the supplementation of soybean oil, and the 2.5% soybean oil treatments higher than those of 5.0 % soybean oil treatments (Table 5.6). The higher DMI of 2.5% soybean oil treatments caused higher nitrogen intake. In addition, the second experiment in chapter 4 showed that the supplementation soybean oil significantly increase nitrogen absorption and retention. Therefore, even though the first experiment in chapter 3 showed that there were no effects on nitrogen balance due to addition of probiotics,

the combining supplementation of soybean oil and probiotics showed obvious synergistic effects on nitrogen absorption.

5.6.7 Supplementation of soybean oil and probiotics reduced C8 to C16 plasma saturated fatty acids; the C8 to C14 fatty acids were numerically greater for supplementation of 2.5% than 5.0% soybean oil

Up to now, there were not any research detailed the effect of probiotics on plasma fatty acid profiles at all. A similar research in Maltese goat kids found that the lactobacilli treatment significantly lowered the levels of blood non-essential fatty acid (NEFA) ($P<0.001$) and triglycerides ($P<0.05$), but did not mention the plasma fatty acid profiles (Chiofalo et al., 2004). One study on beef cattles showed than the proportions of 14:0 ($P=0.01$), 16:0 ($P=0.001$) in the rumen content significantly decreased. Another study carried out by Yeom et al. (2003) showed that the goat plasma C14 to C17 fatty acids were lower for soybean oil supplementation than those of medium-chain triglycerides-product supplementation, and particularly the C15:0, 17:0 and C17:1 significantly decreased ($P<0.05$). Bouattour et al. (2008) did not observed evident changes in milk fat short chain fatty acids for including soybean oil in diary goats, however, the C10 to C17 fatty acids significantly decreased. C14:0, C15, C16:0 are hypercholesteremic and associated with the increased incidence of arteriosclerosis and coronary heart disease (Noakes et al., 1996). Thereupon, the decreases of C10 to C17 fatty acids in animals' plasma or product are good for human and the animals.

Theoretically, the fatty acid profiles of soybean oil were characterized as high C18:2 (48.36%) and C18:1 (24.67%) (Table 5.4), undetected or low C8 to C17 fatty acids. Thereupon, the 5.0% soybean oil not responsible for higher C8 to C16 plasma

saturated fatty acids than 2.5% soybean oil. Meanwhile, the larger C18:2 and C18:1 for higher level (5.0%) soybean oil competed more hydrogenase with the C8 to C16 fatty acids, these reasons may account for the reductions of C8 to C16 plasma saturated fatty acids, and accounted for the higher C8 to C14 fatty acids for supplementations of 2.5% soybean oil than 5.0% soybean oil.

5.6.8 Supplementation of soybean oil and probiotics tended to increase the goat plasma C18:0 and C18:2n6c fatty acids, significantly increased C18:1n9c, C18:1n9t, and C18:3n3; furthermore, these fatty acids were higher for 5.0% soybean oil and 5.0 g/h/d probiotics supplementations.

Long before, Moore et al. (1968) already observed that intraruminal infusion of linoleic acid in sheep raised the content of linoleic acid in plasma lipids. Recently, Zhang et al. (2005) observed that C18:1trans-11, C18:0, C18:1cis-9, C18:2cis-9,12, and total 18-Cfatty acids of sheep rumen digesta DM significantly increased ($P < 0.01$) owing to administration of soybean oil. Jenkins et al. (1994) reported that soybean oil feeding increased the linoleic acid concentration in subcutaneous fat of sheep. In dairy cows fed high intakes of linoleic acid, the content of linoleic acid in plasma and milk was evidently increased (Loor et al., 2002; Petit, 2002). Bickerstaffe et al. (1972) demonstrated that about 90% of dietary linoleic acid was hydrogenated in the rumen of goats. LeDoux et al. (2002) reported that different intakes of linoleic acid did not influence the contents of linoleic acid in goat milk. Contrary to the case of milk, Yeom et al. (2003) found soybean versus medium-chain triglycerides-product significantly raised the goat plasma linoleic acid (C18:2n-6) content ($P < 0.05$). The findings of the present study were resulted from the high content of unsaturated C18 fatty acids in soybean oil (Table 5.4), and the more

efficient or complete hydrogenation of these fatty acids caused by the supplemental probiotics, and more of these fatty acids went into the blood of the animals. The results of the present study may indicates that C18:2n6c fatty acids are more preferentially transformed by microorganism than C18:1n9t, and C18:3n3 fatty acids in the rumen of goats.

5.6.9. The plasma C18:c9,t11 and C18:t10,c12 CLA for goats received additional soybean oil and probiotics ranged from 1.24 to 1.36% and undetectable level to 0.21%, increasing with highly significance (P<0.01); the plasma CLA were greater for supplementation of 5.0% soybean oil and 5.0 g/h/d probitics; there were significant synergistic effects on plasma CLA isomers between soybean oil and probiotics

Beaulieu et al. (2002) elucidated that the CLA in rumen content significantly increased with soybean oil. Zhang et al. (2005) observed sheep rumen ingesta cis9, trans11-CLA significantly increased due to supplementation of soybean oil (P<0.01). More recently, Bouattour et al. (2008) elaborated extremely (P<0.001) increases of *Cis*-9, *trans*-11 C18:2 CLA isomer in milk of goats was caused by supplementation of soybean oil. Moreover, the supplementation of probiotics constructed a healthier and more favorable rumen setting for ruminal microbial growing and activity. Besides, the soybean oil was characterized as high C18:2n6c, and the *lactobacillus acidophilus* that adopted in the present study itself has been well documented to produce CLA from linoleic acid and linoleni acid (Kishino et al., 2002; Julia et al., 2006). The aforementioned reasons ensure the significantly increase of the CLA and the higher CLA contents for the supplementations of 5.0% soybean oil as well as 5.0 g/h/d probiotics, and cause the significant synergistic effects between soybean oil and probiotics on plasma CLA isomers.

