EFFECTS OF FORAGE SPECIES AND FEEDING SYSTEM ON CONJUGATED LINOLEIC ACID (CLA) CONTENT IN GOAT'S MILK

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ผลของชนิดพืชอาหารสัตว์และระบบการให้อาหารต่อปริมาณ CONJUGATED LINOLEIC ACID (CLA) ในนมแพะ

นายศรัณย์พงศ์ ทองเรือง

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรดุษฎีบัณฑิต
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ศรัณย์พงศ์ ทองเรือง : ผลของชนิดพืชอาหารสัตว์และระบบการให้อาหารต่อปริมาณ CONJUGATED LINOLEIC ACID (CLA) ในนมแพะ (EFFECTS OF FORAGE SPECIES AND FEEDING SYSTEM ON CONJUGATED LINOLEIC ACID (CLA) CONTENT IN GOAT'S MILK) อาจารย์ที่ปรึกษา: รองศาสตราจารย์ ดร.ปราโมทย์ แพงคำ. 146 หน้า.

วัตถุประสงค์ของงานวิจัยนี้ คือ ผลของชนิดพืชอาหารสัตว์และระบบการเลี้ยงสัตว์แบบตัดสดมาให้สัตว์กิน เปรียบเทียบกับแบบปล่อยแทะเล็มต่อปริมาณ Conjugated linoleic acid (CLA) ในนมแพะ

การทดลองที่ 1 ทำการทดลองหาผลผลิต คุณค่าทางโภชนะ และกรดไขมันไลโนเลอิกและกรดไขมันไลโนเลนิก ของพืชอาหารสัตว์จำนวน 6 ชนิด แบ่งเป็นหญ้า 3 ชนิด ได้แก่ หญ้ากินนีสีม่วง หญ้าเนเปียร์จักรพรรดิ์ และหญ้าเนเปียร์ปากช่อง 1 และถั่ว 3 ชนิด ได้แก่ ถั่วฮามาต้า ถั่วไมยรา และกระถิน ตัดหญ้าที่อายุ 45 วัน และถั่วอายุ 60 วัน ผลการทดลอง พบว่า หญ้าเนเปียร์ปากช่อง 1 ให้ผลผลิตน้ำหนักแห้ง และผลผลิตกรดไขมันไลโนเลอิกและกรดไขมันไลโนเลนิกสูงกว่าหญ้าชนิดอื่นอย่างมีนัยสำคัญทางสถิติ ($P<0.01$) ส่วนกระถินให้ผลผลิตน้ำหนักแห้ง และผลผลิตกรดไขมันไลโนเลอิกและกรดไขมันไลโนเลนิกสูงกว่าถั่วชนิดอื่นอย่างมีนัยสำคัญทางสถิติ ($P<0.01$) ในการศึกษาการย่อยได้ในกระเพาะรูเมนโดยวิธีหย่อนถุงไนล่อนของแพะนมเพศผู้น้ำหนัก 30 ± 3 กิโลกรัม จำนวน 3 ตัว พบว่า หญ้าและถั่วมีประสิทธิภาพในการย่อยได้ในกระเพาะรูเมนของวัตถุแห้ง โปรตีน Neutral Detergent Fiber (NDF) กรดไขมันไลโนเลอิก และกรดไขมันไลโนเลนิกไม่แตกต่างกันแต่สูงกว่าฟางข้าวอย่างมีนัยสำคัญทางสถิติ ($P>0.05$)

การทดลองที่ 2 ทำการศึกษาผลของชนิดของพืชอาหารสัตว์ที่แตกต่างกันต่อจำนวนประชากรจุลินทรีย์ *Butyrivibrio fibrisolvens*, *Fibrobacter succinogenes* และ total bacteria ในกระเพาะรูเมน จากการศึกษาในแพะนมเพศผู้เจาะกระเพาะ จำนวน 3 ตัว น้ำหนัก 33 ± 3 กิโลกรัม วางแผนการทดลองแบบ 3 × 3 ลาตินสแควร์ โดยแบ่งการทดลองออกเป็น 2 การทดลอง คือ การทดลองเปรียบเทียบหญ้า 3 ชนิดและ การทดลองเปรียบเทียบถั่ว 3 ชนิด ผลการทดลองพบว่า ชนิดของพืชอาหารสัตว์ ไม่ว่าจะเป็นการทดลองในหญ้าหรือการทดลองในถั่ว ไม่มีความแตกต่างกันอย่างมีนัยสำคัญทางสถิติต่อจำนวนประชากรจุลินทรีย์ทั้ง 3 ชนิดในกระเพาะรูเมน

การทดลองที่ 3 ทำการศึกษาผลของชนิดพืชอาหารสัตว์และระบบการเลี้ยงต่อปริมาณ CLA ในน้ำนมแพะและผลต่อจำนวนประชากรจุลินทรีย์ *B. fibrisolvens*, *F. succinogenes* และ total bacteria ในกระเพาะรูเมน โดยใช้แพะนมลูกผสมพันธุ์ซาแนน (>75%) น้ำหนักเฉลี่ย 35 ± 3 กิโลกรัม จำนวน 20 ตัว แบ่งออกเป็น 2 การทดลอง คือ การทดลองที่ 3.1 เปรียบเทียบการเลี้ยงแบบ

ตัดสดกับแบบปล่อยแทะเล็มแบบหมุนเวียนของแพะที่ได้รับหญ้าเนเปียร์ปากช่อง 1 ซึ่งเป็นหญ้าที่ให้ผลจากการทดลองดีที่สุดจากการทดลองที่ 1 และ 2 และการทดลองที่ 3.2 เปรียบเทียบการเลี้ยงแบบตัดสดกับแบบปล่อยแทะเล็มแบบหมุนเวียนของแพะที่ได้รับกระถินซึ่งเป็นถั่วที่ให้ผลจากการทดลองดีที่สุดจากการทดลองที่ 1 และ 2 ผลการทดลองพบว่า แม่แพะรีดนมที่ปล่อยแทะเล็มทั้งการทดลองกับหญ้าเนเปียร์ปากช่อง 1 และการทดลองกับกระถิน มีปริมาณการกินได้ ผลผลิตน้ำนม ไขมันนม ของแข็งรวม ปริมาณ CLA ชนิด Cis-9,Trans-11 CLA และชนิด Trans-10,Cis-12 CLA และโอเมก้า-3 สูงกว่ากลุ่มที่ตัดสดและนำไปให้กินทั้งหญ้าและถั่ว อย่างมีนัยสำคัญทางสถิติ ($P<0.05$)

สาขาวิชาเทคโนโลยีการผลิตสัตว์
ปีการศึกษา 2558

ลายมือชื่อนักศึกษา_____
ลายมือชื่ออาจารย์ที่ปรึกษา_____
ลายมือชื่ออาจารย์ที่ปรึกษาร่วม_____
ลายมือชื่ออาจารย์ที่ปรึกษาร่วม_____

SARANPONG THONGRUANG : EFFECTS OF FORAGE SPECIES AND FEEDING SYSTEM ON CONJUGATED LINOLEIC ACID (CLA) CONTENT IN GOAT'S MILK. THESIS ADVISOR : ASSOC. PROF. PRAMOTE PAENGKOUM, Ph.D., 146 PP.

FORAGE SPECIES/FEEDING SYSTEM/ CONJUGATED LINOLEIC ACID/ GOAT'S MILK

The aim of this research was to study the effects of forage species and the feeding system on conjugated linoleic acid (CLA) content in goat's milk.

The first experiment was carried out to investigate dry matter (DM), crude protein (CP), neutral detergent fiber (NDF), fat, linoleic acid (C18:2n6) and alpha-linolenic acid (C18:3n3) yields, chemical compositions, %C18:2n6, %C18:3n3 and ruminal degradability of 6 forage species, including 3 grasses and 3 legumes. In the grass experiment, Napier Pak Chong 1 was higher ($P<0.01$) in DM, CP, NDF, fat, C18:2n6 and C18:3n3 yields, %CP, %C18:2n6 and %C18:3n3 than Chinese Pennisetum and Purple Guinea. In the legume experiment, Leucaena was higher ($P<0.01$) in DM, CP, NDF, fat, C18:2n6 and C18:3n3 yields, %CP, %C18:2n6 and %C18:3n3 than Hamata and Hedge Lucern.

Potential and effective DM, CP, NDF, C18:2n6 and C18:3n3 degradability of Napier Pak Chong 1 were higher ($P<0.05$) than that of rice straw, but not different from those of Chinese Pennisetum and Purple Guinea. Potential and effective DM, CP, NDF, C18:2n6 and C18:3n3 degradabilities of Leucaena were higher ($P<0.05$) than that of rice straw, but not different from those of Hedge Lucern and Hamata.

The second experiment was to investigate the effects of forage species on *Butyrivibrio fibrisolvens*, *Fibrobacter succinogenes* and the total bacteria population in goat's rumen. In the grass experiment, the population of *B. fibrisolvens*, *F. succinogenes* and the total bacteria was not different from three grass species including Purple Guinea, Chinese Pennisetum and Napier Pak Chong 1. In the legume experiment, the population of *B. fibrisolvens*, *F. succinogenes* and the total bacteria was not different from three legume species including Hamata, Hedge Lucern and Leucaena.

The third experiment was to investigate the effects of forage species and the feeding system on CLA and omega-3 contents in goat's milk and on *B. fibrisolvens*, *F. succinogenes* and the total bacteria population in goat's rumen. For Napier Pak Chong 1 grazing milking goats, the population of *B. fibrisolvens*, *F. succinogenes* and the total bacteria was higher ($P<0.05$) than that in Napier Pak Chong 1, cut-and-carry milking goats. For Leucaena grazing milking goats, the population of *B. fibrisolvens*, *F. succinogenes* and the total bacteria was higher ($P<0.05$) than that in Leucaena cut-and-carry milking goats. Napier Pak Chong 1 grazing milking goats had higher ($P<0.01$) c9,t11 CLA, t10,c12 CLA and omega-3 than thosep in Napier Pak Chong 1 cut-and-carry milking goats. Leucaena grazing milking goats had higher ($P<0.05$) c9,t11 CLA, t10,c12 CLA and omega-3 than those in Leucaena cut-and-carry milking goats.

School of Animal Production Technology

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LIST OF ABBREVIATIONS

ADF	=	Acid Detergent Fiber
BUN	=	Blood Urea Nitrogen
BW	=	Body Weight
C18:2, C18:2n6	=	Linoleic acid
C18:3	=	Linolenic acid
C18:3n3	=	alpha-linolenic acid (α-linolenic acid)
c9,t11	=	Cis-9, Tran-11 CLA
CF	=	Crude Fiber
CLA	=	Conjugated linoleic acid
CP	=	Crude Protein
DM	=	Dry Matter
NDF	=	Neutral Detergent Fiber
NRC	=	National Research Council
SEM	=	Standard Error of Means
t10,c12	=	Trans-10, Cis-12 CLA
VFA	=	Volatile Fatty Acid

CHAPTER I

INTRODUCTION

Nowadays consumers prefer healthy foods those prevented illness, organic foods that made from natural with free of chemical also friendly to environment. They would also like to have food from sustainable farming system with happy-life animals. Milk and milk products with high in natural conjugated linoleic acid (CLA) is one of that needs, because CLA has potential to anti-carcinogenic, anti-oxidant, anti-atherosclerosis, positive effects on cardiovascular diseases, anti-diabetic, anti-obesity, immunomodulation and reduces the plasma cholesterol concentrations (O'Shea et al., 1998; O'Shea et al., 2000; Laso et al., 2007). Natural CLA is synthesized from ruminants especially from grazing animals in the grass and legume pasture. Many commercial CLA capsules in the market are chemically synthesized in the laboratory, so still concern for consumer about how safety and how friendly to environment. Consumers prefer natural CLA in milk and dairy products. Natural CLA content in milk varies widely depending on breeds of animal. For example, dairy goats produce higher CLA than dairy cows (Ceballos et al., 2009). CLA contents of animal products vary widely depending on species and/or varieties of feeds (Clapham et al., 2005; Shingfield et al., 2005). Feeding system, such as grazing pasture animals, has greater CLA than animal fed in conventional system (Dewhurst et al., 2003; Dhiman et al., 1999). The aim of this experiment was, therefore, to study the effects of forage species and feeding system on CLA content in goat's milk.

1.1 Research hypothesis

Dairy goats grazing higher herbage linoleic and linolenic acid of tropical forage crops have greater CLA content in milk than those cut and carry.

1.2 Research objectives

1.2.1 To examine forage yields, nutritive values, linoleic and linolenic acid contents of tropical forage species.

1.2.2 To investigate the DM, CP, NDF, linoleic and linolenic acid degradabilities of tropical forage species in goat's rumen.

1.2.3 To examine the effect of tropical forages species on *Butyrivibrio fibrisolvens*, *Fibrobacter succinogenes* and total bacteria population in goat's rumen.

1.2.4 To study on the effect of tropical forages species and feeding system on CLA content in goat's milk and on *Butyrivibrio fibrisolvens*, *Fibrobacter succinogenes* and total bacteria population in goat's rumen.

1.3 Scope and limitation of this study

This study will focus on influence of some tropical grass and legumes and feeding system (cut-and-carry and rotational grazing system) on quantity of CLA producing bacteria *Butyrivibrio fibrisolvens* and *Fibrobacter succinogenes* and also total bacteria in crossbred Saanen goat's rumen by using real-time PCR technique and on CLA content in crossbred Saanen goat's milk.

1.4 Expected results

Dairy goats grazing higher herbage linoleic and linolenic acid contents of tropical forage crops have greater milk's CLA than those cut and carry.

1.5 References

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CHAPTER II

REVIEW OF THE LITERATURE

The definition of Conjugated linoleic acid (CLA), the synthesis of CLA, the advantage of CLA for consumers and also factors affecting CLA content in ruminant will be described.

2.1 The definition of conjugated linoleic acid

CLA standing for Conjugated linoleic acid which is isomer of C18:2, one of the polyunsaturated fatty acid (PUFA), was synthesized in the rumen of ruminants by *Butyrivibrio fibrisolvens*. Natural CLA was found in milk and meat of ruminants. The main isomer of CLA is cis-9, trans-11 octadecadienoic acid also known as rumenic acid (Kramer et al., 1998), another form is trans-10, cis-12 octadecadienoic acid (Parodi, 1977; Britton et al., 1992; Chin et al., 1992; Parodi, 1994). O'Shea et al. (1998) found total CLA in milk being between 2-30 mg/g fat and found cis-9, trans-11 octadecadienoic acid approximately 90% of total CLA.

2.2 CLA synthesis

The presence of CLA in milk fat from ruminants resulted from the isomerization and biohydrogenation of unsaturated fatty acid by rumen bacteria *Butyrivibrio fibrisolvens* as well as the Δ9-desaturase activity in the mammary gland. Linoleic acid and α-linolenic acids in animal feeds are the main precursors of cis-9,

trans-11 CLA and trans-10, cis-12 CLA in milk. The synthesis pathway of CLA starts with isomerization of linoleic acid (cis-9, cis-12 C18:2) from feed to CLA (cis-9, trans-11 C18:2). CLA, an intermediates substrates, can transfer directly to the target tissue and CLA can also be reduced to vaccenic acid (trans-11 C18:1) which is the end product of biohydrogenation. Dewhurst et al. (2003) described that linoleic acid and α-linolenic acid are the predominant unsaturated fatty acids in forages which are the main precursors of c9t11 CLA and t10c12 CLA in milk. Many recent studies showed large effects of special concentrates on levels of fatty acids in milk and meat. Herbage lipids are the cheapest and safest sources of these fatty acids. The forages and pastures are, therefore, important long-term strategy of CLA. The synthesis pathway of CLA also describe in figure 2.1. (Adapted from Griinari and Bauman, 1999)

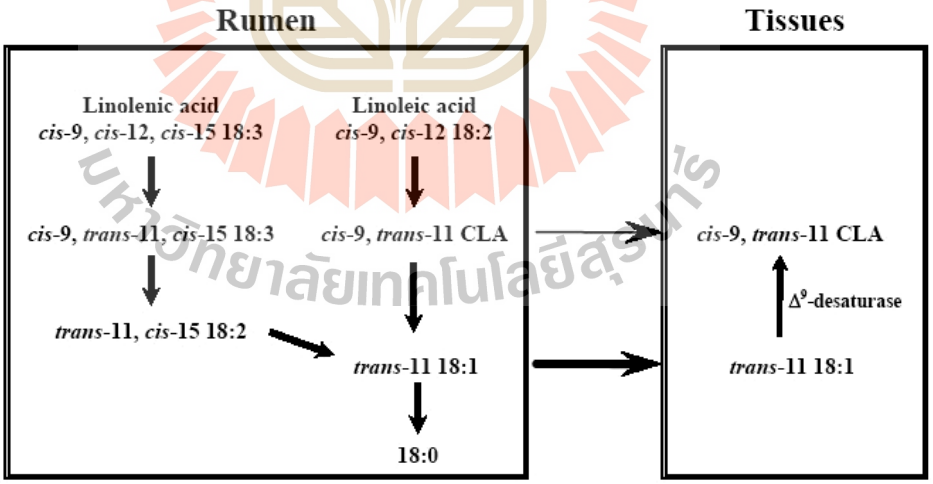

Figure 2.1 The synthesis pathway of CLA in ruminants (Adapted from Griinari and Bauman, 1999).

2.3 Advantage of CLA for consumers

CLA has potential to be anti-carcinogenic, anti-oxidant, anti-atherosclerosis, positive effects on cardiovascular diseases, anti-diabetic, anti-obesity, immunomodulating and reduces the plasma cholesterol concentrations (O'Shea et al., 2000; Laso et al., 2007).

As anti-carcinogenic, CLA could delay or reduce the onset of chemically-induced tumours in various sites including epidermal (Ha et al., 1987; Belury et al., 1996), mammary glands (Ip et al., 1991, 1994; Thompson et al., 1997), digestive tract (Ha et al., 1990; Liew et al., 1995), prostate gland and ovary (Scimeca, 1999) and the others various organ (Shultz et al., 1992; Schonberg and Krokan, 1995; Pariza and Hargraves, 1985). Mechanisms proposed to explain how CLA exerts some of its anticancer activity including conversion to potent cytotoxic lipid peroxidation products in human tumour cell lines; inhibition of nucleotide and protein biosynthesis; interference in oestrogen-regulated mitogenic pathway in breast cancer cells via blockage of cell division at the G0/G1 phase; inhibition of protooncogene expression (c-myc); decreased production of growth stimulatory eicosanoids via the inhibition of arachidonic acid synthesis resulting in alteration of the lipoxygenase and/or cycloxygenase pathways; modulation of cellular host defense systems by stimulation of lymphocyte and macrophage activity and modulation of hepatic lipid composition and metabolism (O'Shea et al., 1998).

Morphological and biological status of the mammary gland as influenced by conjugated linoleic acid. Implication for a reduction in mammary cancer risk (Thompson et al. 1997) using female Sprague Dawley rats being fed 1% CLA diet from weaning until 6 months of age, with the use of the dimethyl-benz[a]anthracene (DMBA) model showed that mammary tumour inhibition of rat fed 1% CLA was

about 57% of control. Milk fat conjugated linoleic acid (CLA) inhibits growth of human mammary MCF-7 cancer cells. Milk enriched in CLA was obtained from cows on pasture supplemented with full fat rapeseeds and full fat soybeans. The result showed that cell number decreased up to 90% ($P<0.05$) (O'Shea et al. 2000).

The benefits of anti-oxidant by CLA are prevention of peroxidation of unsaturated fatty acid (Ha et al., 1990) and prevention of oxidation of linoleic acid (van den Berg et al., 1995; Banni et al., 1996). For anti-atherosclerosis, CLA will decrease LDL (low density lipoprotein) (Lee et al., 1994; Nicolosi et al., 1997). For Anti-obesity, research found that CLA help loss fatty tissue in swine (Dugan et al., 1997) and in rats (Chin et al., 1994; Park et al., 1997) because CLA effective on fat to lean repartitioning and increased lypolysis and decreased lypogenesis.

Laso et al. (2007) reported that CLA in milk works for weight management. Recruited 60 overweight and obese men and women, with body mass index between 25-35 kg per m^2 and age between 35-65 years old were randomly assigned to a daily intake of 500 ml skimmed milk fortified with 3 grams of CLA (Tonalin) or a placebo milk. The result showed that significant 3% body fat mass reduction over 12 weeks was observed, comparing to the same number of people drinking a placebo milk (control). For immunomodulating, CLA actions to increase mitogen induced lymphocyte blasto-genesis. Modulation of cellular host defenses systems by stimulation of lymphocyte and macrophage activity.

2.4 Factors affecting CLA content in ruminants

Naturally, CLA in milk varied greatly depending on breeds of animal and it is often found that dairy goats produced higher CLA than dairy cows (Ceballos et al.,

2009). Animal feeds contain various level of precursor for CLA, depending on species of feeds (Shingfield et al., 2005; Clapham et al., 2005) while feeding system of grazing pasture animals produced greater CLA than animal fed conventional system (Dewhurst et al., 2003; Dhiman et al., 1999).

2.4.1 Factor of animal breeds on CLA content in ruminants

Researcher found that CLA in goats was higher than in cows. Ceballos et al. (2009) worked on composition of goat's and cow's milks produced under similar conditions; comparing concentrate and alfalfa hay, the results showed that Granadina goat's milks (5.23% fat, 13.57% TS and 0.68 g/100g total fatty acid CLA) were significantly higher ($P < 0.05$) than Holstein Friesian cow's milk (3.42% fat, 11.36% TS and 0.45 g/100g total fatty acid CLA). Effect of grazing white clover pasture on milk composition of Holstein and Jersey cows was reported by White et al. (2001) who found that no direct effect of increased grazing in the diet on the short chain FA content in milk, but the content of CLA has a strong positive effect on Holstein milk, but almost none on Jersey milk suggesting that the mammary gland Δ9-desaturase activities of these two cows reacted differently.

2.4.2 Factor of animal feed on CLA content in ruminants

Many recent studies showed large effects of special concentrates and pasture fed on levels of fatty acids in milk and meat. Herbage lipids are the cheapest and safest source of fatty acids produced. Forages and pastures are an important long-term strategy of CLA (Dewhurst et al., 2003). Shingfield et al. (2005) suggested that intake of fatty acid by grazing ruminants would be affected by the forage species consumed; Perennial ryegrass (*Lolium perenne*), 14.2% C18:2, 50.4% C18:3, 20.1% C16:0, Maize (*Zea mays*), 44.8% C18:2, 6.6% C18:3, 17.4% C16:0, 20.3% C18:1. Clapham et al.

(2005) studied 13 species of native forage fatty acid compositions and reported that the average fatty acid compositions of all forages were 2.0-10.3 mg/g DM (16%) linoleic acid (C18:2), 7.0-38.4 mg/g DM (62%) linolenic acid (C18:3) and 2.6-7.5 mg/g DM (16%) palmitic acid (C16:0) while Chicory (*Cichorium intybus*) had highest C18:3 and C18:2, followed by White clover (*Trifolium repens*) and Orchard grass (*Dactylis glomerata*). For tropical forages, Leucaena leaves contains 15.4% palmitic acid (16:0), 19.8% oleic acid (18:1), 51.1% linoleic acid (18:2), 13.6% linolenic acid (18:3) (Lin et al., 1985). *Pennisetum purpureum* cv. Pak Chong I grass was established by Nakhon Ratchasima Animal Feed Research and Development Center, Department of Livestock Development, Ministry of Agriculture and Cooperatives (Keawthong, 2002). This is a crossbred between *Pennisetum purpureum* and *Pennisetum americanum*, perennial, no seeding and short flowering period, height 2-4 meters, high leaf to stem ratio, high yield, protein 15-18% reported by Nakhon Ratchasima Animal Feed Research and Development Center, suitable cutting period at 45 days interval for fresh grass and 60-75 days interval cutting for silage, crude fat 6.2-6.9% (Var. CO-3) (Premaratne and Premalal, 2006) and now it's a favorite grass planting all over the country. Chinese Pennisetum is a crossbred grass between *Pennisetum purpureum* and *Pennisetum alopecuroides*has contained 18.46% crude protein, 1.74% fat, 9.91% ash and 17.7% fiber, 25-35 degree Celsius optimum temperature for growth and 10-15 tons DM per year of production yield.

Addis et al. (2005) milk and cheese fatty acid composition in sheep fed mediterranean forages with reference to conjugated linoleic acid cis-9, trans-11. Mediterranean pastures are based upon self-seeding grasses (e.g., annual ryegrass, *Lolium rigidum* Gaudin) and legumes (e.g., burr medic, *Medicago polymorpha* L.) or

short-lived legumes, like the sulla. These forages are an important component of Mediterranean grazing systems for dairy sheep, showing specific beneficial features in terms of persistence, forage production, forage quality, and animal response. Other species are interesting for dairy sheep grazing systems, namely nonconventional forages palatable to grazing sheep, such the Asteracea *Chrysanthemum coronarium* L. Two experiments were undertaken to evaluate the effect on milk and cheese fatty acid composition of feeding different fresh forages to dairy sheep both in winter (experiment 1, growing stage of the forages, early lactating ewes) and in spring (experiment 2, reproduction stage of the forages, midlactating ewes). Four forage species were compared: annual ryegrass (RY, *Lolium rigidum* Gaudin), sulla (SU, *Hedysarum coronarium* L.), burr medic (BM, *Medicago polymorpha* L.), and a daisy forb (CH, *Chrysanthemum coronarium* L.). The forages were cut twice daily and offered ad libitum to 4 replicate groups of Sarda dairy sheep (groups RY, SU, BM, and CH). The CH forage was particularly rich in linoleic acid in both periods, whereas BM and SU forages were rich in linolenic acid in winter and spring, respectively. This daisy forb is highly productive and can complement grass-based systems as well as other nonconventional daisy forages such as chicory (*Chicorium intybus*) or spineless safflower (*Carthamus tinctorius*). In particular, it has been found that sheep grazing *Chrysanthemum coronarium* during springtime ingest the daisy flowers, putatively raising their intake of CLA precursors, which are concentrated in the flowers. Milk fatty acid composition was affected by the forage in both experiments. Milk conjugated linoleic acid and vaccenic acid contents were higher in CH and BM groups (winter) and CH group (spring) than in the other groups. Table 2.1 showed the result of spring season, plant C18:2 were higher in a daisy forb (0.50% DM) than in the other

groups, while sulla (0.59% DM) was higher in plant C18:3 than in the other groups. Milk CLA was higher ($P < 0.05$) in a daisy forb (2.33 g/100g fatty acid) than burr medic (1.65 g/100g fatty acid) and annual ryegrass (1.43 g/100g fatty acid), while was lowest in sulla (1.12 g/100g fatty acid). No differences were observed when comparing fatty acid profile between milk, 1-d-old cheeses, and 60-d-old cheeses within experimental groups, suggesting that the fatty acid recovery rates during cheese making and ripening were not affected by the feeding regimens. After stepwise discriminant analyses of the pooled data, the milks and cheeses sourced in the different feeding regimens differed among them. Based on these results, we conclude that it is possible to manipulate the fatty acid profile of sheep dairy produce to maximize the content of beneficial fatty acids by the use of appropriate fresh forage-based regimens.

Meinhardt et al. (2004) were study on grazing stockpiled annual ryegrass and cereal rye with a seasonal dairy herd. The results shown that there were no treatment differences in daily milk yield, (67 lb milk/cow/day) or in milk yield per acre (7920 lb/acre of milk). There were significant differences in milk fatty acid composition. CLA (Table 2.1), C18:1 11t and C22:4n6 in milk were elevated when cows grazed annual ryegrass. In late May, milk from cows grazing annual ryegrass contained 14.2 mg CLA/g of fat, 30 % more CLA than milk from cows grazing cereal rye (9.0 mg CLA/g of fat). Results of this study show that both cereal rye and annual ryegrass are practical forages for late winter-early spring on pasture based dairy operation. While cereal rye was a better late winter forage, annual ryegrass persisted longer into the spring season. This study also stresses the importance of further research on forage species and fatty acid content and fall management of accumulated forages.

Table 2.1 Effect of C18:2, C18:3 in individual forage plants on CLA in milk of ruminants.

Forage species	Fatty acids in plants (%DM)		CLA in Milk	References
	C18:2	C18:3		
Daisy forb	0.50	0.19	2.33[a] g/100g fat	Addis et al. (2005)
Burr Medic	0.29	0.34	1.65[b] g/100g fat	
Sulla	0.14	0.59	1.12[c] g/100g fat	
Annual Ryegrass	0.12	0.44	1.43[bc] g/100g fat	
Annual Ryegrass	-	-	14.2[a] mg/g fat	Meinhardt et al. (2004)
Cereal Rye	-	-	9.06[b] mg/g fat	

[a,b,c] Means within column with different superscripts differ ($P < 0.05$).

Effect of C18:2, C18:3 in individual forage plants on CLA and vaccenic acids in rumen by Buccioni et al. (2009) study on effect of three species of herbage (*Medicago sativa, Lolium multiflorum, Avena sativa*) on *in vitro* ruminal production of conjugated linoleic and vaccenic acids. Little information is available about the effect of different forage species on the rumen biohydrogenation process. The aim of the present work is to compare the *in vitro* production of CLA and C18:1 isomers after incubation of three different herbage species in rumen liquor from sheep. Pasture herbage samples of lucerne (*Medicago sativa*; MS), ryegrass (*Lolium multiflorum*; LM) and oats (*Avena sativa*; AS) were submitted to *in vitro* fermentation with sheep rumen inoculum. Samples were collected at 2, 4, 6 and 8 hours of fermentation. The

fatty acid profile of MS was characterised by 11.62 (g/100 g of lipid extract) of linoleic acid (LA) and 27.08 (g/100 g of lipid extract) of α-linolenic acid (LNA), whereas LA in the other two herbages was 6.60 (g/100 g of lipid extract) and 6.95 (g/100 g of lipid extract) in AS and LM, respectively; LNA was 52.20 (g/100 g of lipid extract) and 54.49 (g/100 g of lipid extract) in AS and LM, respectively. The crude fat content of botanical species was respectively 11.90 (g/100g DM) for AS, and 15.77 (g/100g DM) for LM and 26.17 (g/100g DM) for MS. Rumenic acid (RA, cis-9, trans-11 CLA) was the predominant CLA isomer and the maximum yield was attained with AS after 6 hours of fermentation (0.81 g/100 g of lipid extract); RA concentration remained quite low with the other two herbages. The concentration of the other isomer (trans-10, cis-12 CLA) was always very low; the maximum yield (0.09 g/100 g of lipid extract) was reached after 6 hours with AS. The maximum yield of vaccenic acid (VA, trans-11 C18:1) was reached after 8 hours with MS (2.64 g/100 g of lipid extract) (Table 2.2). This herbage also produced the highest amount of trans-10 C18:1 at 6 and 8 hours (0.17 g/100 g of lipid extract). AS appeared to have induced the highest amounts of RA relative to the other two forages. The differences in conjugated dienes and C18:1 isomers content during fermentation could be due not only to different amounts of LA or LNA in the herbage, but also to different releasing times of FA from the plant substrate.

Table 2.2 Effect of C18:2, C18:3 in individual forage plants on CLA and vaccenic acids (VA) in rumen.

Forage species	Fatty acids in plants		CLA in rumen (g/100g fat)	VA in rumen (g/100g fat)
	C18:2	C18:3		
Oats	6.60 g/100g fat	52.20 g/100g fat	0.81[a]	1.72[b]
Ryegrass	6.95 g/100g fat	54.49 g/100g fat	0.01[c]	0.45[c]
Lucerne	11.62 g/100g fat	27.08 g/100g fat	0.07[b]	2.64[a]

[a,b,c] Means within column with different superscripts differ ($P < 0.05$).

Source: Buccioni et al. (2009)

Botanical composition has potential to increase CLA level in milk of ruminant by mixing of high linoleic acid plants. Wu et al (1997) were worked on Paddocks containing Red clover compared to all grass paddocks support high CLA levels in milk**.** The concentration of CLA in milk before and during grazing did not differ between primiparous and multiparous cows. Grazing increased the concentration nearly 2 fold (10.8 vs. 5.8 mg/g of fat, $P<0.001$) without changing milk fat content (Table 2.3). Pasture provided approximately 60% of the total feed intake (due to feeding of the supplemental mix). Energy supplements are usually fed to grazing cows for maximum milk yields. Concentration of CLA in milk was approximately 50% (14.0 vs. 9.2 mg/g) higher ($P<0.001$) for cows grazing mixed red clover and grasses than those grazing grasses only. Grazing lactating cows increased concentration of CLA in milk. The concentration was further increased when the pasture contained red clover. Grazing on pastures containing abundant red clover with minimum concentrate supplementation has potential to produce milk with exceptionally high CLA content.

Different geological locations are important to CLA level. Collomb et al. (2002) studied on composition of fatty acids in cow's milk fat produced in the lowlands, mountains and highlands of Switzerland using high-resolution gas chromatography. The compositions of fatty acids (approx. 70 acids) in 44 summer milk samples from three geographical sites were determined using high-resolution gas chromatography. They observed large differences between Lowlands (600–650 m.), Mountains (900–1210 m.) and Highlands (1275–2120 m.) which are analogous to those observed between winter and summer fats. There were 16 botanical records in the Lowlands, 31 in the Mountains and 55 in the Highlands. The largest relative increases as a function of the altitude of these three sites were those of the concentration of conjugated linoleic acids (0.87, 1.61 and 2.36 g/100g fat) (Table 2.3), especially of the cis-9 trans-11 isomer (0.81, 1.50 and 2.18 g/100g fat), and the fatty acids C18:1 t10+t11 (2.11, 3.66 and 5.10 g/100g fat). There were significant differences in the concentration of fatty acids between the three geographical sites.

Table 2.3 Effect of C18:2, C18:3 in botanical composition on CLA in milk of ruminants.

Forage species	**Botanical composition**	**CLA in milk**	**References**
100% grass	Kentucky bluegrass, Quack grass and Smooth bromegrass	9.2[b] mg/g fat	Wu et al. (1997)
20% red clover 80% grass	Red clover and Same species of grass	14.0[a] mg/g fat	
Highlands (1275-2120 m)	55 plant species	2.36[a] g/100g fat	Collomb et al. (2002)
Mountains (900-1210 m)	31 plant species	1.61[b] g/100g fat	
Lowlands (600-650 m)	16 plant species	0.87[c] g/100g fat	

[a,b,c] Means within column with different superscripts differ ($P < 0.05$).

Mel'uchová et al. (2008) worked on seasonal variations in fatty acid composition of pasture forage plants and CLA content in ewe milk fat. The relations between fatty acid (FA) composition of pasture forage plants and the content of conjugated linoleic acid (CLA) as a total content of cis-9, trans-11 + trans-7, cis-9 + trans-8, cis-10 CLA isomers in ewes' milk fat during natural pasture season (April–September) were investigated (Figure 2.2). The extracts of ewes' milk fat samples as well as the pasture samples were analyzed for fatty acid composition by capillary gas chromatography with flame ionization and mass spectrometric detection. The investigated pasture is composed of three botanical families: *Poaceae*, *Fabaceae*, and *Herbaceae*. The *Poaceae* was a dominant botanical family throughout the pasture season. α-Linolenic, linoleic, and palmitic acids were predominant in pasture plants, and their contents varied during pasture season. The most abundant and most varied fatty acid compound in pasture plants was α -linolenic acid. Its content significantly decreased from 62% to 39% (of total FA) ($P < 0.001$) from May to August, and subsequently it slightly (57%) increased from August to September ($P < 0.05$), compared with the beginning of pasture season. Similarly, the content of CLA in ewes' milk fat decreased from 2.4% in May to 1.3% in August ($P < 0.001$), and subsequently it rose to 2.6% in September ($P < 0.001$). The α-linolenic/linoleic acid ratio in the pasture sample decreased from 4.36 in May to 1.97 in August ($P < 0.001$), and subsequently it increased to 3.14 in September ($P < 0.001$); thus, it reached the level approaching to that at the beginning of pasture season. The pasture seasonal variations in the ratio were directly proportional to the corresponding content of CLA and indirectly proportional to the ratio in ewes' milk fat. The results suggest that the seasonal variations in CLA content in ewes' milk fat are related primarily to the seasonal variation in linoleic and α -linolenic acid content in grass lipids.

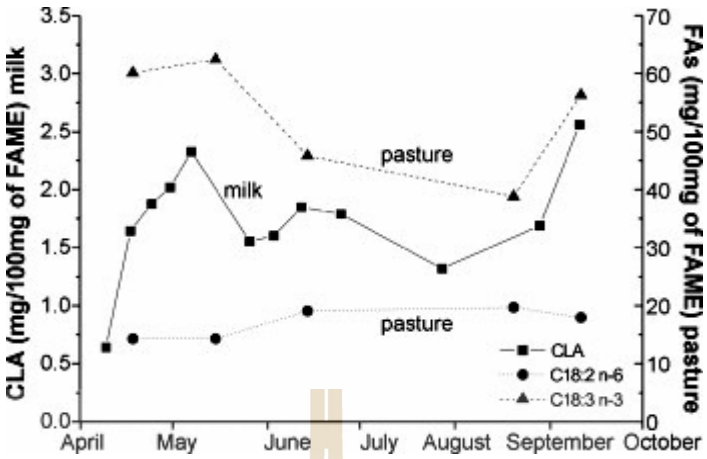

Figure 2.2 Variations in the content of CLA in ewe milk fat, and content of α–linolenic and linoleic acids during pasture season in average pasture samples. C18:3n-3, α-linolenic acid; C18:2n-6, linoleic acid (Mel'uchová et al., 2008).

2.4.3 Factor of feeding system on CLA content in ruminants

Dhiman et al. (1999) in the research trial at University of Wisconsin found that the control cows fed a typical grain/TMR had 5.5 mg/g Fat CLA, cows fed hay with no grain had 14 mg/g Fat CLA and cows fed fresh pasture (direct-grazed) with no grain had 23 mg/g Fat CLA, highest in CLA, and the research also found "high levels of CLA in fresh pasture fed cows. If the forage was allowed to wilt, such as with hay (wilted) silage, the CLA percentage in the milk would drop by one-third. Dhiman et al. (1999) also stated that normal milk processing did not change the CLA percentage in a dairy product. Analysis of current supermarket dairy products found a CLA percentage of 4.5 mg/g in milk, 3.6 mg/g in ice cream, and only 4.8 mg/g in cheese. These were one-fifth the amount of dairy products made from the milk of direct-grazed cows. Fresh forage (pasture) increased CLA higher than hay and silage. The lower content of

CLA in milk of stall-fed goats was related to the loss of precursor fatty acids during the hay-making process (Aii et al., 1988). The other approach to increase the delivery of plant-derived PUFA into ruminant products is to reduce losses through lipolysis and oxidation during field wilting or rumen biohydrogenation (Dewhurst et al., 2003). Further research is needed to establish the relative importance of plant and microbial processes and develop strategies to reduce losses (Dewhurst et al., 2003). Tudisco et al. (2010) studied on the influence of organic systems on milk fatty acid profile and CLA in goats; Treatment 1 housed, alfalfa hay, Treatment 2 grazing pasture from 9.00 am to 4.00 pm; pasture contained 60% legumes (*Trifolium alexandrinum*, Vicia spp.) 40% grass (*Bromus catharticus, Festuca arundinacea, Lolium perenne*) and supplemented with concentrate up to 700g/head/day, maximum at 40% DMI. The results revealed that average milk yield did not affected by the treatments. Organic system (Treatment 2) had highly influenced on CLA [c9t11 CLA (0.810 vs. 0.542 g/100g of fat), t10c12 CLA (0.041 vs. 0.024 g/100g of fat) and Total CLA (0.87 vs. 0.58 g/100g of fat)] than Treatment 1 ($P<0.01$) Comparing organic with conventional dairy milk, Ellis et al. (2005) reported higher proportion ($P<0.01$) of linoleic (C18:2) acid in organic milk, while conventional milk had higher ($P<0.01$) proportion of oleic acid. The red clover has the potential to increase CLA content more than grass paddocks alone (Wu et al., 1997).

In conclusion, forage plants with higher in linoleic acid could elevate CLA content in lactating ruminants. Manipulate the fresh pasture botanical composition could increase naturally occurring CLA in milk of lactating ruminants while feeding system of grazing pasture animals produced greater CLA than animal fed conventional system.

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CHAPTER III

EXPERIMENT I

STUDY ON FORAGE YIELDS, NUTRITIVE VALUES, LINOLEIC AND LINOLENIC ACID CONTENTS AND RUMEN DEGRADABILITIES OF TROPICAL FORAGE

3.1 Abstract

This experiment was carried out to investigate Dry Matter (DM), Crude Protein (CP), Neutral Detergent Fiber (NDF), fat, linoleic (C18:2) and linolenic acid (C18:3) yields, chemical compositions, linoleic and linolenic acid contents (% of total fatty acid), year round yields and DM, CP, NDF, C18:2n6 and C18:3n3 degradabilities in goat's rumen of 6 forage species including three grasses; Purple Guinea (*Panicum maximum* TD58), Chinese Pennisetum (*Pennisetum purpureum* x *Pennisetum alopecuroides*), and Napier Pak Chong 1 (*Pennisetum purpureum* x *Pennisetum americanum*), and three legumes; Hamata (*Stylosanthes hamata*), Hedge Lucern (*Desmanthus virgatus*), and Leucaena (*Leucaena leucocephala*). The result showed that Napier Pak Chong 1 had significantly higher ($P < 0.05$) DM, CP, NDF, fat, linoleic acid and linolenic acid yields, %CP, %linoleic acid and %linolenic acid (% of total fatty acid) than Chinese Pennisetum and Purple Guinea. Leucaena had

significantly higher ($P < 0.05$) DM, CP, NDF, fat, linoleic acid and linolenic acid yields, %CP, %linoleic acid and %linolenic acid (% of total fatty acid) than Hamata and Hedge Lucern.

The effect of forage species on DM, CP, NDF, C18:2n6 and C18:3n3 degradabilities in goat's rumen. Three male ruminally fistulated crossbred Saanen Goats (approximately 30 ± 3 kg body weight) were used as replicates to determine DM, CP, NDF, linoleic and linolenic acid degradabilities of 6 forage species.The results showed that, for grass species, potential DM, CP, NDF, C18:2n6 and C18:3n3 degradabilities (A+B) and effective degradabilities at flow rate of 0.02/h of Napier Pak Chong 1 were significantly higher ($P < 0.05$) than that of rice straw, but not significantly different from those of Chinese Pennisetum and Purple Guinea. For legume species, potential DM, CP, NDF, C18:2n6 and C18:3n3 degradabilities (A+B) and effective degradabilities at flow rate of 0.02/h of Leucaena were significantly higher ($P < 0.05$) than that of rice straw, but not significantly different from those of Hedge Lucern and Hamata.

Key words: tropical forage species, yields, nutritive values, linoleic acid, linolenic acid, degradability, goat

3.2 Introduction

Conjugated linoleic acid (CLA) contents in milk vary widely depending on species and/or varieties of feeds (Clapham et al., 2005; Shingfield et al., 2005). Linoleic acid (C18:2) and α-linolenic acid in animal feeds are the main precursors of cis-9, trans-11 CLA and trans-10, cis-12 CLA in milk. α-Linolenic and linoleic acids are the predominant unsaturated fatty acids in forages (Harfoot and Hazlewood, 1988),

with α-linolenic acid concentrations as high as 50-75% of the total lipid fraction (Hawke, 1973).

Managing the fatty acid composition of grazing ruminant diets could lead to meat and milk products that have higher CLA concentration, but forage fatty acid dynamics must be more fully understood for a range of forages before grazing systems can be specified (Clapham et al., 2005). In terms of good forage, the higher linoleic and linolenic acid forage should also have high yield and nutritive value as well to meet the nutrient requirement of the animals, so the aim of this study is investigation on forage yield, nutritive values, linoleic and linolenic acid and also year round yield of tropical forage species.

Ruminal degradabilities of tropical grasses and legumes by nylon bag techniques, used to provide estimates of the rate and extent of disappearance of feed constituents from the rumen (Quin et al., 1938; Rodriguez, 1968; Mehrez and Orskov, 1977). Utilization of roughage sources especially for tropical grasses and legumes using nylon bag technique will show in potential degradability and effective degradability of the species of tropical forage plants.

3.3 Objective

3.3.1 To examine forage yields, nutritive values, linoleic and linolenic acid contents of tropical forage species.

3.3.2 To investigate the DM, CP, NDF, linoleic and linolenic acid degradabilities of tropical forage species in goat's rumen.

3.4 Materials and methods

3.4.1 Study on forage yields, nutritive values, linoleic and linolenic acid contents of tropical forage species

3.4.1.1 Forage crops and planting Three grass species and three legume species were planted in experimental plots (5 x 5 m^2) and harvested at 45 days for grasses and 60 days for legumes after re-growth cutting. Each forage comprised 3 plots giving a total of 6 x 3 =18 plots; three grasses were Purple Guinea (*Panicum maximum* TD58), Chinese Pennisetum (*Pennisetum purpureum* x *Pennisetum alopecuroides*), and Napier Pak Chong 1 (*Pennisetum purpureum* x *Pennisetum americanum*), and three legumes were Hamata (*Stylosanthes hamata*), Hedge Lucern (*Desmanthus virgatus*), and Leucaena (*Leucaena leucocephala*). The soil is a sandy loam, Korat soil series (Oxic Paleustals).

Approximately 312.5 kg/ha of NPK 15-15-15 fertilizer and approximately 62.5 kg/ha of NPK 46-0-0 fertilizer were applied to all grass plots before planting and after each cutting interval respectively, while approximate 187.5 kg/ha of NPK 12-24-12 fertilizer was applied to all legume plots before planting. Planting space for all forages were 50 x 50 cm^2, seedling for Purple Guinea and all three legumes, stem stick for Chinese Pennisetum and Napier Pak Chong 1. Irrigation was managed by sprinkler every 5 days interval or when necessary to ensure optimal soil moisture conditions for pasture growth (Prasanpanich, 2002).

Forage cutting intervals were at day-45 for grasses and day-60 for legumes (all year round of cutting interval in 2014). Grasses and legumes were cut at 15 cm height and first cuttings were at day-120 and day-180 after planting, respectively. Forage yields were measured by using quadrat technique (size 50 x 50 cm^2) then converted to

kilograms per hectare (kg DM/ha). Data were analyzed as mean with standard deviation and used for calculation of paddock size for herbage allowance.

3.4.1.2 Laboratory analysis For forage yields of each cutting interval, the forage yield was measured in the area of 0.25 m^2 and then hand-clipped and weighed. Each subsample was dried in a hot-air oven at 105°C for 48 hours to determine dry matter (DM) content.

For forage's nutritive value analysis, the forages were freeze-dried, then ground to pass through a 1 mm^2 mesh screen and analyzed for chemical compositions. Total N was determined using the Kjeldahl method and crude protein (CP) was calculated by multiplying the N content by 6.25, Ether extract (EE) and ash contents were quantified by AOAC (1995). Neutral detergent fiber (NDF) and acid detergent fiber (ADF) estimated by the methods described by Van Soest et al. (1991).

Fatty acids in grasses and legumes were extracted using a modified method used by Folch et al. (1957) and Metcalfe et al. (1966). Before the extraction, after cutting the fresh forage samples were immediately frozen at -20°C and subsequently freeze-dried, then ground to pass through a 1 mm^2 mesh screen. Fifteen gram of each sample was homogenized for 2 min with 90 ml of chloroform-methanol (2:1) (Nissel AM-8 Homogenizer, Nihonseikikaisha, LTD., Japan). Each sample was further homogenized for 2 min with 30 ml of deionized water and 5 ml of 0.58% NaCl was added. The under layer of fatty acid methyl esters (FAME) was removed and placed in screw-cap test tube and stored at -20°C until methylation. Fatty acid methel esters (FAME) were prepared by the procedure described by Ostrowska et al. (2000). The procedure involved placing approximately 30 mg of the extracted oil into a 15 ml reaction tube fitted with a teflon-lined screw cap. One and a half ml of 0.5 M sodium

hydroxide in methanol was added. The tubes were flushed with nitrogen, capped, heated at 100°C for 5 min with occasional shaking and then cooled to room temperature. One ml of C17:0 internal standard (2.00 mg/mL in hexane) and 2 ml of boron trifluoride in methanol were added and heated at 100°C for 5 min with occasional shaking and 10 ml of deionized water were added. The solution was transferred to a 40 ml centrifuged tube and 5 ml of hexane were added for FAME extraction. The solution was centrifuged at 2,000 g, at 10°C for 20 min and then the hexane layer was dried over sodium sulfate and transferred into vial for analyzing by gas chromatography (GC) (7890A GC System, Agilent Technology, USA) equipped with a 100 m x 0.25 mm x 0.2 μm film fused silica capillary column (SP1233, Supelco Inc, Bellefonte, PA, USA). Injector and detector temperatures were 250°C. The column temperature was kept at 70°C for 4 min, then increased at 13°C/min to 175°C and held at 175°C for 27 min, then increased at 4°C/min to 215°C for 17 min, then increased at 4°C/min to 240°C and held at 240°C for 10 min.