5.6.10 Supplementation of soybean oil and probiotics significantly decreased the plasma very long-chain fatty acids (P<0.05); there were evident synergistic impacts between soybean oil and probiotics on C20:3n6 (P=0.05), C20:4n6 (P=0.09), and C20:5n3 (P=0.04)

Yeom et al. (2003) dated that soybean versus medium-chain triglycerides-product significantly decrease eicosapentaenoic (C20:5n-3), docosahexaenoic (C22:6n-3) and arachidonic acid (C20:4n-6) in plasma lipids by highly significance. Their findings indicated that the long-chain fatty acids in soybean oil versus medium-chain fatty acids in medium-chain triglycerides-product inhibited microbial activity in the rumen. Beaulieu et al. (2002) elucidated that the 20:0 (P=0.001), and 24:0 (P=0.001) in cattle ruminal contents decreased linearly with increasing dietary soybean oil. Furthermore, Yeom et al. (2003) demonstrated that the presence of soybean oil decreased C20:5n-3 of goat plasma by highly significance. Again, the presence of probiotics created a healthier and more favorite ruminal setting, and it was this healthier and more favorite ruminal setting responsible for the stimulation of microbial activity in the rumen to transfer the very long chain fatty acids with higher efficiency.

5.6.11 Ratios of PUFA/SFA and n−6/n−3 in the plasma ranged from 0.57 to 0.69 and 5.87 to 8.64, increasing with soybean oil and probiotics treatments (PUFA/SFA: P>0.05; n−6/n−3: P<0.05)

Zhang et al. (2005) confirmed that SFA, UFA, MUFA and PUFA significantly increased (P＜0.01). Bouattour et al. (2008) found increased (P<0.001) total unsaturated FA concentrations and monounsaturated FA (21.8 vs. 29.3%) and PUFA (3.73 vs. 4.15%) contents in the goat milk due to addition of soybean oil. Supplementations of different ratio of n-6 to n-3 fatty acids dietary to growing lamb,

the rumen content total saturated fatty acids ranged from 62.9-69.9%, the total mono-unsaturated fatty acids ranged from 17.1-21.9%, the total poly-unsaturated fatty acids ranged from 13.8-16.1%. In addition, the PUFA/SFA ratio and n-6/n-3 ratio ranged form 0.20-0.25 and 3.5-9.1 (Kim et al. 2007). In present study, the plasma TSFA, TMUSFA, TPUSFA, Tn6, and Tn-3 fatty acid were not significantly changed.

More recently, an upper daily intake limit of 6.67 g/d of linoleic acid and a minimum daily intake of 2.87 g/d of n-3 FA (linolenic, eicosapentaenoic, and docosahexaenoic acids) were proposed to be dequate for human adults (Simopoulos et al., 1999). This indicates an n-6:n-3 ratio of 2.3:1 would be optimal for human. The present study found the n−6/n−3 in goat' plasma ranged from 5.87 to 8.64. Still, this ratio range lower than the typical Western-type foods that have an average n-6:n-3 ratio of 10:1 (Kris-Etherton et al., 2000).

5.6.12 Supplementation of probiotics significantly increased KPH fat ($P<0.05$), but did not significantly affect other slaughter attributes; the KPH fat ranged from 1.5-2.1%, dressing percentage range from 43.0-47.2%, LD area ranged from 11.3-13.3 cm^2

Development of fat in goats occurs very late and only reaches appreciable levels when the animals are near or at their mature body weight (Owen et al., 1978, 1983). Moreover, most of the fat is deposited in the visceral rather than carcass depots (Webb et al., 2005). Thereby, the kidney, pelvic and heat (KPH fat) percentage were used to elevate the fat content of goat carcass other than the marline score and subcutaneous fat thickness like lamb and beef.

Beaulieu et al. (2002) dated that administration of 5.0% soybean oil in beef cattle diet, the KPH fat ranged from 2.35-2.60% of carcass, this range was higher than

the finding of the present study (ranged from 1.5-2.1% carcass). Goat meat is leaner than mutton and beef because it incorporates less subcutaneous and intra-muscular fat (Smith et al., 1978). In addition, Dhanda et al. (2003) detailed that the KPH fat for Boer x Angora, Boer x Feral, Boer x Saanen, Feral x Feral, Saanen x Angora and Saanen x Feral goat types ranged 0.90-1.46% of empty body weight, dressing percentage were 51.0-54.0%. If calculating into empty body weight, the results of the present on KPH fat were similar to the findings of Dhanda et al. (2003), but the dressing percentage (43.0-47.2%) were lower than their findings. Some previous studies had confirmed that the dressing percentage of goat kids ranged from 46–48% in different goat breeds (Colomer-Rocher et al., 1992; Hogg et al., 1992), and the differences between various goat genotypes were significant Van Niekerk and Casey (1988). Dhanda et al. (2003) reported a lower LD area (cm2) 9-12.1 range than the findings of the present study (11.3-13.3 cm^2).