3.4.2 Study on DM, CP, NDF, linoleic and linolenic acid degradabilities of tropical forage species in goat's rumen

3.4.2.1 Animals and feeding Three male ruminally fistulated crossbred Saanen goats (approximately 30 ± 3 kg body weight) were used as replicates to determine DM, CP, NDF, linoleic and linolenic acid degradabilities of 6 forage species (Purple Guinea, Chinese Pennisetum, Napier Pak Chong 1, Hamata, Hedge Lucern and Leucaena). The study was devided into 2 experiments by type of forages (grass and legume), Experiment 1 (3 treatments from 3 grasses : control (rice straw), treatment 1: Purple Guinea, treatment 2: Chinese Pennisetum and treatment 3 : Napier Pak Chong 1), Experiment 2 (3 treatments from 3 legumes : control (rice straw),

treatment 1: Hamata, treatment 2: Hedge Lucern and treatment 3: Leucaena).

Each experiment used three male ruminally fistulated crossbred Saanen goats and they were randomly assigned to receive 4 treatments. Each experimental period was 28 days of which the first 14 days were used as adjustment period to the experimental diets. Goats were housed in individual pen and feed *ad libitum* roughage (fresh grass mixed for Experiment 1; fresh legumes mixed for Experiment 2) and all goats were fed approximately 450 g/d of 16% CP concentrate (1.5% BW). The diets were offered in two equal meals at 0800 and 1600 h. Animals had free access to water and trace mineralized salt. They were dewormed at the start by Ivomectin injection treated against intestinal helminthes and intramuscular injected with vitamin AD_3E.

3.4.2.2 Ruminal disappearance study Six freeze-dried forage species samples from experimental 1.1 were ground through a 2 mm screen for *in sacco* ruminal disappearance determination. Approximately 3.0 g of feed sources were weighed into a previously dried (60^{o}C) and tared bag. Bags were made of polyester cloth (3 x 8 cm) with approximately pore size 45 μm. Bags were tied to a weighed chain and placed in the ventral rumen sac of each fistulated goat for 0 (pre feeding), 2, 4, 8, 16, 24, 48, 72, and 96 h of incubation, and were then removed and washed in water and then freeze-dried for 48 h. During each time pH and temperature were measured immediately using a portable pH and temperature meter. The bags were weighed and tested according to the procedure described by Ørskov and McDonald (1979). After weighing each bad individually, the residues were subjected to DM, CP, NDF, linoleic and linolenic acid determination. The degradability value was obtained by subjecting nutrient losses at arbitrary of time using NEWAY program (Chen, 1996) by using the equation below.

Data for ruminal disappearance characteristics of DM, CP, NDF, C18:2 and C18:3 were fitted to the exponential equation following the procedure described by Ørskov and McDonald (1979) and using the NEWAY program (Chen, 1996).

$$P = A + B\,(1 - e^{-ct})$$

Where, P = disappearance rate at time t (%), A = the intercept of the degradation curve at time zero (%), B = the fraction of DM, CP, NDF, C18:2 and C18:3 which will be degraded when give sufficient time for digestion in the rumen (%), c = a rate constant of disappearance of fraction b (h^{-1}), and t = time of incubation (h).

The effective degradability (ED) of DM, CP, NDF, C18:2 and C18:3 were, therefore, calculated using the following equation.

$$ED = A + B\,(c / (c + k))$$

Where k assuming the rate of particulate outflow from the rumen, k is 0.02 h^{-1} by equation of Ørskov and McDonald (1979).

3.4.2.3 Laboratory Analysis Total N was determined using the Kjeldahl method and crude protein (CP) was calculated by multiplying the N content by 6.25, ether extract (EE) contents were quantified by AOAC (1995). Neutral detergent fiber (NDF) estimated by the methods described by Van Soest et al. (1991).

Fatty acids in residues were extracted using a modified method used by Folch et al. (1957) and Metcalfe et al. (1966). Fifteen gram of each sample was homogenized for 2 min with 90 ml of chloroform-methanol (2:1) (Nissel AM-8 Homogenizer, Nihonseikikaisha, LTD., Japan). Each sample was the further homogenized for 2 min with 30 ml of deionized water and 5 ml of 0.58% NaCl was added. The under layer of fatty acid methyl esters (FAME) was removed and placed in screw-cap test tube and stored at -20°C until methylation. Fatty acid methel ester (FAME) were prepared by

the procedure described by Ostrowska et al. (2000). The procedure involved placing approximately 30 mg of the extracted oil into a 15 ml reaction tube fitted with a teflon-lined screw cap. One and a half ml of 0.5 M sodium hydroxide in methanol was added. The tubes were flushed with nitrogen, capped, heated at 100°C for 5 min with occasional shaking and then cooled to room temperature. One ml of C17:0 internal standard (2.00 mg/mL in hexane) and 2 ml of boron trifluoride in methanol were added and heated at 100°C for 5 min with occasional shaking and 10 ml of deionized water were added. The solution was transferred to a 40 ml centrifuged tube and 5 ml of hexane were added for FAME extraction. The solution was centrifuged at 2,000 g, at 10°C for 20 min and then the hexane layer was dried over sodium sulfate and transferred into vial for analyzing by gas chromatography (GC) (7890A GC System, Agilent Technology, USA) equipped with a 100 m x 0.25 mm x 0.2 μm film fused silica capillary column (SP1233, Supelco Inc, Bellefonte, PA, USA). Injector and detector temperatures were 250°C. The column temperature was kept at 70°C for 4 min, then increased at 13°C/min to 175°C and held at 175°C for 27 min, then increased at 4°C/min to 215°C for 17 min, then increased at 4°C/min to 240°C and held at 240°C for 10 min.

3.4.3 Statistical analysis

All data were statistically analyzed as completely randomized design (CRD) using ANOVA procedure of SAS (SAS, 2001).

3.4.4 Experimental location

The experiment was conducted at Suranaree University of Technology's goat farm, the Center for Scientific and Technological Equipment, Building 1 and 10,

Suranaree University of Technology, Nakhon Ratchasima. The soil is a sandy loam, Korat soil series (Oxic Paleustals).

3.4.5 Experimental period

The experiment of forage yields, nutritive values, linoleic and linolenic acid contents of tropical forage species was from May 2013 to December 2014. The experiment of DM, CP, NDF, linoleic and linolenic acid degradabilities of tropical forage species in goat's rumen was from January 2015 to April 2015.

3.5 Results and discussions

3.5.1 Yield, year round yield, chemical composition, linoleic and linolenic acid content of grasses

The DM, CP, NDF, fat, linoleic (C18:2) and linolenic acid (C18:3) yields, chemical compositions, linoleic and linolenic acid contents (% of total fatty acid) of 3 grasses including Purple Guinea, Chinese Pennisetum and Napier Pak Chong 1 in one year round production are shown in Table 3.1. Total all year round dry matter yield from 8 cutting interval was significantly higher ($P < 0.01$) in Napier Pak Chong 1 (65,957.90 kg/ha) than Chinese Pennisetum (53,975.61 kg/ha) and Purple Guinea (26,159.20 kg/ha), respectively. The average DM yield of two Napier cultivars in this experiment were similar to Wijitphan et al. (2009), who cut napier grass at 15 cm height with 35 days cutting interval and 50 x 80 cm planting space (71,403.10 kgDM/ha). Crude Protein (CP) and NDF yields were significantly higher ($P < 0.01$) in Napier Pak Chong 1 (9,593.54; 43,433.28 kg/ha) than Chinese Pennisetum (5,215.46; 36,077.40 kg/ha) and Purple Guinea (2,303.09; 18,358.53 kg/ha), respectively.

Fat, linoleic acid (C18:2n6) and linolenic acid (C18:3n3) yields was significantly higher ($P<0.01$) in Napier Pak Chong 1 (2,269.93; 281.95; 842.93 kg/ha) than Chinese Pennisetum (1,715.43; 184.35; 499.40 kg/ha) and Purple Guinea (665.95; 70.47; 98.04 kg/ha), respectively.

Percentage of DM was significantly higher ($P<0.01$) in Purple Guinea (25.82%) compared with Chinese Pennisetum (19.65%) and Napier Pak Chong 1 (19.07%), but there was no significantly difference among Chinese Pennisetum and Napier Pak Chong 1. Percentage of CP were significantly higher ($P<0.01$) in Napier Pak Chong 1 (13.99%) than Chinese Pennisetum (11.56%) and Purple Guinea (8.63%), respectively. This 13.99% CP of Napier Pak Chong 1 in the experiment was slightly lower than those 15.9% CP reported by Keawthong (2002) at Nakhon Ratchasima Animal Feed Research and Development Center. This could be due to the minimal fertilizer and minimal irrigation level used for this experiment. The average CP percentage of two napier cultivars of the current study were similar to the results of Santos et al. (2001) and Nakamanee et al. (1996), who worked with three napier caltivars in Chainat province, Thailand and obtained 8.9% CP. This CP percentage of Napier Pak Chong 1 and Chinese Pennisetum are higher than CP level in roughage that affects to animal's intake. Milford and Minson (1966) stated that the 7% CP was a critical level in the forage. If the CP content of the grass is less than 7%, it will affect on limit animal production due to low voluntary intake, lower rate of digestibility and negative nitrogen balance. The minimum level of protein in feed, to have adequate rumen fermentation, must contain at least 7% CP content (Minson, 1981).

There were no significant differences in CF, NDF and ADF of Purple Guinea (32.43; 70.18 and 43.65%, respectively), Chinese Pennisetum (33.57; 66.81 and

42.34%, respectively) and Napier Pak Chong 1 (30.98; 65.85 and 42.13%, respectively). While ash percentage were also not significant differences in Purple Guines (10.04%), Chinese Pennisetum (12.50%) and Napier Pak Chong 1 (12.22%). However, the fractions of NDF and ADF, which normally increase with age of the plant. The forage quality of this study were higher than animal requirement which recommended by NRC (2001), the forage crops should have ADF and NDF not less than 17% and 33%, respectively. The different amount of ADF and NDF in forage crops depend on varieties and age of plant (Preston and Leng, 1987)

There were no significant differences in fat percentage between Napier Pak Chong 1 (3.29%) and Chinese Pennisetum (3.10%), but they had significantly greater than Purple Guinea (2.50%). The C18:2n6 percent of total fatty acid was significantly greater ($P < 0.05$) in Napier Pak Chong (12.60% of total fatty acid) than Chinese Pennisetum (10.86% of tatal fatty acid) and Purple Guinea (10.69% of tatal fatty acid), but there was no significant difference among Chinese Pennisetum and Purple Guinea. In addition C18:3n3 percent of total fatty acid was significantly higher ($P < 0.01$) in Napier Pak Chong 1 (36.52% of tatal fatty acid) than Chinese Pennisetum (28.68% of tatal fatty acid) and Purple Guinea (14.44% of tatal fatty acid).

Table 3.2 - 3.6 showed DM, CP, fat, linoleic and linolenic acid yields of Purple Guinea, Chinese Pennisetum and Napier Pak Chong 1 grasses in one year round with 45 days cutting interval. The results of this study showed that the distribution of all yields of all grass all year round were varied, depend on seasonal of the year, rainy season (the 6^{th} cut), a greater DM yield was obtained. However, among treatment showed that Napier Pak Chong 1 (14,087.10 kg/ha) and Chinese Pennisetum (12,006.67 kg/ha) had significantly higher ($P < 0.05$) DM yield than Purple Guinea

(4,458.50 kg/ha), but there was no significantly different between Napier Pak Chong 1 and Chinese Pennisetum, whereas in dry season (the 1st cut), a lower DM yield was obtained, but between the treatment showed that Napier Pak Chong 1 (3,806.50 kg/ha) had significantly higher ($P < 0.05$) DM yield than Chinese Pennisetum (3,206.67 kg/ha) and Purple Guinea (2,478.42 kg/ha) respectively. The current results are in agreement with Wijitphan et al. (2009) who reported that King Napier grass planted with the same soil series and climate had higher yield when cutting in September until November and there was lower yield in January.

This trend was also happened to the distribution of CP, fat, linoleic and linolenic yields, which mean all yields were greater in late-rainy season (the 6th cut) and also in early-winter season (the 7th cut), and they had lower yield in dry season (mostly in the 1st and 2nd cut).

Table 3.1 Forage yields, nutritive values, C18:2n6 and C18:3n3 content of 3 grasses (Purple Guinea, Chinese Pennisetum and Napier Pak Chong 1)

	Purple Guinea	**Chinese pennisetum**	**Napier Pak Chong 1**	**SEM**	**Pr > F**
Yield (kg/ha/year; 8 cuttings)					
DM yield	26,159.20^{c}	53,975.61^{b}	65,957.90^{a}	1,587.107	< 0.01
CP yield	2,303.09^{c}	5,215.46^{b}	9,593.54^{a}	123.102	< 0.01
NDF yield	18,358.53^{c}	36,077.40^{b}	43,433.28^{a}	1,161.302	< 0.01
Fat yield	665.95^{c}	1,715.43^{b}	2,269.93^{a}	44.429	< 0.01
C18:2n6 yield	70.47^{c}	184.35^{b}	281.95^{a}	4.239	< 0.01
C18:3n3 yield	98.04^{c}	499.40^{b}	842.93^{a}	15.514	< 0.01
Nutritive value (%)					
DM	25.82^{a}	19.65^{b}	19.07^{b}	0.588	< 0.01
	---------- % on a dry matter basis --------				
CP	8.63^{c}	11.56^{b}	13.99^{a}	0.232	< 0.01
CF	33.57	32.43	30.98	0.802	0.259
NDF	70.18	66.81	65.85	2.101	0.554
ADF	43.65	42.34	42.13	1.498	0.906
Ash	10.04	12.50	12.22	0.443	0.120
Fat	2.50^{b}	3.10^{a}	3.29^{a}	0.074	0.011
C18:2n6 (% of total fatty acid)	10.69^{b}	10.86^{b}	12.60^{a}	0.188	0.011
C18:3n3 (% of total fatty acid)	14.44^{c}	28.68^{b}	36.52^{a}	0.789	< 0.01

Means within a row followed by the different letter are different ($P < 0.05$),

SEM : standard error of means, DM : Dry Matter, CP : Crude Protein, CF : Crude Fiber, NDF : Neutral Detergent Fiber, ADF : Acid Detergent Fiber, C18:2n6 : Linoleic acid, C18:3n3 : α-linolenic acid.

Table 3.2 Year round dry matter yield of 3 grasses (Purple Guinea, Chinese Pennisetum and Napier Pak Chong 1)

Cutting No.	Cutting date	Dry Matter Yield (kg/ha)			SEM	Pr > F
		Purple Guinea	Chinese Pennisetum	Napier Pak Chong 1		
1st Cut	15/2/2014	2,478.42[c]	3,206.67[b]	3,806.50[a]	51.584	< 0.01
2nd Cut	1/4/2014	2,744.00[c]	3,487.10[b]	4,266.67[a]	57.727	< 0.01
3rd Cut	16/5/2014	2,689.34[c]	3,806.50[b]	4,600.00[a]	56.689	< 0.01
4th Cut	30/6/2014	3,387.60[c]	9,489.34[b]	11,668.78[a]	69.375	< 0.01
5th Cut	14/8/2014	3,466.67[b]	11,508.00[a]	13,774.58[a]	538.806	< 0.01
6th Cut	28/9/2014	4,458.50[b]	12,006.67[a]	14,087.10[a]	401.668	< 0.01
7th Cut	12/11/2014	3,546.67[c]	5,947.60[b]	8,106.67[a]	87.843	< 0.01
8th Cut	27/12/2014	3,388.00[c]	4,523.73[b]	5,647.60[a]	91.789	< 0.01
Total		26,159.20[c]	53,975.61[b]	65,957.90[a]	1,587.107	< 0.01

Means within a row followed by the different letter are different (P < 0.01) and SEM.

Table 3.3 Year round CP yield of 3 grasses (Purple Guinea, Chinese Pennisetum and Napier Pak Chong 1).

Cutting No.	Cutting date	CP Yield (kg/ha)			SEM	Pr > F
		Purple Guinea	Chinese Pennisetum	Napier Pak Chong 1		
1st Cut	15/2/2014	211.16[c]	304.31[b]	520.73[a]	6.129	< 0.01
2nd Cut	1/4/2014	217.05[c]	328.14[b]	557.65[a]	4.490	< 0.01
3rd Cut	16/5/2014	212.73[c]	358.19[b]	601.22[a]	4.512	< 0.01
4th Cut	30/6/2014	320.81[c]	929.01[b]	1,780.66[a]	4.416	< 0.01
5th Cut	14/8/2014	328.29[c]	1,126.63[b]	2,102.00[a]	42.229	< 0.01
6th Cut	28/9/2014	422.22[c]	1,175.45[b]	2,149.69[a]	32.478	< 0.01
7th Cut	12/11/2014	302.18[c]	564.43[b]	1,108.99[a]	9.742	< 0.01
8th Cut	27/12/2014	288.66[c]	429.30[b]	772.59[a]	10.964	< 0.01
Total		2,303.09[c]	5,215.46[b]	9,593.54[a]	123.102	< 0.01

Means within a row followed by the different letter are different (P < 0.01) and SEM.

Table 3.4 Year round fat yield of 3 grasses (Purple Guinea, Chinese Pennisetum and Napier Pak Chong 1).

		Fat Yield (kg/ha)				
Cutting No.	**Cutting date**	**Purple Guinea**	**Chinese Pennisetum**	**Napier Pak Chong 1**	**SEM**	**Pr > F**
1st Cut	15/2/2014	68.65^{c}	110.63^{b}	148.45^{a}	2.005	< 0.01
2nd Cut	1/4/2014	60.09^{b}	92.76^{a}	108.37^{a}	2.633	< 0.01
3rd Cut	16/5/2014	58.90^{c}	101.25^{b}	116.84^{a}	0.999	< 0.01
4th Cut	30/6/2014	85.71^{c}	301.76^{b}	401.41^{a}	1.608	< 0.01
5th Cut	14/8/2014	87.71^{c}	365.95^{b}	473.85^{a}	12.464	< 0.01
6th Cut	28/9/2014	112.80^{c}	381.81^{b}	484.60^{a}	9.619	< 0.01
7th Cut	12/11/2014	98.24^{c}	205.19^{b}	316.16^{a}	3.334	< 0.01
8th Cut	27/12/2014	93.85^{c}	156.07^{b}	220.26^{a}	3.587	< 0.01
Total		665.95^{c}	1,715.43^{b}	2,269.93^{a}	44.429	< 0.01

Means within a row followed by the different letter are different (P < 0.01) and SEM.

Table 3.5 Year round C18:2n6 yield of 3 grasses (Purple Guinea, Chinese Pennisetum and Napier Pak Chong 1).

		C18:2n6 Yield (kg/ha)				
Cutting No.	**Cutting date**	**Purple Guinea**	**Chinese Pennisetum**	**Napier Pak Chong 1**	**SEM**	**Pr > F**
1st Cut	15/2/2014	6.98^{c}	12.01^{b}	18.85^{a}	0.238	< 0.01
2nd Cut	1/4/2014	6.73^{c}	10.30^{b}	13.99^{a}	0.110	< 0.01
3rd Cut	16/5/2014	6.60^{c}	11.24^{b}	15.08^{a}	0.097	< 0.01
4th Cut	30/6/2014	9.17^{c}	32.08^{b}	48.97^{a}	0.149	< 0.01
5th Cut	14/8/2014	9.38^{c}	38.90^{b}	57.81^{a}	1.312	< 0.01
6th Cut	28/9/2014	12.07^{c}	40.59^{b}	59.12^{a}	1.026	< 0.01
7th Cut	12/11/2014	9.99^{c}	22.28^{b}	40.15^{a}	0.386	< 0.01
8th Cut	27/12/2014	9.54^{c}	16.95^{b}	27.97^{a}	0.429	< 0.01
Total		70.47^{c}	184.35^{b}	281.95^{a}	4.239	< 0.01

Means within a row followed by the different letter are different (P < 0.01) and SEM.

Table 3.6 Year round C18:3n3 yield of 3 grasses (Purple Guinea, Chinese Pennisetum and Napier Pak Chong 1).

Cutting No.	Cutting date	C18:3n3 Yield (kg/ha)			SEM	Pr > F
		Purple Guinea	Chinese Pennisetum	Napier Pak Chong 1		
1st Cut	15/2/2014	9.91[c]	33.24[b]	53.59[a]	0.561	< 0.01
2nd Cut	1/4/2014	7.99[c]	24.95[b]	38.52[a]	0.120	< 0.01
3rd Cut	16/5/2014	7.83[c]	27.24[b]	41.52[a]	0.127	< 0.01
4th Cut	30/6/2014	13.34[c]	87.81[b]	152.21[a]	0.251	< 0.01
5th Cut	14/8/2014	13.66[c]	106.49[b]	179.68[a]	3.664	< 0.01
6th Cut	28/9/2014	17.56[c]	111.11[b]	183.76[a]	3.014	< 0.01
7th Cut	12/11/2014	14.19[c]	61.66[b]	114.13[a]	0.880	< 0.01
8th Cut	27/12/2014	13.55[c]	46.90[b]	79.51[a]	0.987	< 0.01
Total		98.04[c]	499.40[b]	842.93[a]	15.514	< 0.01

Means within a row followed by the different letter are different ($P < 0.01$) and SEM.

3.5.2 Yields, year round yields, chemical compositions, linoleic and linolenic acid contents of legumes

The DM, CP, NDF, fat, linoleic (C18:2) and linolenic acid (C18:3) yields, chemical compositions, linoleic and linolenic acid (% of total fatty acid) contents of 3 legumes including Hamata, Hedge Lucern and Leucaena in one year round production are shown in Table 3.7. Total year round DM, CP and NDF yield from 6 cutting intervals with 60 days cutting interval was significantly higher ($P<0.01$) in Leucaena (31,251.05; 7,050.77; 18,822.51 kg/ha) than Hamata (4,774.20; 673.60; 3,117.55 kg/ha) and Hedge Lucern (3,403.72; 615.04; 2,129.37 kg/ha), but there was no significant difference among Hamata and Hedge Lucern.

Fat, linoleic acid (C18:2n6) and linolenic acid (C18:3n3) yield was significantly higher ($P < 0.01$) in Leucaena (1,233.81; 159.92; 231.48 kg/ha) than Hamata (129.65; 13.56; 18.44 kg/har) and Hedge Lucern (113.79; 11.81; 18.52 kg/ha), but there was no significant difference among Hamata and Hedge Lucern.

Percentage of DM was not significantly different among Hamata (28.16%), Hedge Lucern (29.32%) and Leucaena (29.59%). Percentage of CP was significantly higher ($P < 0.01$) in Leucaena (22.68%) than Hedge Lucern (18.07%) and Hamata (14.57%), respectively. Percentage of CF, NDF and ADF were no significant difference in Hamata (29.59%, 65.30% and 40.51%, respectively), Hedge Lucern (28.06%, 62.56% and 37.89%, respectively) and Leucaena (25.92%, 60.23% and 36.69%, respectively). Percentage of ash was not significantly different among Hamata (6.18%), Hedge Lucern (6.64%) and Leucaena (8.45%).

Percentage of fat was significantly higher ($P < 0.01$) in Leucaena (3.89%) and Hedge Lucern (3.39%) compared with Hamata (2.77%), but there was no significant difference between Leucaena and Hedge Lucern. C18:2n6 and C18:3n3 percent of total fatty acid were significantly higher ($P < 0.05$) in Leucaena (13.01; 18.56% of total fatty acid) than Hedge Lucern (10.75; 15.65% of total fatty acid) and Hamata (10.44; 14.14% of total fatty acid), but there was not significantly different between Hedge Lucern and Hamata.