5.6.13 Administered soybean oil and probiotics in goat diet significantly decreased the M. *semimembranosus*, and M. *Triceps humeralisa* color (less redder); the l* and b* color of M. *Longissimus dorsal* were greater for the goats supplemented with 2.5% than 5.0 % soybean oil (P<0.05); there were not any significant interaction between soybean oil and probiotics in the goat meat chroma

The pale or pink muscle color is more favorite by the consumer (Ledward, 1992). The chroma-meter values in the present study were in the similar rang of others (Shorthose, 1989; Warner, 1989; Dhanda et al., 2003). The color of raw meat is largely dictated by the concentration and chemical nature of the haemoproteins present (Dhanda et al., 2003). As shown in Table 5.14, the supplementation of

soybean oil and probiotics significantly changed the EE composition rater than the others, the increase of EE composition will be responsible for the meat color. And the 5.0% soybean oil supplementation reached higher increment of EE composition than 2.5% soybean oil supplementation, this may account for the bigger l* and b* color for the 2.5% soybean oil supplementation, and free of interaction of meat chroma for soybean oil and probitics.

5.6.14 Administration of soybean oil and probiotics in diet of goats did not significantly change the M. *semimembranosus*, M. *longissimus dorsi* as well as M. *Triceps humeralis* shear force; there were not any significant synergistic impact on meat shear force for soybean oil and probiotics

The shear force values were in present study ranged from 5387.1 to 6697.1 g/cm^2 for M. *semimembranosus*, ranged from 4269.5 to 5360.9 g/cm^2 for M. *longissimus dorsi*, and ranged from 5534.1 to 6665.2 g/cm^2 for M. *Triceps humeralis*. Babiker et al. (1990) and Dhanda et al. (2003) reported that shear force values were in the range of 3.7–4.6 kg/cm^2 for the various genotypes goats in their study. However, Riley et al., (1989a) reported a much higher shear force (8.5 kg/cm^2) in Angora and Spanish breeds of goats. The differences of reported shear force may due to differences in the age and liveweight of the goats and the types of muscles studied by the researchers. The acceptable range for goat meat was 4.2-5.5 kg/cm^2 (Webb, 2005), this is similar to the results of the present (4269.5 to 665.2 g/cm^2).

5.6.15 Supplementation of soybean oil and probiotics decreased the meat C14:0 (P<0.05), C15:0 (P<0.05), C16:0 (P>0.05), C16:1 (P>0.05) and C17:1(P<0.05) fatty acids centesimal composition

The findings of the present study were similar to those had been confirmed in other ruminants. E.g., Beaulieu et al. (2002) reported that inclusion of soybean oil in the diet of heifers significantly decreased proportions of 16:0 and 16:1 in tissue lipids from the loin and hindquarter. Bouattour et al. (2008) stated that supplementation of soybean oil significantly decreased the C10-C17 fatty acids in goat milk. Noakes et al. (1996) stressed that C14:0, C15, C16:0 were hypercholesteremic and associated with the increased incidence of arteriosclerosis and coronary heart disease. Grundy and Denke,(1990) claimed that Long chain SFA increase plasma cholesterol levels compared with high levels of MUFA and PUFA. Thereupon, the decreases of C10 to C17 fatty acids in goat meat were good for health human and animals.

5.6.16 All C18 fatty acids of the meat were increased due to supplementation of soybean oil and probiotics; there was not any obvious interaction between soybean oil and probiotics on the C18 fatty acids except for CLA isomers

Beaulieu et al. (2002) reported that inclusion of soybean oil in the diet of heifers, the treatment consistently increased the proportion of 18:1-trans isomers by greater than 40% over control values, increased or tended to increase the proportions of 18:0 in lipids. The proportion of 18:3 n–3 was increased or tended to be increased in tissue lipids. In the study of Bouattour et al. (2008), All C18 fatty acids fatty acids of the goat milk also increased significantly. the aforementioned findings in beef and goat mild were similar to the results of the present study. The significant increase of

C18 fatty acids in goat meat were resulted from the high C18 fatty acids contained in the soybean oil, and accordingly higher level of these fatty acids were into the rumen. In addition, this was in concurrent with the healthier and more preferable rumen setting that constructed by the addition of probiotics for higher efficiency of digestion and absorption, all these reasons were responsible for the increase of C18 fatty acids.

5.6.17 C18:c9,t11 and C18:t10,c12 CLA isomers centesimal composition of the meat ranged from 0.94 to 1.15% and 0.04 to 0.08% of total detected fatty acids; the C18:c9,t11 CLA increased 100 to 139.6% (P<0.01); the C18:t10,c12 CLA increased 100 to 300%; there were significant synergistic effect between soybean oil and probiotics on CLA isomers (P<0.05)

The significant increase of CLA in beef and milk due to inclusion of soybean oil in the diet had been sufficiently reported. For example. Beaulieu et al. (2002) reported that inclusion of soybean oil in the diet of heifers increased the proportion of CLA10,12 in tissue lipids from the forequarter and hindquarter significantly. Bouattour et al. (2008) reported highly significant increase of CLA in goat mild owing to supplementation of soybean oil. The supplementation of soybean oil cause significantly increase of CLA, because soybean contains high linoleic acid, and the linoleic acid is the appropriate substrate for ruminal microbe to synthesize CLA. In addition, the lactobacillus acidophilus that adopted in the present study itself has been well documented to produce CLA from linoleic acid and linoleni acid (Kishino et al., 2002; Julia et al., 2006). Besides, the increased C18:1nt11 go into the organ and tissue and synthesize CLA under the action of □9desaturase, this responsible for the main increment of the CLA.