Compared linoleic and linolenic acid percentage of the tropical forage to the temperate grass and legumes, Shingfield et al. (2005) reported that Perennial ryegrass (*Lolium perenne*) had 14.2% C18:2 and 50.4% C18:3, Maize (*Zea mays*) had 44.8% C18:2 and 6.6% C18:3 of total fatty acid, Buccioni et al. (2009) found that Lucerne (*Medicago sativa*) was characterised by 11.62 (% of total fatty acid) of linoleic acid

and 27.08 (% of total fatty acid) of α-linolenic acid, whereas linoleic acid in the other two herbages was 6.60 and 6.95% of total fatty acid in Oats (*Avena sativa*) and Ryegrass (*Lolium multiflorum*), respectively; α-linolenic acid was 52.20 (% of total fatty acid) and 54.49 (% of total fatty acid) in Oats and Ryegrass, respectively. For another tropical forage experiment, Lin et al. (1985) showed that Leucaena leaves contains 51.1% linoleic acid (18:2) and 13.6% linolenic acid (18:3) of total fatty acid. Maize and Leucaena leaves were higher in linoleic acid than linolenic acid as well as safflower oil, sunflower oil and corn oil, etc. that rich in linoleic acid and used as feed supplement for increasing CLA, but for this experiment were used whole browse or bush of Leucaena for further cut-and-carry versus grazing pasture experiment, so Leucaena in this experiment had lower in linoleic acid compared to those linoleic acid in Leucaena leaves but for linolenic acid were higher than those Leucaena leaves. While Perennial ryegrass had higher in C18:2, Oats and Ryegrass had lower in C18:2 compared to all those of tropical grasses in the experiment including Purple Guinea, Chinese Pennisetum and Napier Pak Chong 1, but Perennial ryegrass, Oats and Ryegrass had higher in C18:3 compared to those of Purple Guinea, Chinese Pennisetum and Napier Pak Chong 1. Also, Lucerne were lower in C18:2 but higher in C18:3 compared to that of Leucaena in this experiment, it may be concluded that some temperate grass and legumes species were not different in C18:2 but mostly higher in C18:3 compared to those tropical grass and legumes.

Table 3.8 - 3.12 showed DM, CP, Fat, linoleic and linolenic year round yields of Hamata, Hedge Lucern and Leucaena legumes in one year round with 60 days cutting interval. The results of this study showed that the distribution of all yields of all legumes all year round were varied, depend on seasonal of the year, rainy season (the 4th

and 5th cut), a greater DM yield was obtained.

However, among treatments showed that in the 4th cut Leucaena (6,646.16 kg/ha) had significantly higher (P<0.05) DM yield than Hamata (1,415.10 kg/ha) and Hedge Lucern (1,185.60 kg/ha), but there was no significantly difference between Hamata and Hedge Lucern, whereas in dry season, a lower DM yield was obtained. This trended was also happened to the distribution of CP, Fat, linoleic and linolenic yields.

Table 3.7 Forage yields, nutritive values, C18:2n6 and C18:3n3 contents of 3 legumes (Hamata, Hedge Lucern and Leucaena)

	Hamata	Hedge Lucern	Leucaena	SEM	Pr > F
Yield (kg/ha/year) 6 cutting interval					
DM yield	4,774.20^{b}	3,403.72^{b}	31,251.05^{a}	526.514	< 0.01
CP yield	673.60^{b}	615.04^{b}	7,050.77^{a}	79.272	< 0.01
NDF yield	3,117.55^{b}	2,129.37^{b}	18,822.51^{a}	834.298	< 0.01
Fat yield	129.65^{b}	113.79^{b}	1,233.81^{a}	29.260	< 0.01
C18:2n6 yield	13.56^{b}	11.81^{b}	159.92^{a}	5.936	< 0.01
C18:3n3 yield	18.44^{b}	18.52^{b}	231.48^{a}	8.717	< 0.01
Nutritive value (%)					
DM	28.16	29.32	29.59	1.241	0.882
	-------- % on a dry matter basis --------				
CP	14.57^{c}	18.07^{b}	22.68^{a}	0.518	< 0.01
CF	29.59	28.06	25.92	1.147	0.468
NDF	65.30	62.56	60.23	1.631	0.177
ADF	40.51	37.89	36.69	1.228	0.476
Ash	8.45	6.64	6.18	0.643	0.376
Fat	2.77^{b}	3.39^{a}	3.89^{a}	0.071	< 0.01
C18:2n6 (% of total fatty acid)	10.44^{b}	10.75^{b}	13.01^{a}	0.404	0.040
C18:3n3 (% of total fatty acid)	14.14^{b}	15.65^{b}	18.56^{a}	0.471	0.023

Means within a row followed by the different letter are different (P < 0.05) and SEM.

Table 3.8 Year round dry matter yield of 3 legumes (Hamata, Hedge Lucern and Leucaena)

Cutting No.	Cutting date	Dry Matter Yield (kg/ha)			SEM	Pr > F
		Hamata	Hedge Lucern	Leucaena		
1st Cut	2/3/2014	456.00[b]	134.12[c]	3,797.64[a]	67.155	< 0.01
2nd Cut	1/5/2014	450.12[b]	236.00[b]	5,198.18[a]	188.136	< 0.01
3rd Cut	30/6/2014	648.65[b]	464.00[b]	5,609.07[a]	65.585	< 0.01
4th Cut	29/8/2014	1,415.10[b]	1,185.60[b]	6,646.16[a]	103.638	< 0.01
5th Cut	28/10/2014	1,335.00[b]	988.00[b]	6,400.00[a]	129.099	< 0.01
6th Cut	27/12/2014	469.33[b]	396.00[b]	3,600.00[a]	81.478	< 0.01
Total		4,774.20[b]	3,403.72[b]	31,251.05[a]	526.514	< 0.01

Means within a row followed by the different letter are different ($P < 0.01$) and SEM.

Table 3.9 Year round CP yield of 3 legumes (Hamata, Hedge Lucern and Leucaena)

Cutting No.	Cutting date	CP Yield (kg/ha)			SEM	Pr > F
		Hamata	Hedge Lucern	Leucaena		
1st Cut	2/3/2014	71.46[b]	25.58[c]	899.28[a]	14.879	< 0.01
2nd Cut	1/5/2014	66.03[b]	40.05[b]	1,130.60[a]	38.944	< 0.01
3rd Cut	30/6/2014	95.16[b]	78.74[b]	1,219.97[a]	13.127	< 0.01
4th Cut	29/8/2014	189.06[b]	215.54[b]	1,502.03[a]	19.470	< 0.01
5th Cut	28/10/2014	178.36[b]	179.62[b]	1,446.40[a]	23.316	< 0.01
6th Cut	27/12/2014	73.54[b]	75.52[b]	852.48[a]	17.922	< 0.01
Total		673.60[b]	615.04[b]	7,050.77[a]	79.272	< 0.01

Means within a row followed by the different letter are different ($P < 0.01$) and SEM.

Table 3.10 Year round fat yield of 3 legumes (Hamata, Hedge Lucern and Leucaena)

Cutting No.	Cutting date	Fat Yield (kg/ha)			SEM	Pr > F
		Hamata	Hedge Lucern	Leucaena		
1st Cut	2/3/2014	13.22[b]	4.59[c]	138.99[a]	1.969	< 0.01
2nd Cut	1/5/2014	12.60[b]	8.14[b]	190.25[a]	5.373	< 0.01
3rd Cut	30/6/2014	18.16[b]	16.01[b]	205.29[a]	1.903	< 0.01
4th Cut	29/8/2014	37.08[b]	39.01[b]	289.11[a]	3.578	< 0.01
5th Cut	28/10/2014	34.98[b]	32.51[b]	278.40[a]	4.263	< 0.01
6th Cut	27/12/2014	13.61[b]	13.54[b]	131.76[a]	2.401	< 0.01
Total		129.65[b]	113.79[b]	1,233.81[a]	29.260	< 0.01

Means within a row followed by the different letter are different (P < 0.01) and SEM.

Table 3.11 Year round C18:2n6 yield of 3 legumes (Hamata, Hedge Lucern and Leucaena)

Cutting No.	Cutting date	C18:2n6 Yield (kg/ha)			SEM	Pr > F
		Hamata	Hedge Lucern	Leucaena		
1st Cut	2/3/2014	1.32[b]	0.52[c]	19.76[a]	0.251	< 0.01
2nd Cut	1/5/2014	1.37[b]	0.89[b]	21.44[a]	0.498	< 0.01
3rd Cut	30/6/2014	1.97[b]	1.74[b]	23.14[a]	0.174	< 0.01
4th Cut	29/8/2014	3.88[b]	3.88[b]	39.15[a]	0.352	< 0.01
5th Cut	28/10/2014	3.66[b]	3.23[b]	37.70[a]	0.425	< 0.01
6th Cut	27/12/2014	1.36[b]	1.55[b]	18.74[a]	0.302	< 0.01
Total		13.56[b]	11.81[b]	159.92[a]	5.936	< 0.01

Means within a row followed by the different letter are different (P < 0.01) and SEM.

Table 3.12 Year round C18:3n3 yield of 3 legumes (Hamata, Hedge Lucern and Leucaena)

Cutting No.	Cutting date	C18:3n3 Yield (kg/ha)			SEM	Pr > F
		Hamata	Hedge Lucern	Leucaena		
1st Cut	2/3/2014	1.70[b]	0.69[c]	25.78[a]	0.263	< 0.01
2nd Cut	1/5/2014	1.92[b]	1.20[b]	32.59[a]	0.652	< 0.01
3rd Cut	30/6/2014	2.77[b]	2.36[b]	35.17[a]	0.230	< 0.01
4th Cut	29/8/2014	5.30[b]	6.67[b]	57.82[a]	0.515	< 0.01
5th Cut	28/10/2014	5.00[b]	5.56[b]	55.68[a]	0.614	< 0.01
6th Cut	27/12/2014	1.75[b]	2.05[b]	24.44[a]	0.319	< 0.01
Total		18.44[b]	18.52[b]	231.48[a]	8.717	< 0.01

Means within a row followed by the different letter are different (P<0.01) and SEM.

3.5.3 Rumen degradability of grass

Goat's rumen environment expressed by the level of pH and temperature, the average ruminal pH was 6.8 and ruminal temperature was 38.8°C. There were no significant differences in these values among times of incubation. The results were similar to the values reported by Chanjula et al (2003) and Promkot and Wanapat (2003) in which ruminal pH and temperatures ranged from 6.5 to 7.0, and 39.0-41.0°C, respectively. These ranges are considered to be optimum for the microbial digestion of fiber and for protein as well (Hoover, 1986; Firkins, 1996; Wanapat, 1990).

For grass species experiment, effect of study of ruminal degradability of three grass species including Purple Guinea, Chinese Pennisetum and Napier Pak Chong 1 and also Rice Straw as a control, the results showed that (Table 3.13) potential dry

matter degradability (A+B) and dry matter effective degradability at flow rate of 0.02/h (which was for roughage determination) of Napier Pak Chong 1 (75.7; 46.3%) was significantly higher ($P<0.05$) than that of rice straw (60.4; 30.3%), but not significantly different from those of Chinese Pennisetum (71.5; 44.0%) and Purple Guinea (70.7; 43.7%), respectively.

Potential crude protein degradability (A+B) and crude protein effective degradability at flow rate of 0.02/h of Napier Pak Chong 1 (89.4; 66.1%) was significantly higher ($P<0.05$) than that of rice straw (71.4; 52.1%), but not significantly different from those of Chinese Pennisetum (86.3; 64.3%) and Purple Guinea (81.3; 61.8%), respectively (Table 3.14).

Table 3.15 showed the potential neutral detergent fiber (NDF) degradability (A+B) and of Napier Pak Chong 1 (76.0%) was significantly higher ($P<0.05$) than that of rice straw (62.5%), but not significantly different from those of Purple Guinea (71.2%) and Chinese Pennisetum (72.2%). Neutral detergent fiber effective degradability at flow rate of 0.02/h of Napier Pak Chong 1 (47.4%) was significantly higher ($P<0.05$) than those of Purple Guinea (45.1%) and rice straw (33.4%), respectively, but not significantly different with that of Chinese Pennisetum (47.1%).

Fatty acid which were the precursor for CLA synthesis, potential linoleic acid (C18:2n6) degradability (A+B) of Napier Pak Chong 1 (95.0%) was significantly higher ($P<0.05$) than that of rice straw (86.3%), but not significantly different from those of Purple Guinea (94.1%) and Chinese Pennisetum (94.7%) (Table 3.16). Linoleic acid effective degradability at flow rate of 0.02/h of Napier Pak Chong 1 (71.9%) was significantly higher ($P<0.05$) than those of Purple Guinea (66.6%) and rice straw (62.2%), but not significantly different from that of Chinese Pennisetum (67.7%).

The results of potential linolenic acid (C18:3n3) degradability (A+B) of Napier Pak Chong 1 (97.9%) was significantly higher ($P<0.05$) than that of rice straw (87.0%), but not significantly different from those of Purple Guinea (93.3%) and Chinese Pennisetum (93.8%) (Table 3.17). Linolenic acid effective degradability at flow rate of 0.02/h of Napier Pak Chong 1 (82.9%) was significantly higher ($P<0.05$) than those of Purple Guinea (75.8%) and rice straw (63.0%), respectively, but not significantly different from that of Chinese Pennisetum (78.4%).

Table 3.13 Dry matter degradability (%) and effective of 3 grasses.

	Rice Straw	Purple Guinea	Chinese pennisetum	Napier Pak Chong 1	SEM
DM degradability (%) at hr					
2	12.3	15.3	16.6	17.3	1.963
4	15.4	16.6	18.7	20.3	1.896
8	16.1	19.2	21.4	23.4	3.182
16	19.1^{b}	31.6^{a}	32.8^{a}	33.1^{a}	1.145
24	23.4^{b}	36.8^{a}	39.1^{a}	42.5^{a}	2.271
48	32.9^{b}	49.4^{a}	52.0^{a}	53.9^{a}	2.254
72	40.8^{b}	58.1^{a}	59.5^{a}	59.5^{a}	2.933
96	46.3^{b}	61.5^{a}	64.1^{a}	67.2^{a}	2.432
A	14.3	15.3	15.3	15.7	1.899
B	46.1^{c}	55.4^{a}	56.2^{a}	60.0^{a}	1.856
c	0.021	0.022	0.024	0.026	0.003
A+B	60.4^{b}	70.7^{a}	71.5^{a}	75.7^{a}	2.261
Effective degradability at flow rate					
0.02	30.3^{b}	43.7^{a}	44.0^{a}	46.3^{a}	1.241
0.05	21.1^{b}	31.5^{a}	32.1^{a}	32.2^{a}	1.533
0.08	18.0^{b}	25.9^{a}	26.2^{a}	26.5^{a}	1.774

Means within a row followed by the different letter are different ($P<0.05$) and SEM.

A = The intercept of the degradation curve at time zero (%), B = The fraction of DM, CP, NDF, C18:2n6 and C18:3n3 which will be degraded when given sufficient time for digestion in the rumen (%), c = A rate constant of disappearance of fraction B (h^{-1}), A+B = Potential degradability (%).

Table 3.14 CP degradability (%) and effective of 3 grasses.

	Rice Straw	Purple Guinea	Chinese pennisetum	Napier Pak Chong 1	SEM
CP degradability (%) at hr					
2	20.5^{b}	22.6^{b}	22.6^{b}	32.1^{a}	1.754
4	26.4^{b}	29.2^{b}	29.8^{b}	45.5^{a}	2.291
8	27.4^{c}	31.9^{bc}	36.8^{b}	50.1^{a}	2.213
16	29.4^{c}	36.8^{bc}	40.6^{b}	57.4^{a}	2.885
24	50.1^{b}	57.6^{a}	63.0^{a}	67.8^{a}	2.358
48	59.6^{b}	73.4^{a}	74.3^{a}	78.1^{a}	1.752
72	64.6^{b}	78.2^{a}	80.3^{a}	82.3^{a}	1.585
96	71.5^{b}	82.6^{a}	84.7^{a}	87.8^{a}	1.276
A	11.8	15.3	15.3	15.7	1.482
B	59.6^{c}	66.0^{b}	70.9^{a}	73.7^{a}	1.451
c	0.033	0.033	0.043	0.043	0.006
A+B	71.4^{b}	81.3^{a}	86.3^{a}	89.4^{a}	1.782
Effective degradability at flow rate					
0.02	52.1^{b}	61.8^{a}	64.3^{a}	66.1^{a}	2.512
0.05	40.5^{b}	45.4^{ab}	49.0^{ab}	55.6^{a}	2.361
0.08	35.1^{b}	37.7^{b}	41.2^{b}	50.3^{a}	2.452

Means within a row followed by the different letter are different ($P<0.05$) and SEM. A = The intercept of the degradation curve at time zero (%), B = The fraction of DM, CP, NDF, C18:2n6 and C18:3n3 which will be degraded when given sufficient time for digestion in the rumen (%), c = A rate constant of disappearance of fraction B (h^{-1}), A+B = Potential degradability (%).

Table 3.15 NDF degradability (%) and effective of 3 grasses.

	Rice Straw	Purple Guinea	Chinese pennisetum	Napier Pak Chong 1	SEM
NDF degradability (%) at hr					
2	12.6	15.6	15.7	17.8	1.961
4	15.9	19.0	19.6	21.3	2.172
8	16.6	20.9	22.7	24.0	2.715
16	20.5^{b}	31.9^{a}	35.2^{a}	35.4^{a}	1.424
24	24.7^{b}	39.5^{a}	40.2^{a}	46.0^{a}	2.361
48	35.1^{b}	52.6^{a}	52.7^{a}	57.1^{a}	2.123
72	43.2^{b}	60.3^{a}	61.9^{a}	62.5^{a}	1.802
96	52.4^{b}	65.2^{a}	65.7^{a}	71.6^{a}	2.645
A	14.9	15.3	15.3	15.7	0.581
B	47.6^{b}	55.9^{a}	56.9^{a}	60.3^{a}	2.174
c	0.018	0.023	0.029	0.036	0.007
A+B	62.5^{b}	71.2^{a}	72.2^{a}	76.0^{a}	2.383
Effective degradability at flow rate					
0.02	33.4^{c}	45.1^{b}	47.1^{a}	47.4^{a}	1.762
0.05	22.1^{b}	32.8^{a}	34.5^{a}	33.8^{a}	1.583
0.08	18.8^{b}	27.7^{a}	28.6^{a}	28.5^{a}	1.551

Means within a row followed by the different letter are different ($P<0.05$) and SEM.
A = The intercept of the degradation curve at time zero (%), B = The fraction of DM, CP, NDF, C18:2n6 and C18:3n3 which will be degraded when given sufficient time for digestion in the rumen (%), c = A rate constant of disappearance of fraction B (h^{-1}), A+B = Potential degradability (%).

Table 3.16 Linoleic acid degradability (%) and effective of 3 grasses.

	Rice Straw	Purple Guinea	Chinese pennisetum	Napier Pak Chong 1	SEM
C18:2n6 degradability (%) at hr					
2	22.1^{b}	26.6^{b}	32.8^{a}	37.5^{a}	1.662
4	33.8	39.7	40.9	43.0	3.153
8	36.4^{c}	43.4^{b}	43.8^{b}	50.6^{a}	1.633
16	52.1	53.6	55.4	59.7	2.744
24	61.7^{b}	62.4^{b}	63.6^{b}	67.9^{a}	1.372
48	74.1^{b}	74.4^{b}	76.8^{ab}	79.7^{a}	1.534
72	81.6^{b}	85.0^{ab}	85.6^{ab}	87.6^{a}	1.981
96	87.5^{b}	92.4^{a}	92.4^{a}	94.2^{a}	1.574
A	11.8	15.3	15.3	15.7	1.581
B	74.5^{b}	78.8^{a}	79.4^{a}	79.3^{a}	1.123
c	0.027	0.030	0.032	0.035	0.006
A+B	86.3^{b}	94.1^{a}	94.7^{a}	95.0^{a}	1.623
Effective degradability at flow rate					
0.02	62.2^{b}	66.6^{b}	67.7^{ab}	71.9^{a}	1.561
0.05	50.3^{b}	53.1^{b}	53.2^{b}	58.3^{a}	1.641
0.08	42.5^{c}	46.7^{bc}	47.3^{b}	52.1^{a}	1.582

Means within a row followed by the different letter are different (P<0.05) and SEM.
A = The intercept of the degradation curve at time zero (%), B = The fraction of DM, CP, NDF, C18:2n6 and C18:3n3 which will be degraded when given sufficient time for digestion in the rumen (%), c = A rate constant of disappearance of fraction B (h^{-1}), A+B = Potential degradability (%).

Table 3.17 Linolenic acid degradability (%) and effective of 3 grasses.

	Rice Straw	Purple Guinea	Chinese pennisetum	Napier Pak Chong 1	SEM
C18:3n3 degradability (%) at hr					
2	21.7^{c}	27.1^{b}	32.4^{a}	35.3^{a}	1.631
4	24.9^{c}	32.2^{b}	39.3^{a}	42.4^{a}	1.193
8	33.0^{c}	43.3^{b}	45.6^{ab}	52.5^{a}	2.654
16	44.4^{d}	53.0^{c}	62.9^{b}	69.7^{a}	1.502
24	67.1^{c}	71.8^{bc}	75.3^{b}	83.9^{a}	1.941
48	73.4^{c}	83.0^{b}	84.3^{b}	89.6^{a}	1.324
72	81.0^{b}	91.0^{a}	92.3^{a}	93.0^{a}	1.912
96	87.2^{b}	94.8^{a}	95.5^{a}	95.6^{a}	1.593
A	11.5	15.3	15.3	15.7	1.583
B	75.5^{b}	78.0^{ab}	78.5^{ab}	82.2^{a}	1.431
c	0.024	0.027	0.051	0.066	0.015
A+B	87.0^{b}	93.3^{a}	93.8^{a}	97.9^{a}	1.541
Effective degradability at flow rate					
0.02	63.0^{c}	75.8^{b}	78.4^{ab}	82.9^{a}	1.552
0.05	46.5^{c}	62.8^{b}	64.3^{b}	73.6^{a}	1.763
0.08	39.6^{c}	55.0^{b}	57.0^{b}	68.1^{a}	1.382

Means within a row followed by the different letter are different (P<0.05) and SEM.
A = The intercept of the degradation curve at time zero (%), B = The fraction of DM, CP, NDF, C18:2n6 and C18:3n3 which will be degraded when given sufficient time for digestion in the rumen (%), c = A rate constant of disappearance of fraction B (h^{-1}), A+B = Potential degradability (%).

3.5.4 Rumen degradability of legume

For legume species experiment, Table 3.18 - 3.22 presented effect of ruminal degradability of three legume species including Hamata, Hedge Lucern and Leucaena and also rice straw as a control. The results showed that potential dry matter degradability (A+B) of Leucaena (69.7%) was significantly higher ($P<0.05$) than that of rice straw (58.4%), but not significantly different from those of Hedge Lucern (65.8%) and Hamata (63.4%). Dry matter effective degradability at flow rate of 0.02/h of Leucaena (51.2%) was significantly higher ($P<0.05$) than those of Hamata (42.1%) and rice straw (31.5%), respectively, but not significantly different from that of Hedge Lucern (48.0%) (Table 3.18).

Potential crude protein degradability (A+B) and crude protein effective degradability at flow rate of 0.02/h of Leucaena (80.2; 63.5%) was significantly higher ($P<0.05$) than those of Hamata (74.8; 57.0%) and rice straw (66.4; 52.1%), respectively, but not significantly different from that of Hedge Lucern (76.5; 60.3%) (Table 3.19). These similar results in Leucaena have also been reported by Paengkoum and Traiyakun (2011) who were study on ruminal and intestinal digestibility of leucaena (*Leucaena leucocephala*) and Jack fruit (*Artocarpus heterophyllus*) foliage using *In sacco* and three-step techniques, using 6-8 weeks old of leucaena foliages, samples of about 10-30 cm from the growing points of plant, using three crossbred (Thai native × Anglo-Nubian) goats, the results showed that leucaena foliage (89.1% DM, 18.9% CP, 51.6% NDF and 20.1% ADF) had potential DM degradation (A+B) 74.10% and DM effective degradability at outflow rate of 0.05 h^{-1} 53.20%. While potential CP degradation (A+B) of Leucaena foliages was 78.10% and CP effective degradability at outflow rate of 0.05 h^{-1} was 55.00%.