5.6.18 The meat PUFA/SFA and n−6/n−3 ratios ranged for 0.15 to 0.19 and 2.09-2.78

Wendell et al. (2005) detected the similar PUFA/SFA ratios range (0.09-0.15), but much higher n−6/n−3 ratios range (9-14) for goat meat. Webb et al. (2005) summarized the PUFA/SFA ratios of goat meat ranged from 0.16 to 0.49, and n−6/n−3 ratio 3.09 to 5.5. The results of the present study were lower than their summary. The presence of a lower n-6/ n-3 ratio associated with decreased risk of coronary diseases (Andrade et al., 1995; Shantha & Napolitano, 1992). The Health Department of England (HMSO, 1994) recommends 4.0 as the maximum ratio of n-6:n-3. The findings of the present study in accordance whit the recommendation of HMSO (1994).

5.7 Conclusions

The administration of soybean oil (2.5 and 5.0% of concentrate) and probiotics (2.5 and 5.0 g/h/d) in diet of growing goats did not significantly affected DMI, significantly increased ADG and feed efficiency. There were distinct interaction between soybean oil and probiotics on increase of ADG and feed conversion.

Supplementation of probiotics significantly increased digestibility of DM and OM, there were significant synergistic impacts for soybean oil and probiotics on DM and OM digestibility. The dietary digestion was slightly greater for goats fed with 2.5% soybean oil than those fed with 5.0% soybean oil. There were significant interaction on DM and OM digestibility for soybean oil and probiotics.

The pH ranged form 6.21 to 6.53 and were higher for 5.0% soybean oil than 2.5% soybean oil supplementation ($P>0.05$). There were not significant synergistic

effects on NH3-N and PUN for soybean oil and probiotics.

The total VFA and propoinic molar proportion were greater for goats supplemented with 5.0% soybean oil than 2.5% soybean oil, there were significant synergistic effect on the total VFA for soybean oil and probiotics, but the synergistic effect did not exist in acetic, propoinic, and butyric molar compositions.

The number of ruminal protozoa and total viable bacteria for growing goats supplemented with soybean oil and probiotics ranged from 1.04 to 1.95 x106 and 1.35 to 2.57 x 1010 per ml rumen fluid. The numbers were significantly greater for supplementation of 5.0 g/h/d probiotics than the control and those of 2.5% soybean oil treatments. There were not any significant interaction in rumimal protozoal and bacterial number between soybean oil and probiotics.

The dietary nitrogen intake, absorption, and retention for goats supplemented with 2.5% soybean oil were higher than those of 5.0% soybean oil and the control. There were not synergistic effects on dietary nitrogen intake and excretion for soybean oil and probiotics, but here were obvious synergistic effect on nitrogen absorptions.

Supplementations of soybean oil and probiotics reduced C8 to C16 plasma saturated fatty acids. The C8 to C14 fatty acids for supplementations of 2.5% soybean oil numerically were greater than those of 5.0% soybean oil supplementations.

C17 and all C18 fatty acids increased due to presences of soybean oil and probiotics. The C17 and all C18 fatty acids were greater for the goats supplemented with 5.0 g/h/d probiotics and for those of 5.0% soybean oil.

C18:c9,t11 and C18:t10,c12 CLA for goats received additional soybean oil and probiotics increased with highly significance ($P<0.01$). The C18:c9,t11 ranged from 1.24 to 1.36%, they increased 134.0 to 156.6%. The C18:t10,c12 ranged form

undetectable level to 0.21%. The CLA isomers were greater for supplementations of 5.0% soybean oil and 5.0 g/h/d probiotics. There were significant synergistic impact between soybean oil and probiotics on increase of CLA isomers.

The supplementations of soybean oil and probiotics significantly decreased the plasma very long-chain fatty acids. There were significant synergistic impacts between soybean oil and probiotics on C20:3n6, C20:4n6, and C20:5n3.

The ratios of PUFA/SFA and n−6/n−3 ranged from 0.57 to 0.69 and 5.87 to 8.64. The ratios increased with soybean oil and probiotics treatments. There were not any significant interactions between soybean oil and probiotics in TSFA, inTMUSFA, in TPUSFA, in Tn6, in total Tn3, and in DFA.

Supplementations of probiotics significantly increased weights of kidney, pelvic, and heart fat (KPH fat), and percentage of KPH fat on carcass, but did not significantly affect other slaughter attributes. There was not obvious interaction between soybean oil and probiotics on the growing goats slaughter attributes.

The administered soybean oil and probiotics in feed significantly decreased the M. semimembranosus, and M. Triceps humeralis a* color (less redder). The l* and b* color of M. Longissimus dorsal for the goats supplemented with 2.5% soybean oil were greater than those of 5.0 % soybean oil supplementations and the control. There were not any significant interaction between soybean oil and probiotics in the goat meat chroma.

Administration of soybean oil and probiotics in diet of goats did not significantly change the *M. semimembranosus*, M. *longissimus dorsi* as well as M. *Triceps humeralis* shear force, and there were not any significant synergistic impact on meat shear force for soybean oil and probiotics.

The ether extracts of M. semimembranosus, M. longissimus dorsi, and M. Triceps humeralissample blend (W:W:W=1:1:1) for the goats receiving additional soybean oil and probiotics significantly increased, but the OM, DM, and CP did not increased significantly. There were not significant interactions between soybean oil and probiotics on the meat chemical compositions.

supplementations of soybean oil and probiotics significantly decreased M. semimembranosus, M. longissimus dorsi, and M. Triceps humeralismixed samples C14:0, C15:0, C16:0, C16:1 and C17:1 fatty acid centesimal compositions. Supplementations of 5.0% soybean oil were mildly greater than those of 2.5% soybean oil, the soybean oil and probiotics displayed synergistic impact on reduction of C15:0 but not for others.

All C18 fatty acids of the meat samples were increased due to supplementations of soybean oil and probiotics, but there was not any obvious interaction between soybean oil and probiotics on the C18 fatty acids except for CLA isomers.

The meat C18:c9,t11 and C18:t10,c12 CLA isomers centesimal composition ranged from 0.48 to 1.15% and 0.02 to 0.08% of total detected fatty acids. The C18:c9,t11 CLA increased 100 to 139.6%. The C18:t10,c12 CLA increased 100 to 300%. There was significant synergistic effect of soybean oil and probiotics on CLA isomers.

Supplementations of soybean oil and probiotics significantly decreased C20:0 and C22:0, significantly C20:3n. There were obvious interactions between soybean and probiotics on C20:0, C22:0, C20:2 and C20:3n. Administration of soybean oil and probiotics in goat feed significantly increased total CLA isomers, total n-6, and total

poly-unsaturated fatty acids; tended to increased total saturated, total n-3, total mono-unsaturated, and desirable fatty acids. Supplementation of 5.0% soybean oil significantly increased the ratios of poly-unsaturated fatty acids to total saturated fatty acids, significantly decreased the ratios of total n-6 fatty acids to n-3 fatty acids. A remarkable interaction between soybean oil and probiotics existed in total CLA isomers, total n-6 fatty acids, total saturated fatty acids, and total n-3 fatty acids (mg/g lipid).

The goat meat total CLA isomers and desirable fatty acids ranged from 5.36-8.17 mg/g lipid and 597.6 to 665.5 mg/g lipid owing to administrations of soybean oil and probiotics, those of 5.0% soybean oil supplementations were significantly higher than the control and administration of 2.5% soybean oil.

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CHAPTER VI

OVERALL DISCUSSION AND IMPLICATION

Nowadays, public requirements for food quality and safety, environmental deterioration and pollution, together with animal welfare have become the keystones that should be considered in animal agriculture. Goats are widely distributed around the world and goat meat is one of the crucial nutrition source of human (Webb et al., 2005). The production of goat meat must be of wholesomeness, and be free of pathogens and toxins, and should in compliance with the aforementioned keystones. In addition, since goat meat contains high CLA (McMillin et al., 2005) and the biological effects of CLA (such as immonumodulation, anti-atherosclerosis, anti-diabetes, and so on) have be well been documented (An, 2006), there is incentive for the production of goat meat containing increased proportions of CLA perceived as healthy and functional food (Kramer et al., 2006). This study aimed at utilizations of high bio-availability and nuisance free supplements to improve growth performance, ruminal metabolism, and in the high-light to increase the meat CLA content of stall-fed growing goats fed with whole plant corn silage.

The study demonstrated that administration of probiotics (*S. cerevisiae* and *L. acidophilus*) increased DMI, ADG, and feed conversion, optimized rumen metabolism, and increased dietary digestion. These findings resulted from the healthier

more favorable rumen setting and the subsequent improvement of nutrients digestion and absorption (Klaenhammer, 1998; Fonty et al, 2006). Moreover, these findings were in consisted with those of others. e.g., Kumagai et al. (2004), Han et al. (2008) Fadel Elseed et al. (2007), Feng et al. (2008) and other many researchers have stressed the improvement of growth performance and optimization of the ruminal metabolism.

Supplementation of probiotics increased plasma CLA and desirable fatty acids, optimized ratios of PUFA: SFA and n6:n3. Korniluk et al. (2007) asserted that addition of Se-yeast to the diets of rats that enriched in CLA isomers increased the yield of CLA isomers accumulation in the spleens and pancreas in comparison with those fed the diet enriched in only CLA isomers. CLA in *Lactobacillus* cultures are clearly stressed as early as1988 by Fairbank et al. Later Pariza and Yang (1999) developed a method for production of cis-9, trans-11 CLA by utilizing of Lactobacillus. The ability of converting linoleic acid to CLA of probiotic compounds had been approved by many people (Kishino et al., 2002; Julia et al., 2006). Furthermore, Ogawa et al. (2001) emphasized that *Lactobacillus acidophilus* converts linoleic acid to CLA under microaerobic conditions other than aerobic conditions, and supplementation of yeast scavenges the oxygen in rumen (Yoon et al., 1995), thus, supplemented *S.cerevisiae* and *L. acidophilus* together resulted in increase of CLA.

Supplementation of soybean oil and sunflower oil significantly increased ADG and feed efficiency without affect on DMI, rumen metabolism, and dietary

digestion. There were sufficient studies asserted that a certain (less than 5% diet DM) supplementation of soybean and sunflower oil positively increased the host animal's productivity performances without negative effect on DMI, rumen metabolism, and dietary digestion (Bouattour et al., 2008; Chilliard et al., 2006; Mir et al., 2002).