In Table 3.20, potential neutral detergent fiber (NDF) degradability (A+B) of Leucaena (80.4; 56.2%) was significantly higher ($P<0.05$) than those of Hamata (70.0; 47.8%) and rice straw (61.5; 34.4%), respectively, but not significantly different from that of Hedge Lucern (78.1; 52.0%).

Legume herbage C18:2n6 and C18:3n3 digestibilities, potential linoleic acid (C18:2n6) degradability (A+B) of Leucaena (97.7%) was significantly higher ($P<0.05$) than those of Hamata (92.0%) and rice straw (84.3%), respectively, but not significantly different from that of Hedge Lucern (95.2%) (Table 3.21). Linoleic acid effective degradability at flow rate of 0.02/h of Leucaena (73.1%) was significantly higher ($P<0.05$) than that of rice straw (65.2%), but not significantly different from those of Hamata (72.9%) and Hedge Lucern (73.0%)

Potential linolenic acid (C18:3n3) degradability (A+B) of Leucaena (98.8%) was significantly higher ($P<0.05$) than those of Hamata (92.7%) and rice straw (83.6%), respectively, but not significantly different from that of Hedge Lucern (95.8%) (Table 3.22). Linolenic acid effective degradability at flow rate of 0.02/h of Leucaena (73.3%) was significantly higher ($P<0.05$) than that of rice straw (62.6%), but not significantly different from those of Hamata (70.2%) and Hedge Lucern (71.3%).

Different roughage degradability in the rumen of all 3 grass species and all 3 legumes compare with rice straw (rice straw's chemical composition showed in Tabel 4A), could be attributed to their chemical composition, especially CP and NDF contents, which could be more easily attacked by rumen microorganism (Mahadeevan et al., 1980). There is a decrease in the proportion of CP and increase in the concentration of cellulose, hemicelluloses and lignin, which are normally associated

with a depression in dry matter digestibility. The cell wall content and the magnitude and nature of lignification of these call walls are amongst the most important factors which govern the degradability and the rate of passage of forage. This experiment showed that Rice Straw was lower in crude protein and higher in neutral detergent fiber and acid detergent fiber and was lowest in DM and CP degradability. This pattern was also supported by Promkot and Wanapat (2003) who reported that palm seed meal was low in protein and high in NDF, and lowest in DM and CP digestibility.

Preston (1986) reported that the rate of degradation (c) was an important parameter in the assessment of the fermentation in the rumen. In this experiment the rate of degradation (c) of DM, CP, NDF, C18:2 and C18:3 did not different between treatments but Napier Pak Chong 1 grass and Leucaena showed the highest number as compared to those of the other treatments.

Table 3.18 Dry matter degradability (%) and effective of 3 legumes.

	Rice Straw	Hamata	Hedge Lucern	Leucaena	SEM
DM degradability (%) at hr					
2	11.8^{b}	20.6^{a}	21.9^{a}	23.0^{a}	1.212
4	14.9^{b}	22.2^{a}	22.9^{a}	25.3^{a}	1.143
8	15.3^{b}	27.7^{a}	31.3^{a}	31.5^{a}	1.914
16	18.0^{d}	29.9^{c}	37.0^{b}	43.0^{a}	1.991
24	22.4^{c}	36.3^{b}	46.0^{a}	46.3^{a}	1.731
48	30.4^{c}	47.0^{b}	56.5^{a}	59.8^{a}	1.352
72	38.9^{c}	56.1^{b}	60.4^{ab}	65.9^{a}	2.364
96	45.1^{c}	57.3^{b}	61.9^{b}	68.5^{a}	1.873
A	13.3	14.7	15.7	17.8	1.681
B	45.1	48.7	50.1	51.9	2.381
c	0.019	0.026	0.036	0.037	0.007
A+B	58.4^{b}	63.4^{ab}	65.8^{a}	69.7^{a}	2.172
Effective degradability at flow rate					
0.02	31.5^{c}	42.1^{b}	48.0^{a}	51.2^{a}	1.772
0.05	22.1^{c}	32.3^{b}	38.1^{a}	39.5^{a}	0.863
0.08	19.0^{c}	28.4^{b}	33.4^{a}	33.8^{a}	0.883

Means within a row followed by the different letter are different ($P<0.05$) and SEM.
A = The intercept of the degradation curve at time zero (%), B = The fraction of DM, CP, NDF, C18:2n6 and C18:3n3 which will be degraded when given sufficient time for digestion in the rumen (%), c = A rate constant of disappearance of fraction B (h^{-1}), A+B = Potential degradability (%).

Table 3.19 CP degradability (%) and effective of 3 legumes.

	Rice Straw	Hamata	Hedge Lucern	Leucaena	SEM
CP degradability (%) at hr					
2	20.0	23.9	23.9	24.3	1.611
4	27.8^{b}	28.6^{b}	28.7^{b}	35.8^{a}	1.223
8	31.3^{c}	34.4^{bc}	38.2^{ab}	42.4^{a}	1.674
16	39.7^{b}	40.6^{b}	51.8^{a}	54.8^{a}	1.401
24	49.4^{c}	54.0^{b}	56.1^{b}	60.9^{a}	1.434
48	58.1^{b}	67.5^{a}	67.9^{a}	69.9^{a}	1.862
72	63.5^{b}	72.2^{a}	74.8^{a}	76.3^{a}	1.462
96	69.8^{c}	73.6^{bc}	75.9^{ab}	79.5^{a}	1.715
A	10.8	14.7	15.7	17.8	2.781
B	55.6	60.1	60.8	62.4	2.663
c	0.030	0.032	0.036	0.043	0.005
A+B	66.4^{c}	74.8^{b}	76.5^{ab}	80.2^{a}	1.291
Effective degradability at flow rate					
0.02	52.1^{c}	57.0^{b}	60.3^{ab}	63.5^{a}	1.632
0.05	40.5^{c}	43.6^{bc}	47.1^{b}	55.3^{a}	1.953
0.08	35.1^{c}	37.5^{bc}	40.4^{ab}	47.5^{a}	2.485

Means within a row followed by the different letter are different ($P<0.05$) and SEM.
A = The intercept of the degradation curve at time zero (%), B = The fraction of DM, CP, NDF, C18:2n6 and C18:3n3 which will be degraded when given sufficient time for digestion in the rumen (%), c = A rate constant of disappearance of fraction B (h^{-1}), A+B = Potential degradability (%).

Table 3.20 NDF degradability (%) and effective of 3 legumes.

	Rice Straw	Hamata	Hedge Lucern	Leucaena	SEM
NDF degradability (%) at hr					
2	12.1^{b}	22.9^{a}	23.8^{a}	24.6^{a}	1.994
4	15.3^{b}	25.8^{a}	26.2^{a}	27.3^{a}	1.116
8	15.9^{b}	32.0^{a}	34.4^{a}	34.5^{a}	1.837
16	19.4^{c}	37.3^{b}	40.1^{ab}	46.3^{a}	2.884
24	23.7^{c}	41.6^{b}	48.9^{ab}	49.9^{a}	2.615
48	32.7^{c}	52.3^{b}	59.7^{a}	65.5^{a}	2.213
72	41.5^{c}	61.2^{b}	65.3^{ab}	70.7^{a}	2.385
96	51.3^{c}	67.0^{b}	68.6^{ab}	75.2^{a}	2.416
A	13.9	14.7	15.7	17.8	1.792
B	47.6^{c}	55.3^{b}	62.4^{ab}	62.6^{a}	2.413
c	0.017	0.019	0.029	0.034	0.006
A+B	61.5^{c}	70.0^{b}	78.1^{a}	80.4^{a}	1.441
Effective degradability at flow rate					
0.02	34.4^{c}	47.8^{b}	52.0^{ab}	56.2^{a}	2.432
0.05	23.1^{b}	36.5^{a}	41.1^{a}	42.8^{a}	2.367
0.08	19.8^{b}	32.2^{a}	36.0^{a}	36.8^{a}	2.531

Means within a row followed by the different letter are different ($P<0.05$) and SEM.
A = The intercept of the degradation curve at time zero (%), B = The fraction of DM, CP, NDF, C18:2n6 and C18:3n3 which will be degraded when given sufficient time for digestion in the rumen (%), c = A rate constant of disappearance of fraction B (h^{-1}), A+B = Potential degradability (%).

Table 3.21 Linoleic acid degradability (%) and effective of 3 legumes.

	Rice Straw	Hamata	Hedge Lucern	Leucaena	SEM
C18:2n6 degradability (%) at hr					
2	26.0^{c}	32.4^{b}	35.2^{ab}	38.1^{a}	1.831
4	29.5^{b}	39.3^{a}	40.7^{a}	42.9^{a}	1.875
8	43.3^{b}	48.0^{a}	48.0^{a}	50.4^{a}	1.392
16	53.0^{c}	61.1^{b}	63.8^{ab}	67.4^{a}	1.985
24	61.8^{c}	67.2^{b}	67.5^{b}	71.5^{a}	1.237
48	73.4^{b}	82.2^{a}	82.4^{a}	82.9^{a}	1.466
72	81.0^{b}	89.6^{a}	90.1^{a}	91.3^{a}	1.452
96	87.2^{b}	93.4^{a}	94.6^{a}	95.0^{a}	1.211
A	10.8	14.7	15.7	17.8	2.446
B	73.5^{b}	77.3^{a}	79.5^{a}	79.9^{a}	1.184
c	0.030	0.031	0.037	0.066	0.014
A+B	84.3^{c}	92.0^{b}	95.2^{ab}	97.7^{a}	1.323
Effective degradability at flow rate					
0.02	65.2^{b}	72.9^{a}	73.0^{a}	73.1^{a}	1.264
0.05	51.2^{b}	58.3^{a}	58.8^{a}	58.9^{a}	1.183
0.08	44.3^{b}	49.7^{a}	51.9^{a}	52.4^{a}	1.447

Means within a row followed by the different letter are different ($P<0.05$) and SEM.
A = The intercept of the degradation curve at time zero (%), B = The fraction of DM, CP, NDF, C18:2n6 and C18:3n3 which will be degraded when given sufficient time for digestion in the rumen (%), c = A rate constant of disappearance of fraction B (h^{-1}), A+B = Potential degradability (%).

Table 3.22 Linolenic acid degradability (%) and effective of 3 legumes.

	Rice Straw	Hamata	Hedge Lucern	Leucaena	SEM
C18:3n3 degradability (%) at hr					
2	31.0	32.2	34.1	35.0	1.421
4	36.2^{b}	38.3^{ab}	38.9^{ab}	40.8^{a}	1.225
8	42.7^{b}	45.5^{ab}	47.8^{a}	48.7^{a}	1.317
16	52.6^{b}	55.2^{ab}	57.8^{a}	61.0^{a}	1.134
24	61.6^{c}	65.9^{ab}	62.7^{bc}	66.9^{a}	1.142
48	73.4^{b}	77.2^{ab}	83.4^{a}	87.6^{a}	1.561
72	80.3^{c}	86.7^{b}	90.3^{ab}	93.1^{a}	1.445
96	87.1^{c}	92.5^{b}	94.6^{ab}	96.5^{a}	1.163
A	11.1	14.7	15.7	17.8	2.316
B	72.4^{b}	78.0^{a}	80.1^{a}	81.0^{a}	1.426
c	0.026	0.027	0.034	0.036	0.006
A+B	83.6^{c}	92.7^{b}	95.8^{ab}	98.8^{a}	1.692
Effective degradability at flow rate					
0.02	62.6^{b}	70.2^{a}	71.3^{a}	73.3^{a}	1.304
0.05	52.3^{b}	56.6^{a}	56.1^{a}	57.1^{a}	1.367
0.08	43.7^{b}	49.3^{a}	49.4^{a}	50.1^{a}	1.823

Means within a row followed by the different letter are different ($P<0.05$) and SEM.
A = The intercept of the degradation curve at time zero (%), B = The fraction of DM, CP, NDF, C18:2n6 and C18:3n3 which will be degraded when given sufficient time for digestion in the rumen (%), c = A rate constant of disappearance of fraction B (h^{-1}), A+B = Potential degradability (%).

3.6 Conclusions

Napier Pak Chong 1 had significantly higher ($P<0.05$) in DM yield, CP yield, NDF yield, Fat yield, Linoleic acid yield, Linolenic acid yield, %CP, %Linoleic acid and %Linolenic acid (% of total fatty acid) than Chinese Pennisetum and Purple Guinea. Leucaena had significantly higher ($P<0.05$) in DM yield, CP yield, NDF yield, Fat yield, Linoleic acid yield, Linolenic acid yield, %CP, %Linoleic acid and %Linolenic acid (% of total fatty acid) than Hamata and Hedge Lucern.

For grass species, potential DM, CP, NDF, C18:2n6 and C18:3n3 degradabilities (A+B) and effective degradabilities at flow rate of 0.02/h of Napier Pak Chong 1 were significantly higher ($P<0.05$) than that of rice straw, but not significantly different from those of Chinese Pennisetum and Purple Guinea. For legume species, potential DM, CP, NDF, C18:2n6 and C18:3n3 degradabilities (A+B) and effective degradabilities at flow rate of 0.02/h of Leucaena were significantly higher ($P<0.05$) than that of rice straw, but not significantly different from those of Hedge Lucern and Hamata.

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CHAPTER IV

EXPERIMENT II

EFFECTS OF FORAGE SPECIES ON *Butyrivibrio fibrisolvens*, *Fibrobacter succinogenes* AND TOTAL BACTERIA POPULATION IN GOAT'S RUMEN

4.1 Abstract

The objective of this experiment was to investigated the effects of forage species on *Butyrivibrio fibrisolvens*, *Fibrobacter succinogenes* and total bacteria population in goat's rumen. Three male ruminally fistulated crossbred Saanen goats (approximately 33 ± 3.0 kg body weight) were used as replicates to determine content of *B. fibrisolvens*, *F. succinogenes* and total bacteria in goat's rumen by effect of 6 forage species. The results showed that in grass experiment, *B. fibrisolvens*, *F. succinogenes* and total bacteria of goats were not significantly different between three grass species including Purple Guinea, Chinese Pennisetum and Napier Pak Chong 1 at h0, h2, h4 and h6. While in legume experiment, population of ruminal *B. fibrisolvens*, *F.succinogenes* and total bacteria of goats were not significantly different between three legume species including Hamata, Hedge Lucern and Leucaena at h0, h2, h4 and h6.

Key words: grass, legume, goat, *Butyrivibrio fibrisolvens*, *Fibrobacter succinogenes*, total bacteria

4.2 Introduction

Conjugated linoleic acid (CLA) which is isomer of C18:2, one of the polyunsaturated fatty acid (PUFA), was synthesised in the rumen of ruminants by *Butyrivibrio fibrisolvens*. Natural CLA was found in milk and meat of ruminants. The main isomer of CLA is cis-9, trans-11 octadecadienoic acid also known as rumenic acid (Kramer et al., 1998), another form is trans-10, cis-12 octadecadienoic acid (Parodi, 1977; Britton et al., 1992; Chin et al., 1992; Parodi, 1994). O'Shea et al. (1998) found total CLA in milk being between 2-30 mg/g fat and found cis-9, trans-11 octadecadienoic acid about 90% of total CLA. The presence of CLA in milk fat from ruminants resulted from the isomerization and biohydrogenation of unsaturated fatty acid by rumen bacteria *B. fibrisolvens* as well as the Δ9-desaturase activity in the mammary gland. Linoleic acid and α-linolenic acids in animal feeds are the main precursors of cis-9, trans-11 CLA and trans-10, cis-12 CLA in milk. The synthesis pathway of CLA starts with isomerization of linoleic acid (cis-9, cis-12 C18:2) from feed to CLA (cis-9, trans-11 C18:2). CLA, an intermediates substrates, can transfer directly to the target tissue and CLA can also be reduced to vaccenic acid (trans-11 C18:1) which is the end product of biohydrogenation. Dewhurst et al. (2003) described that linoleic acid and α-linolenic acid are the predominant unsaturated fatty acids in forages which are the main precursors of c9t11 CLA and t10c12 CLA in milk. Many recent studies showed large effects of special concentrates on levels of fatty acids in milk and meat. Herbage lipids are the cheapest and safest sources of these fatty acids. The forages and pastures are, therefore, important long-term strategy of CLA.

4.3 Objective

The objective of this experiment was to investigate the effects of forage species on *B. fibrisolvens, F. succinogenes* and total bacteria population in goat's rumen

4.4 Materials and methods

4.4.1 Animals and treatments

Three male ruminally fistulated crossbred Saanen goats (approximately 33 ± 3 kg body weight) were used as replicates to determine content of *B. fibrisolvens, F. succinogenes* and total bacteria in goat's rumen by effect of 6 forage species (Purple Guinea, Chinese Pennisetum, Napier Pak Chong 1, Hamata, Hedge Lucern and Leucaena). The study was devided into 2 experiments by type of forages (grass and legume), Experiment 1 (3 treatments from 3 grasses : Purple Guinea, Chinese Pennisetum and Napier Pak Chong 1), and Experiment 2 (3 treatments from 3 legumes : Hamata, Hedge Lucern and Leucaena).

Goats were housed in individual pen and feed *ad libitum* roughage and 16% CP concentrate at 1.5% BW. The diets were offered in two equal meals at 0800 and 1600 h. Animals had free access to water and trace mineralized salt. They were dewormed at the start by Ivomectin injection, treated against intestinal helminthes and intramuscular injected with vitamin AD_3E.

Each experiment used three male ruminally fistulated crossbred Saanen Goats and they were randomly assigned in 3 × 3 Latin Square Design to receive 3 treatments. Each experiment was conducted in three periods; each period lasted 28 days of which the first 7 days were used as adjustment period to the experimental diets. Overall experimental period was 84 days. At the end of each period, rumen content of each

animal was collected at 0, 2, 4 and 6 h post-feeding in the morning and were used for DNA extraction of *B. fibrisolvens*, *F. succinogenes* and total bacteria using real-time PCR technique, (LightCycler® Nano System version 1.0.1, Roche).

Metabolism trial seven days collection was conducted for nutrient utilization in goats. The metabolic cages were specially designed with a facility for separate collection of feces and urine. The animals were kept in metabolic cages for 3 days, prior to actual collection of 7 days to acclimatize the animals to the new surroundings. The appropriate aliquots of feed offered, residue left, feces were preserved for chemical analysis. Body weight of the animals was recorded before and after the metabolism trials.

Measurement data of feed offered and residues were obtained. For further analysis, about 10% of feces (fresh weight) from each goat was taken daily and accumulated in a deep freezer at -20^{o}C until the end of the experiment. Feces from seven days were thoroughly mixed and then samples were taken and dried at 60^{o}C for 12 hours. Dried samples were ground with a mortar and pestle, the determination of dry matter (DM) was done by drying at 105^{o}C for 24h, ash content was assayed by incinerating samples at 550^{o}C, and organic matter (OM) could therefore be obtained. Nitrogen (N) was determined by the Macro Kjeldahl technique (AOAC, 1985) and crude protein calculated as N x 6.25. Neutral detergent fiber (NDF) and acid detergent fiber (ADF) determination were analyzed according to following the procedure described by Van Soest et al. (1991).

Blood samples were taken from the jugular vein at 0 (prior to morning feeding), 2, 4 and 6 hours post feeding. Then, the blood samples were prior to plasma separation by centrifugation (3,000 xg for 15 min) and plasma samples were then stored at -20^{o}C for determining blood urea nitrogen (BUN) concentration.

4.4.2 Laboratory analyses

For forage's nutritive value analysis, the forages were freeze-dried, then ground to pass through a 1 mm^2 mesh screen and analyzed for chemical composition. Total N was determined using the Kjeldahl method and crude protein (CP) was calculated by multiplying the N content by 6.25, ether extract (EE) and ash contents were quantified by AOAC (1995). Neutral detergent fiber (NDF) and acid detergent fiber (ADF) were estimated by the methods described by Van Soest et al. (1991).

Fatty acids in grasses and legumes were extracted using a modified method used by Folch et al. (1957) and Metcalfe et al. (1966). Before the extraction, after cutting then the fresh forage samples were immediately frozen at -20°C and subsequently freeze-dried, then ground to pass through a 1 mm^2 mesh screen. Fifteen gram of each sample was homogenized for 2 min with 90 ml of chloroform-methanol (2:1) (Nissel AM-8 Homogenizer, Nihonseikikaisha, LTD., Japan). Each sample was the further homogenized for 2 min with 30 ml of deionized water and 5 ml of 0.58% NaCl was added. The under layer of fatty acid methyl esters (FAME) was removed and placed in screw-cap test tube and stored at -20°C until methylation. Fatty acid methel ester (FAME) were prepared by the procedure described by Ostrowska et al. (2000). The procedure involved placing approximately 30 mg of the extracted oil into a 15 ml reaction tube fitted with a teflon-lined screw cap. One and a half ml of 0.5 M sodium hydroxide in methanol was added. The tubes were flushed with nitrogen, capped, heated at 100°C for 5 min with occasional shaking and then cooled to room temperature. One ml of C17:0 internal standard (2.00 mg/mL in hexane) and 2 ml of boron trifluoride in methanol were added and heated at 100°C for 5 min with occasional shaking and 10 ml of deionized water were added. The solution was

transferred to a 40 ml centrifuged tube and 5 ml of hexane were added for FAME extraction. The solution was centrifuged at 2,000 g, at 10°C for 20 min and then the hexane layer was dried over sodium sulfate and transferred into vial for analyzing by gas chromatography (GC) (7890A GC System, Agilent Technology, USA) equipped with a 100 m x 0.25 mm x 0.2 μm film fused silica capillary column (SP1233, Supelco Inc, Bellefonte, PA, USA). Injector and detector temperatures were 250°C. The column temperature was kept at 70°C for 4 min, then increased at 13°C/min to 175°C and held at 175°C for 27 min, then increased at 4°C/min to 215°C for 17 min, then increased at 4°C/min to 240°C and held at 240°C for 10 min.

Community DNA was extracted from 1.5 ml aliquots of rumen fluid and digesta by the RBB+C method described by Yu and Morrison (2004). In brief, the cell lysis is achieved by bead-beating in the presence of 4% (w/v) sodium dodecyl sulfate (SDS), 500 mM NaCl, and 50 mM EDTA. The buffer should also protect the released DNA from degradation by DNases, which are very active in the rumen and gastrointestinal sample. After bead-beating, most of the impurities and the SDS are removed by precipitation with ammonium acetate and then the nucleic acids are removed by precipitation with isopropanol. Genomic DNA can then purified via sequential digestion with RNase A and proteinase K, and the DNA are purified.

Real-time PCR, species specific PCR primers (*B. fibrisolvens*, *F. succinogenes* and total bacteria) used to amplify 16S rDNA regions (target DNA) were chosen from Kobayashi et al. (2000) for *B. fibrisolvens* (FR-27: 5'-AGAGTTTGATCCTGGCTCA GGA-3', Prb-156: 5'-CACGTTGTCATGCAACATCGT-3', 213 bp), and Denman and McSweeney (2006) for Total bacteria (Forward: 5'-CGGCAACGAGCGCAACCC-3' and Reverse: 5'-CCATTGTAGCACGTGTGTAGCC-3') and *F. succinogenes* (Forward:

5'-GTTCGGAATTACTGGGCGTAAA-3' and Reverse: 5'-CGCCTGCCCCTGAACT ATC-3'). Real-time PCR amplification and detection were performed using a LigthCycler Nano (LightCycler® Nano System version 1.0.1, Roche).

PCR conditions for *F. succinogenes* were as follows: 30 s at 94°C for denaturing, 30 s at 60°C for annealing, and 30 s at 72°C for extension (48 cycles), except for 9 min of denaturation in the first cycle and 10 min of extension in the last cycle. Amplification of 16S rDNA for the other two species was carried out similarly, except at an annealing temperature of 55°C. To determine the specificity of amplification, an analysis of the product melting curve was performed after the last cycle of each amplification. A sample-derived standard was prepared from the treatment pool set of community DNA, instead of amplifying the target genes from individual community DNA samples and then pooling the PCR products. Then the PCR product was purified and quantified using spectrophotometry. For each sample-derived standard, the copy number concentration was calculated based on the length of the PCR product and the mass concentration. Ten-fold serial dilutions were made in Tri-EDTA prior to real-time PCR. In total, three real-time PCR standards were prepared. The conditions of the real-time PCR assays of target genes were the same as those of the regular PCR described earlier. The LightCycler® Nano System was used for real-time PCR amplification. All PCRs were performed in duplicate.