Supplementations of soybean oil and sunflower oil significantly decreased plasma C15:0 fatty acid, tended to increase total saturated fatty acids, and significantly enhanced plasma CLA content. These results could be a consequence of the higher levels of PUFA (C18:2 and C18:3), on the two substances (Bouattour et al., 2008), and a consequence of the devoid or very low level of C14:0, C15:0, and other middle chain fatty acids.

The administration of soybean oil together with probiotics in diet of growing goats significantly increased ADG and feed efficiency. There were distinct interaction between soybean oil and probiotics on the increase of ADG and feed conversion. Ringo et al. (1998) and Kaste et al. (2007) had testified the synergistic effects of PUFA and lactobacilli on colonization of fish and piglets intestine with lactobacilli. The present study testified that there were synergistic effects between soybean oil and probiotics on ADG and feed efficiency in growing goats.

Combining supplementation of soybean oil and probiotics decreased the plasma and meat C14:0, C15:0, C16:0, C16:1 and C17:1 fatty acids, increased all C18 fatty acids. The C18:c9,t11 and C18:t10,c12 CLA isomers increased significantly. Significant synergized effect of soybean oil and probiotics on the CLA isomers was

found. *Lactobacillus acidophilus* converts linoleic acid into CLA had been well approved (Ogawa et al., 2001; Kishino et al., 2002; Julia et al., 2006), thereupon, it reaseaable to find the significant synergistic effect of soybean oil and probiotics on the plasma and meat CLA isomers. Again, it was the characteristics of soybean oil (high level of C18 fatty acids, devoid or low level of C14:0 to C17:0) respond for the findings of decreasing C14:0 to C17:0 and increasing C18 fatty acids.

Combining supplementation of soybean oil and probiotics ranged the ratios of poly-unsaturated fatty acids to total saturated fatty acids and total n-6 fatty acids to n-3 fatty acids from 0.15 to 0.19 and 2.09-2.78 in goat meat. These findings were in accordance with Wendell et al. (2005) and the recommends The Health Department of England (HMSO, 1994).

Overall, the suggestions based on the findings of this study were as followed:

1. Supplementations of 2.5 and 5.0 g/h/d 2-dosages of *S. cerevisiae* and *L.acidophilus* blend to growing goats fed with whole plant corn silage significantly optimize rumen metabolism and increase dietary digestion, increase ADG, significantly increase feed efficiency;
2. Supplementations of soybean oil and sunflower oil to the growing goats improve ADG and ruminal metabolism, the supplementation of soybean oil is better than sunflower oil, and the appropriate dosages for soybean oil are 2.5 and 5.0% of supplemental concentrate;
3. Supplementations of 2.5 and 5.0% soybean oil together with 2.5 and 5.0 g/h/d

probiotics to growing goats, increase ADG and optimize ruminal metabolism;

4. Combining supplementations of soybean oil and probiotics improve goat carcass and meat quality;
5. Combining supplementations of soybean oil and probiotics decrease goat meat undesirable fatty acids such as C15:0, C16:0, increase desirable fatty acids (C18:0+all unsaturated fatty acids), significantly increase goat meat CLA contents; combining supplementations of soybean oil and probiotics push the goat meat become wholesome food;
6. Combining supplementations of soybean oil and probiotics ranged the meat n−6/n−3 ratios in a range of 2.09-2.78;
7. Combining supplementation of soybean oil and probiotics pushing the goat meat becomes healthy and functional food;
8. Synergistic effects between soybean oil and probiotics on the goat meat and carcass quality, on goat meat fatty acid profiles need further study to testify it.

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APPENDIX

Determination of lipid content and fatty acid profiles

Determination of lipid content

Chloroform-methanol (2:1, v/v)

Chloroform

0.58% NaCl solution

Dry N_2 gas

Blender/Homegenizer

250 ml Erlenmeyer flask

a) Weigh 5 g dry meat sample
b) Homogenize with 90 ml chloroform-methanol (2:1, v/v)
c) Add 30 ml chloroform, wait for 5 minutes and filter the solid sample waste away, then add 30 deionized water
d) Add 0.58% NaCl solution 5 ml, wait until the chloroform layer separates from the methanal- water phase
e) Transfer the chloroform phase (below level) to 250 ml flask that is weighed already
f) Evaporate the solvent under stream of dry N_2 gas
g) Determine lipid content

Preparation of sample for fatty acid profiles determination

Methanol

NaOH

0.5 M methanol-NaOH (dissolve 2 g NaOH into 1 ml Hexane)

Saturated NaCl solution (26.47 g NaCl into 100 ml DI water at 25 °C)

40 % boron trifluoride (BF3)

International acidinternal standard (heptadecanoic acid C17:0) (2 mg C17:0 dissolved into 1 ml hexane)

AOACS standard fatty acid methyl ester (FAME) mixture

Dry N_2 gas

10 ml test tube that with a butyl rubber septa screw cap

Water bath

Vortex

0.5 and 1 ml pipettes

a) Weigh 30 mg extracted lipid into the test tube
b) Add 1.5 ml 0.5 M methanol-NaOH, follow by shaking vigorously and putting into water bath and fix at 100 °C for 5 minutes
c) Cool down in cool water to normal room temperature, follow by drying with N_2 stream
d) Add 40% BF3 1ml (w=w; Supelco Inc., Bellefonte, PA, USA), shake vigorously and put into water bath and fix at 100 °C for 5 minutes
e) Cool down in cool water to normal room temperature, follow by drying with N_2 stream

f) Add International acidinternal standard (heptadecanoic acid C17:0) 1ml, shake vigorously and put into water bath and fix at 100 °C for 5 minutes

g) Cool down in cool water to normal room temperature, add 5 ml saturated NaCl solution follow by shaking vigorously and adding 2ml hexane

h) Wait for 10 minutes and measure 1 ml into a 2 ml vial for GC injection

Table A1 The samples preparation method for plasma urea nitrogen (PUN) analyzing.

Reagents	**Blank**	**Standard**	**Analyzing**
DI water (µl)	20	-	-
Standard (µl)	-	20	-
Samples(µl)	-	-	20
Color reagent(ml)	3.0	3.0	3.0
Acid reagent(ml)	2.0	2.0	2.0

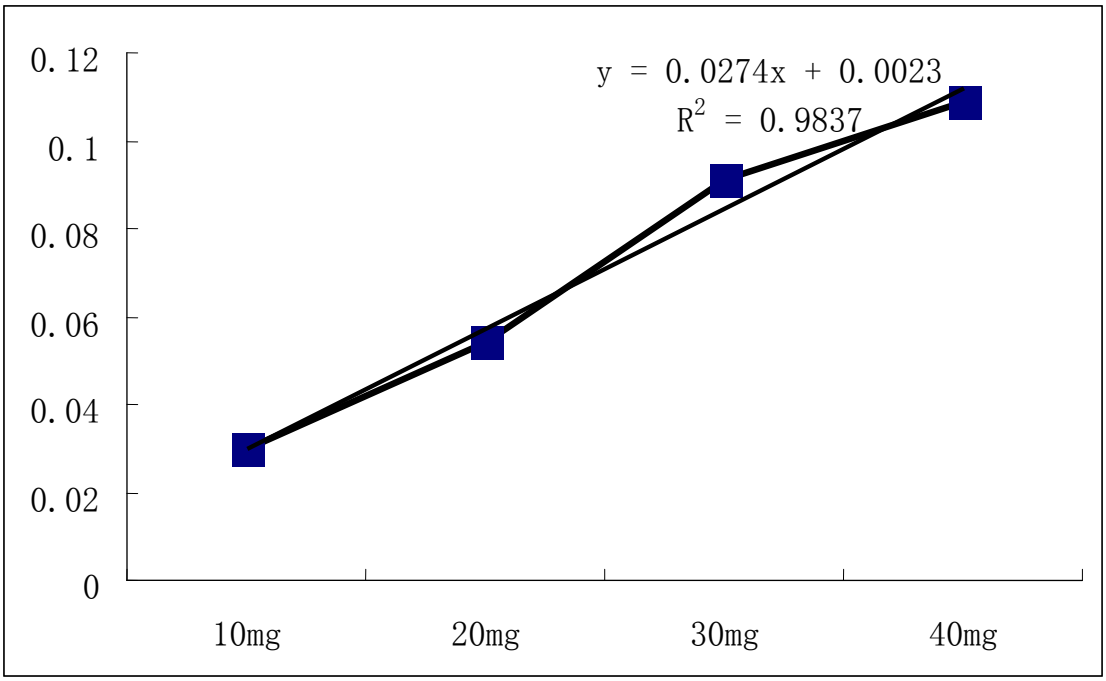


Figure A1 Linear equation and r^2 of the PUN standard

Table A2 The profiles of external standard adopted in the GC analysis.

fatty acid	Time(min)	Area(pA*S)	Amt/Area	Amount (mg/ml)
C4:0	13.66	223.57	1.79E-03	4.00E-01
C6:0	15.79	269.16	1.49E-03	4.00E-01
C8:0	18.04	303.09	1.32E-03	4.00E-01
C10:0	20.19	325.34	1.23E-03	4.00E-01
C11:0	21.31	163.14	1.23E-03	2.00E-01
C12:0	22.54	330.57	1.21E-03	4.00E-01
C13:0	23.96	171.34	1.17E-03	2.00E-01
C14:0	25.66	351.74	1.14E-03	4.00E-01
C14:1	27.48	158.71	1.26E-03	2.00E-01
C15:0	27.73	181.88	1.10E-03	2.00E-01
C15:1	30.02	167.01	1.20E-03	2.00E-01
C16:0	30.40	548.71	1.09E-03	6.00E-01
C16:1	32.75	159.90	1.25E-03	2.00E-01
C17:0	33.69	156.84	1.28E-03	2.00E-01
C17:1	36.72	171.37	1.17E-03	2.00E-01
C18:0	38.11	362.08	1.10E-03	4.00E-01
C18:1n9t	40.26	174.52	1.15E-03	2.00E-01
C18:1n9c	41.17	361.96	1.05E-03	4.00E-01
C18:2n6t	43.46	153.04	1.31E-03	2.00E-01
C18:2n6c	45.13	139.86	1.43E-03	2.00E-01