4.4.3 Statistical analysis

All data were statistically analyzed as repeated measurements for a 3 × 3 Latin squares design using ANOVA procedure of SAS (SAS, 2001). Significant differences among treatment were assessed by Duncan's New Multiple Range Test. A significant level of $P<0.05$ was used (Steel and Torries, 1980).

4.4.4 Experimental location

The experiment was conducted at Suranaree University of Technology's goat farm, the Center for Scientific and Technological Equipment, Buildings 1 and 10, Suranaree University of Technology, Nakhon Ratchasima.

4.4.5 Experimental period

The experiment was from May 2015 to August 2015.

4.5 Results and discussions

4.5.1 Feed chemical composition

Chemical composition also linoleic and linolenic acid contents of grasses and legumes experiment were demonstrated in the Table 4.1 and 4.2, this was close to each other for the main composition in experiment I-I, but slightly higher because of the period of the study were covered the rainy season, which were mostly high in all nutritive values. The nutritive value of Napier Pak Chong 1 were closed to the reported of Keawthong (2002) and the nutritive value of Leucaena were slightly higher than studied by Paengkoum and Traiyakun (2011).

4.5.2 Dry matter intake, body weight change and nutrient digestibility

There was no effect of the grass or legume species on dry matter intake (DMI) (g/d), body weight gain (g/d) or BW change (kg) and apparent digestibility (%) in Table 4.3 and Table 4.4. Average total DMI ranged from 1,060.92-1,092.91 g/day, BWG ranged from 62.50-67.50 g/day, DM digestibility ranged from 62.37-65.92%, OM digestibility ranged from 61.37-63.84% and fat digestibility ranged from 85.16-88.96%.

4.5.3 Environmental in rumen and blood urea nitrogen

The effects of grass or legume species on environmental in rumen including ruminal pH, ruminal NH_3-N (mg/dl), total VFA (mM/L), VFA proportions (% Molar) and blood urea nitrogen (BUN) were not significantly different among the treatments (Table 4.5, 4.6, 4.7 and 4.8)

Table 4.1 Chemical composition of grass experimental diets.

Items	Concentrate (16% CP)	Purple Guinea	Chinese pennisetum	Napier Pak Chong 1
DM	94.16	23.28	19.46	19.01
	---------- % on a dry matter basis ----------			
CP	16.57	9.16	12.05	14.12
CF	14.90	32.17	31.23	30.11
NDF	44.24	69.29	65.20	64.34
ADF	24.56	42.19	41.14	41.10
Ash	7.33	10.03	12.46	12.17
Fat	3.44	2.53	3.27	3.34
C18:2n6 (% of total fatty acid)	3.29	11.50	11.85	13.92
C18:3n3 (% of total fatty acid)	0.41	16.72	30.69	38.74

Means within a row followed by the different letter are different ($P < 0.05$),

SEM = standard error of means, DM = dry matter, CP = crude protein, CF = crude fat, NDF = neutral detergent fiber, ADF = acid detergent fiber, C18:2n6 = linoleic acid, C18:3n3 = α-linolenic acid.

Table 4.2 Chemical composition of legume experimental diets.

Items	Concentrate (16% CP)	Hamata	Hedge Lucern	Leucaena
DM	94.16	27.11	28.21	28.14
	---------- % on a dry matter basis ----------			
CP	16.57	15.33	19.17	22.96
CF	14.90	28.28	27.55	24.74
NDF	44.24	64.14	61.23	59.44
ADF	24.56	39.89	36.51	35.33
Ash	7.33	8.02	6.23	6.07
Fat	3.44	2.96	3.58	3.97
C18:2n6 (% of total fatty acid)	3.29	10.95	11.34	14.20
C18:3n3 (% of total fatty acid)	0.41	16.23	17.71	20.49

Means within a row followed by the different letter are different ($P<0.05$),

SEM = standard error of means, DM = dry matter, CP = crude protein, CF = crude fat, NDF = neutral detergent fiber, ADF = acid detergent fiber, C18:2n6 = linoleic acid, C18:3n3 = α-linolenic acid.

Table 4.3 Fatty acid profiles of grass experimental diets.

Fatty acid (% of Total FA)	16% CP Conc.	Purple Guinea	Chinese Pennisetum	Napier Pak Chong 1
C4:0	3.65	5.34	4.67	4.12
C6:0	0.90	1.90	1.55	1.26
C8:0	0.85	1.28	0.92	1.02
C10:0	1.66	1.57	1.16	1.00
C11:0	0.66	1.54	1.97	1.05
C12:0	32.92	2.16	1.89	1.43
C13:0	0.64	1.13	1.07	1.06
C14:0	6.08	1.82	1.90	1.17
C14:1	1.03	2.10	1.32	1.46
C15:0	1.11	2.35	2.26	1.79
C15:1	0.66	2.57	1.49	1.27
C16:0	17.33	11.00	12.16	10.95
C16:1	0.83	2.06	1.36	1.36
C17:1	0.84	1.70	1.62	1.55
C18:0	2.82	2.25	1.86	1.09
C18:1n9	17.11	4.97	3.46	3.58
C18:2n6	3.29	11.50	11.85	13.92
C20:0	0.31	2.02	1.96	1.33
C18:3n6	0.66	3.98	3.60	2.56
C20:1	0.64	2.47	1.39	1.22
C18:3n3	0.41	16.72	30.69	38.74
C21:0	0.68	1.81	1.48	1.19
C18:4n3	0.51	3.52	1.34	1.07
C20:2	0.84	3.56	2.42	1.24
C22:0	0.56	2.02	1.76	1.03
C20:3n6	0.75	2.77	2.17	1.08
C22:1n9	0.71	0.05	0.07	0.07
C20:3n3	0.45	1.99	0.21	0.62
C20:4n6	0.55	1.77	0.25	0.64
C23:0	0.52	0.06	0.16	0.12
SFA[1]	70.70	38.26	36.76	29.62
MUFA[2]	21.83	15.93	10.71	10.51
PUFA[3]	7.47	45.81	52.53	59.87
PUFA/SFA	0.11	1.20	1.43	2.02

[1]SFA = Sum of saturated fatty acid from C4:0-C14:0, C15:0, C16:0, C18:0, C20:0, C21:0, C22:0, C23:0,

[2]MUFA = Sum of monounsaturated fatty acid from C14:1, C15:1, C16:1, C17:1, C18:1n9, C20:1, C22:1n9,

[3]PUFA = Sum of polyunsaturated fatty acid from C18:2n6, C18:3n6, C18:3n3, C18:4n3, C20:2, C20:3n6, C20:3n3, C20:4n6.

Table 4.4 Fatty acid profiles of legume experimental diets.

Fatty acid (% of Total FA)	16% CP Conc.	Hamata	Hedge Lucern	Leucaena
C4:0	3.65	2.69	2.82	1.71
C6:0	0.90	1.39	1.34	1.17
C8:0	0.85	1.92	2.11	1.60
C10:0	1.66	1.56	1.61	1.14
C11:0	0.66	2.12	1.71	1.36
C12:0	32.92	2.16	1.25	1.14
C13:0	0.64	1.49	1.17	1.05
C14:0	6.08	2.39	1.60	1.38
C14:1	1.03	2.89	2.93	2.19
C15:0	1.11	2.14	2.21	1.60
C15:1	0.66	1.61	2.26	2.82
C16:0	17.33	11.07	10.66	10.25
C16:1	0.83	2.38	2.17	2.50
C17:1	0.84	1.31	1.53	1.49
C18:0	2.82	2.25	2.50	2.15
C18:1n9	17.11	3.84	4.18	4.11
C18:2n6	3.29	10.95	11.34	14.20
C20:0	0.31	2.29	2.11	1.67
C18:3n6	0.66	3.23	2.44	3.96
C20:1	0.64	2.34	2.09	1.05
C18:3n3	0.41	16.23	17.71	20.49
C21:0	0.68	2.57	2.28	1.01
C18:4n3	0.51	2.27	2.88	2.97
C20:2	0.84	3.12	3.62	3.97
C22:0	0.56	3.03	2.27	1.23
C20:3n6	0.75	3.62	3.35	4.23
C22:1n9	0.71	1.17	1.87	1.10
C20:3n3	0.45	3.42	2.89	3.40
C20:4n6	0.55	2.51	2.90	2.91
C23:0	0.52	0.04	0.19	0.15
SFA[1]	70.70	39.11	35.84	28.60
MUFA[2]	21.83	15.54	17.03	15.26
PUFA[3]	7.47	45.35	47.13	56.13
PUFA/SFA	0.11	1.16	1.31	1.96

[1]SFA = Sum of saturated fatty acid from C4:0-C14:0, C15:0, C16:0, C18:0, C20:0, C21:0, C22:0, C23:0,

[2]MUFA = Sum of monounsaturated fatty acid from C14:1, C15:1, C16:1, C17:1, C18:1n9, C20:1, C22:1n9,

[3]PUFA = Sum of polyunsaturated fatty acid from C18:2n6, C18:3n6, C18:3n3, C18:4n3, C20:2, C20:3n6, C20:3n3, C20:4n6.

Table 4.5 Dry Matter Intake (DMI) and nutrient intake of goats fed different grass species.

Items	Purple Guinea	Chinese penisetum	Napier Pak Chong 1	SEM	Pr > F
DMI (g/d)					
Concentrate	440	437	446	9.026	0.920
Roughage	620	640	647	17.033	0.808
Total	1,060	1,077	1,092	25.632	0.878
CP intake (g/d)					
Concentrate	72.91	72.36	73.85	1.496	0.920
Roughage	56.79^{c}	77.12^{b}	91.31^{a}	2.160	< 0.01
Total	129.70^{b}	149.48^{ab}	165.16^{a}	3.577	0.019
C18:2n6 intake (g/d)					
Concentrate	0.50	0.49	0.50	0.010	0.925
Roughage	1.80^{c}	2.48^{b}	3.01^{a}	0.069	< 0.01
Total	2.30^{c}	2.97^{b}	3.51^{a}	0.080	< 0.01
C18:3n3 intake (g/d)					
Concentrate	0.06	0.06	0.06	0.002	0.630
Roughage	2.62^{c}	6.42^{b}	8.37^{a}	0.186	< 0.01
Total	2.68^{c}	6.48^{b}	8.43^{a}	0.187	< 0.01

Means within a row followed by the different letter are different ($P < 0.05$) and

SEM : standard error of means.

Table 4.6 Dry Matter Intake (DMI) and nutrient intake of goats fed different legume species.

Items	Hamata	Hedge Lucern	Luecaena	SEM	Pr > F
DMI (g/d)					
Concentrate	441	445	451	9.854	0.928
Roughage	540	562	580	15.346	0.595
Total	981	1,007	1,032	24.855	0.732
CP intake (g/d)					
Concentrate	73.13	73.74	74.68	1.632	0.928
Roughage	82.78^{c}	107.67^{b}	133.17^{a}	3.281	< 0.01
Total	155.91^{b}	181.41^{ab}	207.84^{a}	4.828	0.013
C18:2n6 intake (g/d)					
Concentrate	0.50	0.50	0.51	0.011	0.961
Roughage	1.75^{c}	2.28^{b}	3.27^{a}	0.077	< 0.01
Total	2.25^{c}	2.78^{b}	3.78^{a}	0.088	< 0.01
C18:3n3 intake (g/d)					
Concentrate	0.06	0.06	0.06	0.002	0.296
Roughage	2.59^{c}	3.56^{b}	4.72^{a}	0.114	< 0.01
Total	2.66^{c}	3.62^{b}	4.78^{a}	0.115	< 0.01

Means within a row followed by the different letter are different ($P < 0.05$) and

SEM : standard error of means.

Table 4.7 Body weight change and digestibility of goats fed with different grass.

Items	Purple Guinea	Chinese pennisetum	Napier Pak Chong 1	SEM
BW Change				
BW Change (kg)	1.75	1.79	1.89	0.235
Apparent digestibility (%)				
DM	60.36	61.55	63.84	1.342
OM	62.37	63.58	65.92	1.494
CP	70.12	72.54	74.86	1.641
NDF	71.64	73.49	75.16	1.313
ADF	67.32	68.45	69.57	1.184
Fat	85.16	87.64	88.96	1.343

Means within a row followed by the different letter are different ($P < 0.05$) and

SEM : standard error of means.

Table 4.8 Body weight change and digestibility of goats fed with different legumes.

Items	Hamata	Hedge Lucern	Leucaena	SEM
BW Change				
BW Change (kg)	1.69	1.77	1.82	0.313
Apparent digestibility (%)				
DM	66.21	67.75	68.38	1.052
OM	67.36	69.54	70.23	1.161
CP	74.57	77.92	79.51	1.665
NDF	75.65	78.13	79.73	1.455
ADF	70.36	70.92	74.88	1.543
Fat	87.54	88.46	89.16	1.051

Means within a row followed by the different letter are different ($P < 0.05$) and

SEM : standard error of means.

Table 4.9 Ruminal pH, ruminal ammonia nitrogen (NH_3-N) and blood urea nitrogen (BUN) in goats fed three grass species.

	Purple Guinea	**Chinese pennisetum**	**Napier Pak Chong 1**	**SEM**
Ruminal pH				
0	6.94	6.97	6.96	0.071
2	6.49	6.51	6.59	0.063
4	6.61	6.79	6.74	0.082
6	6.74	6.84	6.80	0.071
Ruminal NH_3-N (mg/dl)				
0	11.75	12.10	12.70	0.515
2	15.80	14.00	13.60	0.873
4	17.50	18.40	19.20	0.772
6	15.10	16.40	15.90	0.864
BUN (mg/dl)				
0	16.21	16.81	17.40	0.485
2	16.77	18.38	17.67	0.644
4	18.40	18.40	18.92	0.516
6	16.80	16.98	16.46	0.397

Means within a row followed by the different letter are different ($P < 0.05$) and SEM : standard error of means.

Table 4.10 Ruminal pH, ruminal ammonia nitrogen (NH_3-N) and blood urea nitrogen (BUN) in goats fed three legume species.

	Hamata	**Hedge Lucern**	**Leucaena**	**SEM**
Ruminal pH				
0	6.80	6.94	6.94	0.061
2	6.64	6.79	6.79	0.063
4	6.79	6.71	6.61	0.075
6	6.76	6.87	6.84	0.081
Ruminal NH_3-N (mg/dl)				
0	12.40	12.90	13.10	0.472
2	16.70	18.50	17.80	0.715
4	19.10	17.40	18.60	0.844
6	15.40	16.30	15.70	0.512
BUN (mg/dl)				
0	16.92	17.17	17.70	0.436
2	17.78	18.43	18.58	0.513
4	19.41	19.88	19.60	0.476
6	17.37	17.40	16.80	0.454

Means within a row followed by the different letter are different ($P < 0.05$) and

SEM : standard error of means.

Table 4.11 Total volatile fatty acid (VFA) and proportion of VFAs in goats fed three grass species.

	Purple Guinea	**Chinese pennisetum**	**Napier Pak Chong 1**	**SEM**
Total VFA (mM/L) (h)				
0	59.45	60.42	61.64	2.471
2	63.54	62.44	65.84	2.463
4	66.45	67.92	67.38	2.742
6	63.12	63.84	62.82	1.676
VFA proportions (% Molar)				
Acetic acid	68.80	69.46	69.85	1.012
Propionic acid	21.02	20.16	20.05	0.441
Butyric acid	10.18	10.38	10.10	0.314

Means within a row followed by the different letter are different ($P < 0.05$) and SEM : standard error of means.

Table 4.12 Total volatile fatty acid (VFA) and proportion of VFAs in goats fed three legume species.

	Hamata	**Hedge Lucern**	**Leucaena**	**SEM**
Total VFA (mM/L) (h)				
0	60.57	61.44	61.85	2.561
2	64.34	63.51	66.64	2.243
4	67.84	68.45	68.57	1.682
6	64.00	64.54	63.87	1.946
VFA proportions (% Molar)				
Acetic acid	65.64	66.54	67.25	1.052
Propionic acid	22.64	21.89	21.11	0.683
Butyric acid	11.72	11.57	11.64	0.254

Means within a row followed by the different letter are different ($P < 0.05$) and SEM : standard error of means.

4.5.4 Effects of grass or legume species on *Butyrivibrio fibrisolvens*, *Fibrobacter succinogenes* and total bacteria population in goat's rumen

The results in grass experiment showed that the population of ruminal *B. fibrisolvens* bacteria of goats was not significantly different between three grass species including Purple Guinea, Chinese Pennisetum and Napier Pak Chong 1 at h0 (1.51, 1.72 and 1.85 × 10^6 copies/ml), h2 (1.73, 1.94 and 1.99 × 10^6 copies/ml), h4 (2.46, 2.53 and 2.81 × 10^6 copies/ml), and h6 (2.51, 2.91 and 2.97 × 10^6 copies/ml) (Table 4.13). The population of ruminal *F. succinogenes* bacteria of goats was not significantly different among three grass species including Purple Guinea, Chinese Pennisetum and Napier Pak Chong 1 at h0 (4.21, 4.46 and 4.51 × 10^6 copies/ml), h2 (4.53, 4.56 and 4.94 × 10^6 copies/ml), h4 (5.24, 5.60 and 5.76 × 10^6 copies/ml), and h6 (5.22, 5.59 and 5.98 × 10^6 copies/ml).

Population of ruminal total bacteria of goats were not significantly different among three grass species including Purple Guinea, Chinese Pennisetum and Napier Pak Chong 1 at h0 (4.08, 4.32 and 5.06 × 10^{10} copies/ml), h2 (4.84, 4.95 and 5.52 × 10^{10} copies/ml), h4 (5.14, 5.67 and 6.30 × 10^{10} copies/ml), and h6 (5.62, 5.79 and 6.58 × 10^{10} copies/ml).

While for legumes experiment, the population of ruminal *B. fibrisolvens* bacteria of goats also was not significantly different among three legume species including Hamata, Hedge Lucern and Leucaena at h0 (1.31, 1.32 and 1.56 × 10^6 copies/ml), h2 (1.76, 1.81 and 1.87 × 10^6 copies/ml), h4 (2.29, 2.30 and 2.66 × 10^6 copies/ml), and h6 (2.73, 2.81 and 3.01 × 10^6 copies/ml) (Table 4.14). The population of ruminal *F. succinogenes* bacteria of goats was not significantly different among three legume species including Hamata, Hedge Lucern and Leucaena at h0 (4.01, 4.05

and 4.31 × 10^6 copies/ml), h2 (4.30, 4.54 and 4.57 × 10^6 copies/ml), h4 (5.06, 5.26 and 5.41 × 10^6 copies/ml), and h6 (5.56, 5.66 and 5.73 × 10^6 copies/ml).

Population of ruminal total bacteria of goats was not significantly different among three legume species including Hamata, Hedge Lucern and Leucaena at h0 (3.12, 3.40 and 4.16 × 10^{10} copies/ml), h2 (3.44, 3.78 and 4.64 × 10^{10} copies/ml), h4 (4.47, 4.75 and 5.30 × 10^{10} copies/ml), and h6 (4.34, 4.97 and 5.40 × 10^{10} copies/ml).

The results of no significantly different of population of rumimal *B. fibrisolvens*, *F. succinogenes* and total bacteria among the treatments of this experiment were similar to the studied by Khaing et al. (2016) reported that fifteen male Boer crossbred goats around six months old of approximately 18.54 ± 1.83 kg of BW were fed with Napier grass compared to whole corn plant silage. The mean concentrations of rumen NH_3-N (mg/dl) were not significantly differences among the treatments, the total VFA production in the rumen fluid of the goats was not significantly different among the treatments. The total bacteria population of rumen content was not significantly different among the treatments, total bacteria of goats fed Napier grass were 10.0 $\log_{10}$ or 1.00 × 10^{10} copies/ml in rumen, while those of goats fed whole corn plant silage were 10.2 $\log_{10}$ or 1.58 × 10^{10} copies/ml in rumen. While the *F. succinogenes* of goats fed Napier grass were showed 6.3 $\log_{10}$ or 2.00×10^6 copies/ml in rumen significantly ($P<0.05$) higher than that of goats fed whole corn plant silage (5.6 $\log_{10}$ or 3.98 × 10^5 copies/ml in rumen), compared to this experiment that used only fresh grass and legumes had greater population of *F. succinogenes* ranged from 4.21-5.98×10^6 copies/ml in rumen of grass experiment and 4.01-5.73×10^6 copies/ml in rumen of legumes experiment. While Metzler-Zebeli et al. (2013) showed the greater number of *F. succinogenes* and total bacteria population in Boer breed, White German Noble

breed and Toggenburg breed goats with fed Meadow grass hay (5.2% CP) 100% had *F. succinogenes* 5.87×10^{9} copies/ml and total bacteria 11.1 $\log_{10}$ or 1.26×10^{11} copies/ml in rumen.

For the numbers of *B. fibrisolvens* bacteria population in Saanen goat's rumen in this study were lower when compare to steers, Guo et al. (2010) showed that four ruminlly cannulated Chinese Luxi steers (BW 559.4 ± 30.1 kg) were used in a crossover design experiment with an experimental period of 28 days. The forage to concentrate ratio of the basal diet was 35:65 on dry matter basis. The daily feeding was fixed at 7.5 kg/head and included Chinese wildrye (10.2% CP of Total feed). Rumen fluid was collected at 07:30 prefeeding, at 11:30 and 17:30 postfeeding on day 27 and 28. A part of the pooled sample from rumen fluid was analysed for species-specific real-time PCR quantification. The numbers of *B. fibrisolvens* bacteria were 4.74 $\log_{10}$ copies/µl or 5.49×10^{7} copies/ml and total bacteria were 10.91 $\log_{10}$ or 8.13×10^{10} copies/ml.

Table 4.13 Rumen microorganisms of goats fed with three grasses including Purple Guinea, Chinese Pennisetum and Napier Pak Chong 1

	h	Purple Guinea	Chinese Pennisetum	Napier Pak Chong 1	SEM	Pr > F
B. fibrisolvens	0	1.51	1.72	1.85	0.079	0.414
(10^6 copies/ml)	2	1.73	1.94	1.99	0.027	0.118
	4	2.46	2.53	2.81	0.040	0.138
	6	2.51	2.91	2.97	0.059	0.177
F. succinogenes	0	4.21	4.46	4.51	0.030	0.056
(10^6 copies/ml)	2	4.53	4.56	4.94	0.095	0.292
	4	5.24	5.60	5.76	0.066	0.191
	6	5.22	5.59	5.98	0.124	0.289
Total bacteria	0	4.08	4.32	5.06	0.099	0.076
(10^{10} copies/ml)	2	4.84	4.95	5.52	0.111	0.200
	4	5.14	5.67	6.30	0.159	0.098
	6	5.62	5.79	6.58	0.172	0.154

Means within a row followed by the different letter are different ($P < 0.05$) and SEM : standard error of means.

Table 4.14 Rumen microorganisms of goats fed with three legumes including Hamata, Hedge Lucern and Luecaena

	h	Hamata	Hedge Lucern	Leucaena	SEM	Pr > F
B. fibrisolvens	0	1.31	1.32	1.56	0.063	0.188
(10^6 copies/ml)	2	1.76	1.81	1.87	0.062	0.267
	4	2.29	2.30	2.66	0.069	0.190
	6	2.73	2.81	3.01	0.057	0.103
F. succinogenes	0	4.01	4.05	4.31	0.058	0.123
(10^6 copies/ml)	2	4.30	4.54	4.57	0.035	0.071
	4	5.06	5.26	5.41	0.147	0.279
	6	5.56	5.66	5.73	0.143	0.133
Total Bacteria	0	3.12	3.40	4.16	0.171	0.287
(10^{10} copies/ml)	2	3.44	3.78	4.64	0.142	0.155
	4	4.47	4.75	5.30	0.119	0.130
	6	4.34	4.97	5.40	0.415	0.569

Means within a row followed by the different letter are different ($P < 0.05$) and

SEM : standard error of means.

4.6 Conclusions

Grass experiment, population of ruminal *B. fibrisolvens* bacteria of goats was not significantly different among three grass species including Purple Guinea, Chinese Pennisetum and Napier Pak Chong 1 at h0, h2, h4 and h6. Population of ruminal *F. succinogenes* bacteria of goats was not significantly different among three grass species including Purple Guinea, Chinese Pennisetum and Napier Pak Chong 1 at h0, h2, h4 and h6. Population of ruminal total bacteria of goats was not significantly different among three grass species including Purple Guinea, Chinese Pennisetum and Napier Pak Chong 1 at h0, h2, h4 and h6.