Profiles of Volatile Fatty Acid Standard Mix

Catalog No.46975-U

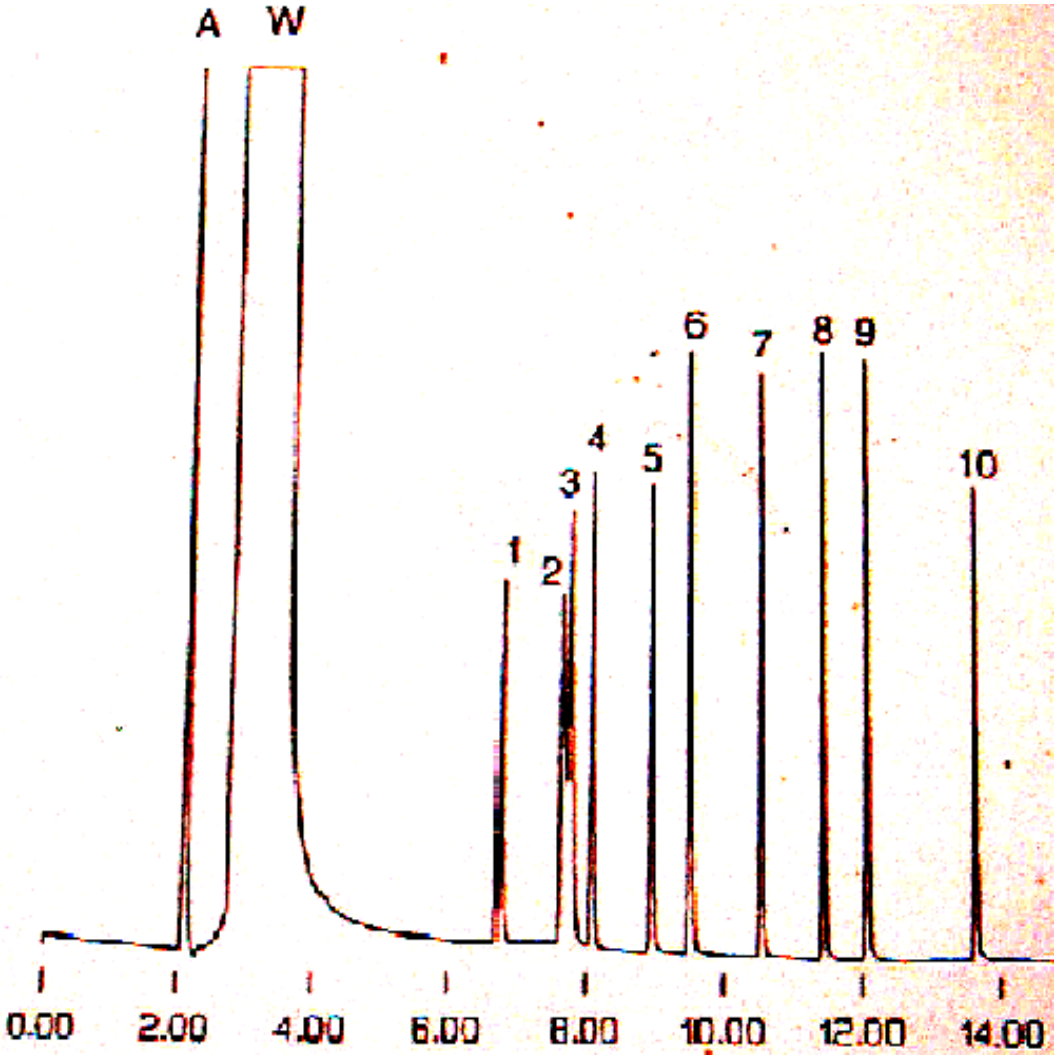


Figure A2 C1-C7 acids at 10Mm in deionized water (A. Air; W. Water; 1. Acetic acid; 2. Formic acid; 3. Prolionic acid; 4. Isobutyric acid; 5. Butyric acid; 6.Isovaleric acid;7. n-Valeric acid; 8.Isocaproic acid; 9. n-Caproic acid; 10.Heptanoic acid)

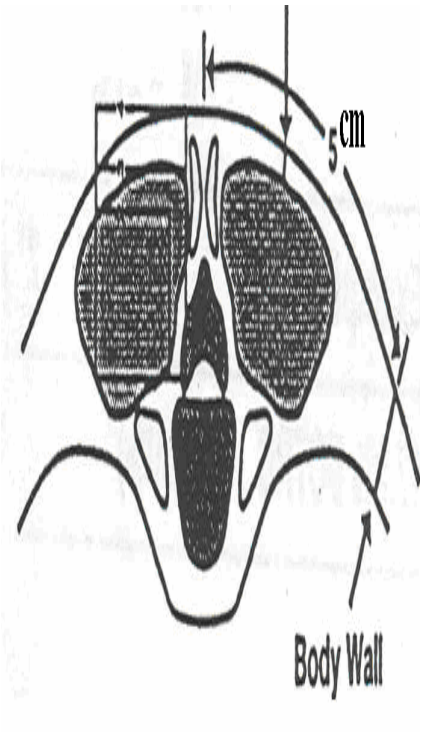
Table A3 The profiles of volatile acids standard adopted in the GC analysis

Fatty acids	**percent purity**	**concentration weight (mM)**	**Supelco lot No**
Formic acid	99.9	10.85	LA80814
Prolionic acid	99.0	10.07	LA59541
Isobutyricacid	99.0	10.39	LA47585
Butyric acid	99.0	9.99	LA72119
Isovaleric acid	99.0	9.99	LA44138
n-Valeric acid	99.0	10.23	LA49523
Isocaproic acid	99.0	10.00	LA84002
n-Caproic acid	99.0	10.23	LA49522
Hexanoic acid	99.0	9.94	LA50426
Heptanoic acid	99.0	10.14	LA49524

Score (15)

Score (13)

Score (11)



Body wall measurement

Figure A3 Leg score and body wall thickness measurement template

BIOGRAPHY

Mr. Yong Han was born on October 15, 1978 in Guizhou province, China. He received Bachelor degree in Animal Science from Guizhou University in 2003. In the same year, he continued his M.S study at Guizhou University.

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