Legume experiment, population of ruminal *B. fibrisolvens* bacteria of goats was not significantly different between three legume species including Hamata, Hedge Lucern and Leucaena at h0, h2, h4 and h6. Population of ruminal *F. succinogenes* bacteria of goats was not significantly different between three legume species including Hamata, Hedge Lucern and Leucaena at h0, h2, h4 and h6. Population of ruminal total bacteria of goats was not significantly different between three legume species including Hamata, Hedge Lucern and Leucaena at h0, h2, h4 and h6.

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CHAPTER V

EXPERIMENT III

EFFECTS OF FORAGE SPECIES AND FEEDING SYSTEMS ON CLA CONTENT IN GOAT'S MILK AND ON *Butyrivibrio fibrisolvens, Fibrobacter succinogenes* AND TOTAL BACTERIA POPULATION IN GOAT'S RUMEN

5.1 Abstract

The objective of this experiment was to investigate the effects of forage species and feeding system on Conjugated linoleic acid (CLA) content in goat's milk and on *Butyrivibrio fibrisolvens*, *Fibrobacter succinogenes* and total bacteria population in goat's rumen. Twenty female crossbred Saanen lactating goats (approximately 35±3.0 kg body weight) in early to mid-lactation stage were used in a completely randomized design (CRD) and sprit into 2 experiment (grass and legume which highest in quality from Experiment I and II). The results showed that Napier Pak Chong 1 grazing milking goats, the average *B. fibrisolvens*, *F. succinogenes* and total bacteria were significantly higher ($P < 0.05$) than that in Napier Pak Chong 1 cut-and-carry milking goats. While Leucaena grazing milking goats, the average *B. fibrisolvens*, *F. succinogenes* and total bacteria were significantly higher ($P < 0.05$) than that in Leucaena cut-and-carry milking

goats. Grazing Napier Pak Chong 1 milking goats had significantly higher ($P < 0.05$) milk yield, milk fat, c9,t11 CLA, t10,c12 CLA and omega-3 than that from cut-and-carry Napier Pak Chong 1 milking goats. Grazing Leucaena milking goats had significantly higher ($P < 0.05$) milk yield, milk fat, c9, t11 CLA, t10, c12 CLA and omega-3 than that from cut-and-carry Leucaena milking goats.

Key words: Napier Pak Chong 1, Leucaena, milk, CLA, omega-3, total bacteria, *Butyrivibrio fibrisolvens*, *Fibrobacter succinogenes*

5.2 Introduction

Conjugated linoleic acid (CLA) which is isomer of C18:2, one of the polyunsaturated fatty acid (PUFA), was synthesised in the rumen of ruminants by *Butyrivibrio fibrisolvens*. Natural CLA was found in milk and meat of ruminants. The main isomer of CLA is cis-9, trans-11 octadecadienoic acid also known as rumenic acid (Kramer et al., 1998), another form is trans-10, cis-12 octadecadienoic acid (Parodi, 1977; Britton et al., 1992; Chin et al., 1992; Parodi, 1994). O'Shea et al. (1998) found total CLA in milk being between 2-30 mg/g fat and found cis-9, trans-11 octadecadienoic acid about 90% of total CLA. The presence of CLA in milk fat from ruminants resulted from the isomerization and biohydrogenation of unsaturated fatty acid by rumen bacteria *B. fibrisolvens* as well as the Δ9-desaturase activity in the mammary gland. Linoleic acid and α-linolenic acids in animal feeds are the main precursors of cis-9, trans-11 CLA and trans-10, cis-12 CLA in milk. The synthesis pathway of CLA starts with isomerization of linoleic acid (cis-9, cis-12 C18:2) from feed to CLA (cis-9, trans-11 C18:2). CLA, an intermediates substrates, can transfer directly to the target tissue and CLA can also be reduced to vaccenic acid (trans-11

C18:1) which is the end product of biohydrogenation. Dewhurst et al. (2003) described that linoleic acid and α-linolenic acid are the predominant unsaturated fatty acids in forages which are the main precursors of c9,t11 CLA and t10,c12 CLA in milk. Many recent studies showed large effects of special concentrates on levels of fatty acids in milk and meat. Herbage lipids are the cheapest and safest sources of these fatty acids. The forages and pastures are, therefore, important long-term strategy of CLA.

Dhiman et al. (1999) in the research trial at University of Wisconsin found that the control cows fed a typical grain/TMR had 5.5 mg/g Fat CLA, Cows fed hay with no grain had 14 mg/g Fat CLA and Cows fed fresh pasture (direct-grazed) with no grain had 23 mg/g Fat CLA, highest in CLA, and the research also found "High levels of CLA in fresh pasture fed cows. If the forage was allowed to wilt, such as with hay (wilted) silage, the CLA percentage in the milk would drop by one-third. Dhiman et al. (1999) also stated that normal milk processing did not change the CLA percentage in a dairy product. Analysis of current supermarket dairy products found a CLA percentage of 4.5 mg/g in milk, 3.6 mg/g in ice cream, and only 4.8 mg/g in cheese. These were one-fifth the amount of dairy products made from the milk of direct-grazed cows. Fresh forage (pasture) increased CLA higher than hay and silage. The lower content of CLA in milk of stall-fed goats was related to the loss of precursor fatty acids during the hay-making process (Aii et al., 1988). The other approach to increase the delivery of plant-derived PUFA into ruminant products is to reduce losses through lipolysis and oxidation during field wilting or rumen biohydrogenation (Dewhurst et al., 2003). Further research is needed to establish the relative importance of plant and microbial processes and develop strategies to reduce losses (Dewhurst et al., 2003). Tudisco et al. (2010) studied on the influence of organic systems on milk fatty acid profile and

CLA in goats; Treatment 1 housed, alfalfa hay, Treatment 2 grazing pasture from 9.00 am to 4.00 pm; pasture contained 60% legumes (*Trifolium alexandrinum*, Vicia spp.) 40% grass (*Bromus catharticus*, *Festuca arundinacea*, *Lolium perenne*) and supplemented with concentrate up to 700g/head/day, maximum at 40% DMI. The results revealed that average milk yield did not affected by the treatments. Organic system (Treatment 2) had highly influenced on CLA [c9, t11 CLA (0.810 vs. 0.542 g/100g of fat), t10, c12 CLA (0.041 vs. 0.024 g/100g of fat) and total CLA (0.87 vs. 0.58 g/100g of fat)] than Treatment 1 ($P<0.01$) Comparing organic with conventional dairy milk, Ellis et al. (2005) reported higher proportion ($P<0.01$) of linoleic (C18:2) acid in organic milk, while conventional milk had higher ($P<0.01$) proportion of oleic acid. The red clover has the potential to increase CLA content more than grass paddocks alone (Wu et al., 1997).

5.3 Objective

The objective of this experiment was to investigate the effects of forage species and feeding system on CLA content in goat's milk and on *Butyrivibrio fibrisolvens*, *Fibrobacter succinogenes* and total bacteria population in goat's rumen.

5.4 Materials and methods

5.4.1 Animals and treatments

Twenty female crossbred Saanen lactating goats (approximately 35 ± 3.0 kg body weight) in early to mid-lactation stage were used in a completely randomized design (CRD) and sprit into 2 experiment (grass and legume which highest in quality from Experiment 1 and 2). The experimental treatments were as follows:

Grass Experiment Treatment 1 : Grass + Cut-and-carry

Treatment 2 : Grass + Rotational grazing system

Legume Experiment Treatment 1 : Legume + Cut-and-carry

Treatment 2 : Legume + Rotational grazing system

All animals were received concentrate diet up to 1.5% of body weight base on nutrient requirement recommended for goat by NRC (2007) and clean drinking water and trace mineralized salt blocks were provided for them. They were dewormed at the start by Ivomectin injection, treated against intestinal helminthes and intramuscular injected with vitamin AD_3E.

Cut-and-carry milking goats were housed, unrestrained, in the barn. Cut-and-carry roughage were available *ad libitum* for cut-and-carry dairy goats at 0800 and 1600 h.

Grazing time for grazing dairying goats were from 0700 to 1900, experiment length was 45 days for grass experiment (rotational grazing system of 5 paddocks and 9 days for each paddock) and experiment length was 60 days for legume experiment (rotational grazing system of 5 paddocks and 12 days for each paddock).

Paddock size of grass and legume pasture were calculated from herbage allowance with additional 30% because of 70% utilization condition. Based on an intake requirement (Maximum 5%; DM basis) for bodyweight maintenance and milk production, herbage allowance and proximate analysis of the experimental forage (Prasanpanich, 2002), it was estimated that paddock size for grazing Napier Pak Chong 1 pasture, herbage production was approximate 10,000 kgDM/ha (Sep - Oct 2015), herbage production/m^2 was 1 kgDM, milking goat (approximate 40 kgBW throughout the period of grazing), roughage intake of 5 + 1.5%BW (40 × 6.5) was 2.6 kgDM,

Napier Pak Chong 1 intake/head/day was 2.6 m^2, Paddock size for 5head/9day (2.6×45) was 117 sq.m. Paddock size for grazing Leucaena pasture, herbage production were approximate 5,000 kgDM/ha (Nov-Dec 2015), herbage production/m^2 were 0.5 kgDM, milking goat (approximate 40 kgBW), roughage intake of 5+1.5%BW (40 × 6.5) was 2.6 kgDM, Leucaena intake/head/day was 5.2 m^2, Paddock size for 5head/12day (5.2 × 60) was 312 sq.m.

Mean pasture intake of the grazed goats was estimated as the difference between the daily pre-grazing and post-grazing pasture estimate of forage availability, replicate by paddocks calculate for means (g DM/head/day), and that of the indoor goats as the difference between the daily herbage offered and the residual uneaten herbage (Prasanpanich, 2002).

Collecting of milk yield every day and analyzed for milk composition by MilkoScan and analyzed for CLA and fatty acid composition by Gas Chromatography (GC).

Rumen content of each animal was collected at 0, 2, 4 and 6 h postfeeding in the morning by suction technique (stomach tube) at last day of experiment were used for DNA extraction of *Butyrivibrio fibrisolvens*, *Fibrobacter succinogenes* and Total bacteria by real-time PCR technique, (LightCycler® Nano System version 1.0.1, Roche).

Blood samples were taken from the jugular vein at 0 (prior to feeding), 2, 4 and 6 hours post feeding. Then, the blood samples were prior to plasma separation by centrifugation (3,000 xg for 15 min) and plasma samples were then stored at -20°C for determining blood urea nitrogen (BUN) concentration.

5.4.2 Laboratory analyses

For forage's nutritive value analysis, the forage were freeze-dried, then ground to pass through a 1 mm^2 mesh screen and analyzed for chemical composition. Total N was determined using the Kjeldahl method and crude protein (CP) was calculated by multiplying the N content by 6.25, Ether extract (EE) and ash contents were quantified by AOAC (1995). Neutral detergent fiber (NDF) and acid detergent fiber (ADF) estimated by the methods described by Van Soest et al. (1991).

Fatty acids in feed and milk were extracted using a modified method used by Folch et al. (1957) and Metcalfe et al. (1966). Fifteen gram of each sample was homogenized for 2 min with 90 ml of chloroform-methanol (2:1) (Nissel AM-8 Homogenizer, Nihonseikikaisha, LTD., Japan). Each sample was the further homogenized for 2 min with 30 ml of deionized water and 5 ml of 0.58% NaCl was added. The under layer of fatty acid methyl esters (FAME) was removed and placed in screw-cap test tube and stored at -20°C until methylation. Fatty acid methel ester (FAME) were prepared by the procedure described by Ostrowska et al. (2000). The procedure involved placing approximately 30 mg of the extracted oil into a 15 ml reaction tube fitted with a teflon-lined screw cap. One and a half ml of 0.5 M sodium hydroxide in methanol was added. The tubes were flushed with nitrogen, capped, heated at 100°C for 5 min with occasional shaking and then cooled to room temperature. One ml of C17:0 internal standard (2.00 mg/mL in hexane) and 2 ml of boron trifluoride in methanol were added and heated at 100°C for 5 min with occasional shaking and 10 ml of deionized water were added. The solution was transferred to a 40 ml centrifuged tube and 5 ml of hexane were added for FAME extraction. The solution was centrifuged at 2,000 g, at 10°C for 20 min and then the hexane layer was dried over sodium sulfate

and transferred into vial for analyzing by GC (7890A GC System, Agilent Technology, USA) equipped with a 100 m x 0.25 mm x 0.2 μm film fused silica capillary column (SP1233, Supelco Inc, Bellefonte, PA, USA). Injector and detector temperatures were 250°C. The column temperature was kept at 70°C for 4 min, then increased at 13°C/min to 175°C and held at 175°C for 27 min, then increased at 4°C/min to 215°C for 17 min, then increased at 4°C/min to 240°C and held at 240°C for 10 min.

DNA extraction, community DNA was extracted from 0.5 ml aliquots of rumen fluid and digesta by the RBB+C method described by Yu and Morrison (2004). In brief, the cell lysis is achieved by bead-beating in the presence of 4% (w/v) sodium dodecyl sulfate (SDS), 500 mM NaCl, and 50 mM EDTA. The buffer should also protect the released DNA from degradation by DNases, which are very active in the rumen and gastrointestinal sample. After bead-beating, most of the impurities and the SDS are removed by precipitation with ammonium acetate and then the nucleic acids are removed by precipitation with isopropanol. Genomic DNA can then purified via sequential digestion with RNase A and proteinase K, and the DNA are purified.

Real-time PCR, species specific PCR primers (*B. fibrisolvens*, Total bacteria and *F. succinogenes*) used to amplify 16S rDNA regions (target DNA) were chosen from Kobayashi et al. (2000) for *B. fibrisolvens* (FR-27: 5'-AGAGTTTGATCCTGGC TCAGGA-3', Prb-156: 5'-CACGTTGTCATGCAACATCGT-3', 213 bp), and Denman and McSweeney (2006) for Total bacteria (Forward: 5'-CGGCAACGAGCGCAACCC -3' and Reverse: 5'-CCATTGTAGCACGTGTGTAGCC-3') and *F. succinogenes* (Forward: 5'-GTTCGGAATTACTGGGCGTAAA-3' and Reverse: 5'-CGCCTGCCCCTGAACT ATC-3'). Real-time PCR amplification and detection were performed using a LigthCycler Nano (LightCycler® Nano System version 1.0.1, Roche).

PCR conditions for *F. succinogenes* were as follows: 30 s at 94°C for denaturing, 30 s at 60°C for annealing, and 30 s at 72°C for extension (48 cycles), except for 9 min of denaturation in the first cycle and 10 min of extension in the last cycle. Amplification of 16S rDNA for the other two species was carried out similarly, except at an annealing temperature of 55°C. To determine the specificity of amplification, an analysis of the product melting curve was performed after the last cycle of each amplification. A sample-derived standard was prepared from the treatment pool set of community DNA, instead of amplifying the target genes from individual community DNA samples and then pooling the PCR products. Then the PCR product was purified and quantified using spectrophotometry. For each sample-derived standard, the copy number concentration was calculated based on the length of the PCR product and the mass concentration. Ten-fold serial dilutions were made in Tri-EDTA prior to real-time PCR. In total, three real-time PCR standards were prepared. The conditions of the real-time PCR assays of target genes were the same as those of the regular PCR described earlier. The LightCycler® Nano System was used for real-time PCR amplification. All PCRs were performed in duplicate.

5.4.3 Statistical analysis

All data were statistically analyzed as Completely Randomized Design using ANOVA procedure of SAS (SAS, 2001). Significant differences among treatment were assessed by Duncan's new multiple range test. A significant level of $P<0.05$ was used (Steel and Torries, 1980).

5.4.4 Experimental location

The experiment was conducted at Suranaree University of Technology's goat farm, the Center for Scientific and Technological Equipment, Buildings 1 and 10,

Suranaree University of Technology, Nakhon Ratchasima.

5.4.5 Experimental period

The experiment was from September 2015 to February 2016.

5.5 Results and discussions

5.5.1 Feed chemical composition and fatty acid profiles

Chemical composition of Napier Pak Chong 1 grass and Leucaena experimental treatments are demonstrated in the Table 5.1 and 5.2, this was close to each other for the main composition in experiment I-I and II, The nutritive value of Napier Pak Chong 1 were closed to the reported of Keawthong (2002) and the nutritive value of Leucaena were slightly higher than studied by Paengkoum and Traiyakun (2011) by younger age at cutting. The fatty acid profiles of Napier Pak Chong 1 and Leucaena presented in Table 5.3 and 5.4, respectively, they were mainly contained C16:0, C18:0, C18:1n9, C18:2n6 and C18:3n3.

5.5.2 Environmental in rumen and blood urea nitrogen

Ruminal pH, ruminal ammonia nitrogen (mg/dl), blood urea nitrogen (BUN), total VFA (mM/L) and VFA proportions (% Molar) of Napier Pak Chong 1 cut-and-carry and grazing milking goats were not significantly different between the treatments (Table 5.5), while for those data showed the same results to Leucaena cut-and-carry and grazing milking goats (Table 5.6). The result of BUN was similar to Prasanpanich et al. (2002), mean BUN levels at the PM feeding in the grazing dairy cows group (24.9 mg/100 ml) were did not differ significantly to those of indoor dairy cows group (23.9 mg/100 ml) but they found the different in AM feeding, outdoor cows showed higher BUN of higher eating activity than their housed early in the morning.

Table 5.1 Chemical composition of grass experimental diets.

		Napier Pak Chong 1	
Items	**21% CP Conc.**	**Cut-and-Carry**	**Grazing**
DM	94.28	19.05	19.02
	---------- % on a dry matter basis ----------		
CP	20.45	14.78	14.51
CF	12.69	31.15	31.81
NDF	45.98	64.84	65.24
ADF	22.84	42.52	42.94
Ash	7.69	11.84	11.75
Fat	2.78	3.81	3.79
C18:2n6 (% of total fatty acid)	3.44	14.51	14.83
C18:3n3 (% of total fatty acid)	0.96	39.25	39.98

DM : Dry Matter, CP : Crude Protein, CF : Crude Fiber,

NDF : Neutral Detergent Fiber, ADF : Acid Detergent Fiber,

C18:2n6 : Linoleic acid, C18:3n3 : α-linolenic acid.

Table 5.2 Chemical composition of legume experimental diets.

Items	21% CP Conc.	Leucaena	
		Cut-and-Carry	**Grazing**
DM	94.28	29.68	29.51
	---------- % on a dry matter basis ----------		
CP	20.45	23.12	23.51
CF	12.69	25.54	25.85
NDF	45.98	60.84	60.43
ADF	22.84	36.38	36.97
Ash	7.69	6.44	6.91
Fat	2.78	3.74	3.85
C18:2n6 (% of total fatty acid)	3.44	14.84	14.92
C18:3n3 (% of total fatty acid)	0.96	20.51	21.01

DM : Dry Matter, CP : Crude Protein, CF : Crude Fiber,

NDF : Neutral Detergent Fiber, ADF : Acid Detergent Fiber,

C18:2n6 : Linoleic acid, C18:3n3 : α-linolenic acid.

Table 5.3 Fatty acid profiles of grass experimental diets.

		Napier Pak Chong 1	
Fatty acid (% of Total FA)	**21% CP Conc.**	**Cut-and-Carry**	**Grazing**
C4:0	3.00	3.42	3.36
C6:0	0.86	1.13	1.11
C8:0	1.17	1.09	1.04
C10:0	1.50	1.00	1.04
C11:0	0.62	1.01	1.04
C12:0	30.83	1.29	1.03
C13:0	0.59	1.02	1.05
C14:0	8.60	1.04	1.01
C14:1	1.02	1.58	1.51
C15:0	1.04	1.23	1.21
C15:1	0.60	1.39	1.36
C16:0	15.61	10.32	10.35
C16:1	0.79	1.50	1.52
C17:1	0.75	1.69	1.73
C18:0	5.03	1.03	1.00
C18:1n9	15.86	3.70	3.67
C18:2n6	3.44	14.51	14.83
C20:0	0.75	1.20	1.15
C18:3n6	0.72	2.70	2.50
C20:1	0.59	1.35	1.21
C18:3n3	0.96	39.25	39.98
C21:0	0.61	1.08	1.06
C18:4n3	0.65	1.20	1.11
C20:2	0.92	1.36	1.39
C22:0	0.51	1.01	1.04
C20:3n6	0.80	1.21	1.23
C22:1n9	0.63	0.09	0.11
C20:3n3	0.54	0.74	0.62
C20:4n6	0.60	0.77	0.66
C23:0	0.44	0.11	0.08
SFA[1]	71.13	26.98	26.58
MUFA[2]	20.25	11.29	11.12
PUFA[3]	8.61	61.73	62.30
PUFA/SFA	0.12	2.29	2.34

[1]SFA = Sum of saturated fatty acid from C4:0-C14:0, C15:0, C16:0, C18:0, C20:0, C21:0, C22:0, C23:0,

[2]MUFA = Sum of monounsaturated fatty acid from C14:1, C15:1, C16:1, C17:1, C18:1n9, C20:1, C22:1n9,

[3]PUFA = Sum of polyunsaturated fatty acid from C18:2n6, C18:3n6, C18:3n3, C18:4n3, C20:2, C20:3n6, C20:3n3, C20:4n6.

Table 5.4 Fatty acid profiles of legume experimental diets.

		Leucaena	
Fatty acid (% of Total FA)	**21% CP Conc.**	**Cut-and-Carry**	**Grazing**
C4:0	3.00	1.70	1.66
C6:0	0.86	1.04	1.02
C8:0	1.17	1.47	1.35
C10:0	1.50	1.01	1.05
C11:0	0.62	1.21	1.13
C12:0	30.83	1.26	1.18
C13:0	0.59	1.18	1.22
C14:0	8.60	1.27	1.22
C14:1	1.02	2.06	2.03
C15:0	1.04	1.47	1.38
C15:1	0.60	2.80	2.82
C16:0	15.61	10.13	10.18
C16:1	0.79	2.37	2.41
C17:1	0.75	1.50	1.53
C18:0	5.03	2.20	2.16
C18:1n9	15.86	4.24	4.20
C18:2n6	3.44	14.84	14.92
C20:0	0.75	1.63	1.65
C18:3n6	0.72	3.81	3.78
C20:1	0.59	1.20	1.16
C18:3n3	0.96	20.51	21.01
C21:0	0.61	1.14	1.02
C18:4n3	0.65	2.85	2.82
C20:2	0.92	3.82	3.77
C22:0	0.51	1.11	1.04
C20:3n6	0.80	4.10	4.13
C22:1n9	0.63	1.23	1.28
C20:3n3	0.54	3.55	3.59
C20:4n6	0.60	3.06	3.09
C23:0	0.44	0.26	0.20
SFA[1]	71.13	28.07	27.44
MUFA[2]	20.25	15.40	15.44
PUFA[3]	8.61	56.54	57.12
PUFA/SFA	0.12	2.01	2.08

[1]SFA = Sum of saturated fatty acid from C4:0-C14:0, C15:0, C16:0, C18:0, C20:0, C21:0, C22:0, C23:0,

[2]MUFA = Sum of monounsaturated fatty acid from C14:1, C15:1, C16:1, C17:1, C18:1n9, C20:1, C22:1n9,

[3]PUFA = Sum of polyunsaturated fatty acid from C18:2n6, C18:3n6, C18:3n3, C18:4n3, C20:2, C20:3n6, C20:3n3, C20:4n6.

Table 5.5 Ruminal pH, ruminal ammonia nitrogen (NH_3-N), blood urea nitrogen (BUN), Volatile Fatty Acid (VFA) and body weight (BW) change of Napier Pak Chong 1 cut-and-carry and grazing milking goats.

	Napier Pak Chong 1		
	Cut-and-Carry	**Grazing**	**SEM**
Ruminal pH	6.90	7.00	0.062
Ruminal NH_3-N (mg/dl)	18.26	20.80	0.891
BUN (mg/dl)	21.80	22.50	0.676
Total VFA (mM/L) (h)	61.33	62.50	1.293
VFA proportions (% Molar)			
Acetic acid	67.90	68.97	1.014
Propionic acid	21.54	20.01	0.647
Butyric acid	10.56	11.02	0.465
BW Change			
BW Change (kg)	6.53	6.80	0.071

Means within a row followed by the different letter are different ($P < 0.05$),

SEM : standard error of means,

C18:2n6 : Linoleic acid, and C18:3n3 : α-linolenic acid.

Table 5.6 Ruminal pH, ruminal ammonia nitrogen (NH_3-N), blood urea nitrogen (BUN), Volatile Fatty Acid (VFA) and body weight (BW) change of Leucaena cut-and-carry and grazing milking goats.

	Leucaena		
	Cut-and-Carry	**Grazing**	**SEM**
Ruminal pH	6.88	7.00	0.063
Ruminal NH_3-N (mg/dl)	19.92	20.97	0.571
BUN (mg/dl)	21.30	22.80	0.831
Total VFA (mM/L) (h)	60.66	61.90	1.382
VFA proportions (% Molar)			
Acetic acid	67.50	68.58	1.034
Propionic acid	21.56	20.10	0.635
Butyric acid	10.94	11.32	0.233
BW Change			
BW Change (kg)	7.96	8.75	0.616

Means within a row followed by the different letter are different ($P < 0.05$),

SEM : standard error of means,

C18:2n6 : Linoleic acid, and C18:3n3 : α-linolenic acid.

5.5.3 Dry matter intake, body weight change

The dry matter intake of concentrate and roughage and the body weight change has been shown in Table 5.7 for Napier Pak Chong 1 grass fed and Table 5.8 for Leucaena legume fed. There were no different of concentrate intake between treatments in both of Napier Pak Chong 1 and Leucaena experiment. However, grazing goats were significantly higher ($P<0.05$) in roughage intake (950.39 vs 788.23 g/d), (815.44 vs 607.25 g/d) and total DMI (1,448.54 vs 1,282.55 g/d), (1,312.76 vs 1,096.84 g/d) than those cut-and-carry goats in Napier Pak Chong 1 and Leucaena experiment, respectively. While, body weight was did not significantly between treatments for both grass and legume experiment, this could be explained that when goats were grazed in the pasture, they were very active, Luginbuhl et al. (1995) stated that goats are very active foragers, able to cover a wide area in search of plant materials.

Table 5.7 Dry matter intake (DMI), Crude Protein (CP), C18:2n6 and C18:3n3 intake of Napier Pak Chong 1 cut-and-carry and grazing milking goats.

Items		Napier Pak Chong 1 grass		SEM	Pr > F
		Cut-and-Carry	Grazing		
DMI (g/d)	Conc.	494	498	3.873	0.620
	Roughage	788[b]	950[a]	7.649	< 0.01
	Total	1,283[b]	1,449[a]	29.538	0.023
CP intake (g/d)	Conc.	101	102	0.700	0.680
	Roughage	116[b]	138[a]	2.728	< 0.01
	Total	217[b]	240[a]	3.098	< 0.01
C18:2n6 intake (g/d)	Conc.	0.47	0.48	0.010	0.724
	Roughage	4.36[b]	5.34[a]	0.069	< 0.01
	Total	4.83[b]	5.82[a]	0.112	< 0.01
C18:3n3 intake (g/d)	Conc.	0.13	0.13	0.006	0.764
	Roughage	11.78[b]	14.39[a]	0.566	0.050
	Total	11.92[b]	14.53[a]	0.418	0.014

Means within a row followed by the different letter are different ($P < 0.05$),

SEM : standard error of means,

C18:2n6 : Linoleic acid, and C18:3n3 : α-linolenic acid.

Table 5.8 Dry matter intake (DMI), Crude Protein (CP), C18:2n6 and C18:3n3 intake of Leucaena cut-and-carry and grazing milking goats.

Items		Leucaena		SEM	Pr > F
		Cut-and-Carry	Grazing		
DMI (g/d)	Conc.	490	497	6.103	0.582
	Roughage	607[b]	815[a]	8.426	< 0.01
	Total	1,097[b]	1,313[a]	31.137	< 0.01
CP intake (g/d)	Conc.	100	102	0.570	0.118
	Roughage	140[b]	192[a]	3.014	< 0.01
	Total	241[b]	293[a]	3.498	< 0.01
C18:2n6 intake (g/d)	Conc.	0.47	0.48	0.010	0.810
	Roughage	3.37[b]	4.68[a]	0.099	< 0.01
	Total	3.84[b]	5.16[a]	0.187	< 0.01
C18:3n3 intake (g/d)	Conc.	0.13	0.13	0.006	0.861
	Roughage	4.66[b]	6.59[a]	0.066	< 0.01
	Total	4.79[b]	6.73[a]	0.070	< 0.01

Means within a row followed by the different letter are different ($P < 0.05$),

SEM : standard error of means,

C18:2n6 : Linoleic acid, and C18:3n3 : α-linolenic acid.

5.5.4 The effects of forage species and feeding system on *Butyrivibrio fibrisolvens, Fibrobacter succinogenes* and total bacteria population in goat's rumen

The population of *B. fibrisolvens*, Napier Pak Chong 1 grazing milking goats had the average *B. fibrisolvens* bacteria at last day h0 was 4.35×10^6 copies/ml rumen content, which was significantly higher ($P<0.05$) than that in Napier Pak Chong 1 cut-and-carry milking goats (2.27×10^6 copies/ml rumen content), at h2 was 4.58×10^6 copies/ml rumen content significantly higher ($P<0.05$) than that in cut-and-carry milking goats (2.41×10^6 copies/ml), at h4 was 5.80×10^6 copies/ml rumen content significantly higher ($P<0.05$) than that in cut-and-carry milking goats (3.64×10^6 copies/ml), at h6 was 5.95×10^6 copies/ml rumen content significantly higher ($P<0.05$) than that in cut-and-carry milking goats (3.78×10^6 copies/ml) (Table 5.9).

For Napier Pak Chong 1 grazing milking goats, the average *F. succinogenes* bacteria at last day h0 was 6.23×10^6 copies/ml rumen content, which was significantly higher ($P<0.05$) than that in Napier Pak Chong 1 cut-and-carry milking goats (4.15×10^6 copies/ml rumen content), at h2 was 6.64×10^6 copies/ml rumen content significantly higher ($P<0.05$) than that in cut-and-carry milking goats (4.78×10^6 copies/ml), at h4 was 7.41×10^6 copies/ml rumen content significantly higher ($P<0.05$) than that in cut-and-carry milking goats (5.58×10^6 copies/ml), at h6 was 7.27×10^6 copies/ml rumen content significantly higher ($P<0.05$) than that in cut-and-carry milking goats (5.58×10^6 copies/ml).

Total bacteria population, Napier Pak Chong 1 grazing milking goats, the average total bacteria at last day h0 was 8.27×10^{10} copies/ml rumen content, which was significantly higher ($P<0.05$) than that in Napier Pak Chong 1 cut-and-carry milking goats (6.34×10^{10} copies/ml rumen content), at h2 was 8.97×10^{10} copies/ml

rumen content significantly higher ($P < 0.05$) than that in cut-and-carry milking goats (6.87×10^{10} copies/ml), at h4 was 9.76×10^{10} copies/ml rumen content significantly higher ($P < 0.05$) than that in cut-and-carry milking goats (7.13×10^{10} copies/ml), at h6 was 9.97×10^{10} copies/ml rumen content significantly higher ($P < 0.05$) than that in cut-and-carry milking goats (7.56×10^{10} copies/ml).

In Leucaena experiment, the average *B. fibrisolvens* bacteria at last day h0 was 4.23×10^{6} copies/ml rumen content, which was significantly higher ($P < 0.05$) than that in Leucaena cut-and-carry milking goats (2.15×10^{6} copies/ml rumen content), at h2 was 4.46×10^{6} copies/ml rumen content significantly higher ($P < 0.05$) than that in cut-and-carry milking goats (2.33×10^{6} copies/ml), at h4 was 5.58×10^{6} copies/ml rumen content significantly higher ($P < 0.05$) than that in cut-and-carry milking goats (3.25×10^{6} copies/ml), at h6 was 5.63×10^{6} copies/ml rumen content significantly higher ($P < 0.05$) than that in cut-and-carry milking goats (3.36×10^{6} copies/ml) (Table 5.10).

For Leucaena grazing milking goats, the average *F. succinogenes* bacteria at last day h0 was 6.46×10^{6} copies/ml rumen content, which was significantly higher ($P < 0.05$) than that in Leucaena cut-and-carry milking goats (4.35×10^{6} copies/ml rumen content), at h2 was 6.63×10^{6} copies/ml rumen content significantly higher ($P < 0.05$) than that in cut-and-carry milking goats (4.80×10^{6} copies/ml), at h4 was 7.25×10^{6} copies/ml rumen content significantly higher ($P < 0.05$) than that in cut-and-carry milking goats (5.33×10^{6} copies/ml), at h6 was 7.36×10^{6} copies/ml rumen content significantly higher ($P < 0.05$) than that in cut-and-carry milking goats (5.95×10^{6} copies/ml).

Leucaena grazing milking goats had the average Total bacteria at last day h0 was 7.37×10^{10} copies/ml rumen content, which was significantly higher ($P < 0.05$) than that in Leucaena cut-and-carry milking goats (5.44×10^{10} copies/ml rumen content), at h2 was 7.76×10^{10} copies/ml rumen content significantly higher ($P < 0.05$) than that in cut-and-carry milking goats (5.94×10^{10} copies/ml), at h4 was 8.26×10^{10} copies/ml rumen content significantly higher ($P < 0.05$) than that in cut-and-carry milking goats (6.54×10^{10} copies/ml), at h6 was 8.69×10^{10} copies/ml rumen content significantly higher ($P < 0.05$) than that in cut-and-carry milking goats (6.92×10^{10} copies/ml).

In conclusion, all of the results showed that grazing milking goats of Napier Pak Chong 1 or Leucaena pasture were significant higher ($P < 0.05$) in *B. fibrisolvens*, *F. succinogenes* and Total bacteria populations than those Napier Pak Chong 1 or Leucaena cut-and-carry milking goats, it can be the results from grazing goats had greater feed intake than that cut-and-carry goats

Table 5.9 Rumen microorganisms of cut-and-carry and grazing Napier Pak Chong 1 grass milking Saanen goats.

		Napier Pak Chong 1 grass feeding system			
	hour	**Cut-and-Carry**	**Grazing**	**SEM**	**Pr > F**
B. fibrisolvens	0	2.27[b]	4.35[a]	0.278	< 0.01
(10^6 copies/ml)	2	2.41[b]	4.58[a]	0.327	0.011
	4	3.64[b]	5.80[a]	0.382	0.022
	6	3.78[b]	5.95[a]	0.329	0.011
F. succinogenes	0	4.15[b]	6.23[a]	0.385	0.027
(10^6 copies/ml)	2	4.78[b]	6.64[a]	0.313	0.018
	4	5.58[b]	7.41[a]	0.355	0.033
	6	5.58[b]	7.27[a]	0.360	0.047
Total bacteria	0	6.34[b]	8.27[a]	0.380	0.035
(10^{10} copies/ml)	2	6.87[b]	8.97[a]	0.397	0.030
	4	7.13[b]	9.76[a]	0.369	< 0.01
	6	7.56[b]	9.97[a]	0.334	< 0.01

Means within a row followed by the different letter are different ($P < 0.05$) and SEM : standard error of means.

Table 5.10 Rumen microorganisms of cut-and-carry and grazing Leucaena legume milking Saanen goats.

		Leucaena legume feeding system			
	hour	**Cut-and-Carry**	**Grazing**	**SEM**	**Pr > F**
B. fibrisolvens	0	2.15^{b}	4.23^{a}	0.276	< 0.01
(10^6 copies/ml)	2	2.33^{b}	4.46^{a}	0.310	< 0.01
	4	3.25^{b}	5.58^{a}	0.442	0.030
	6	3.36^{b}	5.63^{a}	0.365	0.015
F. succinogenes	0	4.35^{b}	6.46^{a}	0.265	< 0.01
(10^6 copies/ml)	2	4.80^{b}	6.63^{a}	0.330	0.024
	4	5.33^{b}	7.25^{a}	0.300	0.013
	6	5.95^{b}	7.36^{a}	0.291	0.042
Total bacteria	0	5.44^{b}	7.37^{a}	0.335	0.021
(10^{10} copies/ml)	2	5.94^{b}	7.76^{a}	0.314	0.020
	4	6.54^{b}	8.26^{a}	0.285	0.017
	6	6.92^{b}	8.69^{a}	0.317	0.023

Means within a row followed by the different letter are different ($P < 0.05$) and SEM : standard error of means.

5.5.5 Milk yield (g/d) and milk compositions (%) of cut-and-carry vs grazing goats of Napier Pak Chong1 and Leucaena experiments

Table 5.11 and 5.12 showed milk yield (g/d) and milk composition (%) of cut-and-carry milking goats were compared with grazing milking goats, in Napier Pak Chong 1 grass and Leucaena experiment, respectively, for grass experiment, the result showed that grazing Napier Pak Chong 1 milking goats had significantly higher ($P<0.05$) milk yield about 1,901.82 g/d more than cut-and-carry Napier Pak Chong 1 milking goats (1,488.55 g/d), while milk fat and total solid, grazing Napier Pak Chong 1 milking goats had significantly higher ($P < 0.05$) (3.85 and 12.58%, respectively) more than cut-and-carry Napier Pak Chong 1 milking goats (3.49 and 12.04%, respectively). However, milk protein, lactose and SNF did not differ between treatments.

In Leucaena legume experiment, the result showed that grazing Leucaena milking goats had significantly higher ($P < 0.05$) milk yield about 2,010.50 g/d more than cut-and-carry Leucaena milking goats (1,573.71 g/d), while milk fat and total solid, grazing Leucaena milking goats had significantly higher ($P < 0.05$) (3.80 and 12.60%, respectively) more than cut-and-carry Leucaena milking goats (3.41 and 12.01%, respectively). However, milk protein, lactose and SNF did not differ between treatments.

The current study was showed the different in milk yield, milk fat and total solid of dairy goats, but Prasanpanich et al. (2002) found that there was no significant difference in milk yield and composition of milk of cows grazed outdoors and cows fed indoors, he were studied about grazing behavior, milk production, liveweight change and health status were studied in 2 groups of 6 Friesian-cross cows grazed outdoor on pasture or housed indoors during mid-lactation in central Thailand. Indoor

cows were housed in an open-sided barn and fed with cut-and-carried pasture. Outdoor cows were strip-grazed on the same guinea grass pasture without any shade and were brought indoors only for milking. All cows were also fed meal concentrate twice daily at milking according to their level of milk production. Milk production (11.9 vs 12.3 kg/d for FCM yield) and composition were similar in both groups. Hoof damage was higher amongst cows housed than in those grazing outdoors. These data suggest that dairy cows will produce satisfactory milk yields when grazed outdoors instead of being housed, as is common in Thailand. This grazing system should result in significant reductions in farm costs.

Compared to current study, goats were smaller than cows and they were rest under the bushed of Napier grass and browse of the leucaena legume under the critical temperature during the day.

Table 5.11 Milk yield (g/d) and milk composition (%) of cut-and-carry and grazing Napier Pak Chong 1 grass of milking Saanen goats.

	Napier Pak Chong 1 grass feeding system			
	Cut-and-Carry	**Grazing**	**SEM**	**Pr > F**
Milk yield (g/d)	1,489[b]	1,902[a]	66.937	0.015
Milk composition (%)				
Milk fat (%)	3.49[b]	3.85[a]	0.041	< 0.01
Milk protein (%)	3.42	3.45	0.044	0.744
Milk lactose (%)	4.33	4.48	0.080	0.366
Solid not fat (%)	8.55	8.73	0.097	0.382
Total solid (%)	12.04[b]	12.58[a]	0.059	< 0.01

Means within a row followed by the different letter are different ($P < 0.05$) and SEM : standard error of means.

Table 5.12 Milk yield (g/d) and milk composition (%) of cut-and-carry and grazing Leucaena legume of milking Saanen goats.

	Leucaena legume feeding system			
	Cut-and-Carry	**Grazing**	**SEM**	**Pr > F**
Milk yield (g/d)	1,574[b]	2,011[a]	52.790	< 0.01
Milk composition (%)				
Milk fat (%)	3.41[b]	3.80[a]	0.055	< 0.01
Milk protein (%)	3.40	3.51	0.069	0.449
Milk lactose (%)	4.42	4.53	0.060	0.385
Solid not fat (%)	8.60	8.81	0.050	0.070
Total solid (%)	12.01[b]	12.60[a]	0.095	0.015

Means within a row followed by the different letter are different ($P < 0.05$) and SEM : standard error of means.

Table 5.13 Effect of cut-and-carry vs grazing system of milking goats fed Napier Pak Chong 1 on fatty acid profile of milk fat.

Fatty acid (% of Total FA)	Napier Pak Chong 1		SEM	Pr > F
	Cut-and-Carry	Grazing		
C4:0	5.79	5.44	0.092	0.095
C6:0	4.25	4.29	0.071	0.785
C8:0	5.69	5.11	0.121	0.043
C10:0	11.43	11.12	0.548	0.784
C12:0	3.08	3.11	0.223	0.948
C13:0	0.29	0.25	0.041	0.636
C14:0	5.39	5.34	0.147	0.880
C14:1	0.53	0.56	0.061	0.836
C15:0	0.49	0.51	0.048	0.840
C15:1	0.32	0.46	0.038	0.119
C16:0	18.33	17.38	0.560	0.421
C16:1	1.59	1.66	0.074	0.647
C17:1	0.02	0.02	0.003	0.471
C18:0	2.69	2.59	0.101	0.636
C18:1n9t	16.37	16.41	0.571	0.973
C18:1n9c	17.20	17.35	0.539	0.893
C18:2n6t	1.59	1.62	0.075	0.847
C18:2n6c	2.11	3.33	0.159	<0.01
C20:0	1.35	1.20	0.056	0.217
C18:3n6	0.05	0.05	0.008	0.868
C20:1	0.02	0.04	0.005	0.290
C18:3n3	0.74	1.15	0.067	<0.01
c9,t11 CLA	0.23	0.39	0.011	<0.01
t10,c12 CLA	0.00	0.15	0.000	<0.01
C21:0	0.12	0.13	0.013	0.877
C20:2	0.04	0.04	0.006	0.874
C22:0	0.13	0.12	0.011	0.647
C20:3n6	0.02	0.02	0.003	0.760
C22:1n9	0.02	0.02	0.005	0.845
C20:3n3	0.02	0.02	0.004	0.823
C20:4n6	0.03	0.04	0.005	0.408
C23:0	0.07	0.08	0.005	0.408
SFA[1]	59.10	56.67	0.604	0.049
MUFA[2]	36.07	36.52	0.565	0.701
PUFA[3]	4.83	6.81	0.205	<0.01

SEM : standard error of means, [1]SFA : Sum of saturated fatty acid from C4:0-C14:0, C15:0, C16:0, C18:0, C20:0, C21:0, C22:0, C23:0, [2]MUFA : Sum of monounsaturated fatty acid from C14:1, C15:1, C16:1, C17:1, C18:1n9t, C18:1n9c, C20:1, C22:1n9, [3]PUFA : Sum of polyunsaturated fatty acid from C18:2n6t, C18:2n6c, C18:3n6, C18:3n3, c9,t11 CLA, t10,c12 CLA, C20:2, C20:3n6, C20:3n3, C20:4n6.

Table 5.14 Effect of cut-and-carry vs grazing system of milking goats fed Leucaena on fatty acid profile of milk fat.

Fatty acid (% of Total FA)	Leucaena		SEM	Pr > F
	Cut-and-Carry	Grazing		
C4:0	5.29	5.31	0.262	0.971
C6:0	5.09	4.65	0.265	0.430
C8:0	4.57	4.22	0.146	0.266
C10:0	11.40	11.24	0.552	0.891
C12:0	3.61	3.32	0.250	0.578
C13:0	0.34	0.24	0.048	0.328
C14:0	5.39	5.40	0.157	0.975
C14:1	0.51	0.57	0.061	0.637
C15:0	0.51	0.68	0.064	0.222
C15:1	0.41	0.67	0.061	0.067
C16:0	17.55	17.49	0.621	0.963
C16:1	1.60	1.41	0.050	0.095
C17:1	0.02	0.02	0.004	0.826
C18:0	2.58	2.89	0.172	0.394
C18:1n9t	16.56	15.83	0.708	0.620
C18:1n9c	17.30	16.62	0.607	0.591
C18:2n6t	1.63	1.78	0.045	0.147
C18:2n6c	2.08	3.24	0.063	<0.01
C20:0	2.16	2.16	0.076	0.858
C18:3n6	0.05	0.05	0.006	0.870
C20:1	0.04	0.04	0.005	0.833
C18:3n3	0.65	1.14	0.069	0.016
c9,t11 CLA	0.25	0.44	0.012	<0.01
t10,c12 CLA	0.00	0.14	0.000	<0.01
C21:0	0.08	0.09	0.005	0.347
C20:2	0.04	0.04	0.006	0.733
C22:0	0.12	0.13	0.011	0.730
C20:3n6	0.02	0.02	0.003	0.371
C22:1n9	0.02	0.02	0.003	0.524
C20:3n3	0.02	0.02	0.003	0.402
C20:4n6	0.03	0.04	0.005	0.536
C23:0	0.08	0.09	0.004	0.481
SFA[1]	58.77	57.91	0.342	0.092
MUFA[2]	36.46	35.18	0.558	0.284
PUFA[3]	4.77	6.91	0.162	<0.01

SEM : standard error of means, [1]SFA : Sum of saturated fatty acid from C4:0-C14:0, C15:0, C16:0, C18:0, C20:0, C21:0, C22:0, C23:0, [2]MUFA : Sum of monounsaturated fatty acid from C14:1, C15:1, C16:1, C17:1, C18:1n9t, C18:1n9c, C20:1, C22:1n9, [3]PUFA : Sum of polyunsaturated fatty acid from C18:2n6t, C18:2n6c, C18:3n6, C18:3n3, c9,t11 CLA, t10,c12 CLA, C20:2, C20:3n6, C20:3n3, C20:4n6.

Table 5.15 CLA and C18:3n3 in milk (% of total fatty acid) of cut-and-carry and grazing Napier Pak Chong 1 grass/ Leucaena legume of milking Saanen goats.

	Forage Species			
	Napier Pak Chong 1 grass feeding system			
	Cut-and-Carry	Grazing	SEM	Pr > F
CLA in milk (% of total fatty acid)				
c9,t11 CLA	0.23[b]	0.39[a]	0.011	< 0.01
t10,c12 CLA	0.00[b]	0.15[a]	0.000	< 0.01
Total CLA	0.23[b]	0.54[a]	0.011	< 0.01
C18:3n3	0.74[b]	1.15[a]	0.067	< 0.01
	Leucaena legume feeding system			
	Cut-and-Carry	Grazing	SEM	Pr > F
CLA in milk (% of total fatty acid)				
c9,t11 CLA	0.25[b]	0.44[a]	0.012	< 0.01
t10,c12 CLA	0.00[b]	0.14[a]	0.000	< 0.01
Total CLA	0.25[b]	0.58[a]	0.012	< 0.01
C18:3n3	0.65[b]	1.14[a]	0.069	0.016

Means within a row followed by the different letter are different ($P < 0.05$) and

SEM : standard error of means.

5.5.6 Milk's CLA and omega-3 (% of total fatty acid) of cut-and-carry and grazing Napier Pak Chong 1 grass/ Leucaena legume of milking Saanen goats

Grazing Napier Pak Chong 1 milking goats had significantly higher (P<0.05) Cis-9, Trans-11 CLA 0.39% of total fatty acid more than cut-and-carry Napier Pak Chong 1 milking goats (0.23% of total fatty acid). Tran-10, Cis-12 CLA, only grazing Napier Pak Chong 1 milking goats had this CLA 0.15% of total fatty acid. Grazing Napier Pak Chong 1 milking goats had significantly higher (P<0.05) total CLA and omega-3 0.54; 1.15% of total fatty acid more than cut-and-carry Napier Pak Chong 1 milking goats (0.23; 0.74% of total fatty acid).

Grazing Leucaena milking goats had significantly higher (P<0.05) Cis-9, Trans-11 CLA 0.44% of total fatty acid more than cut-and-carry Leucaena milking goats (0.25% of total fatty acid). Tran-10, Cis-12 CLA, only grazing Leucaena milking goats had this CLA 0.14% of total fatty acid. Grazing Leucaena milking goats had significantly higher (P<0.05) total CLA and omega-3 0.58; 1.14% of total fatty acid more than cut-and-carry Leucaena milking goats (0.25; 0.65% of total fatty acid).

Since experiment I-I showed that Napier Pak Chong 1 grass had significantly higher (P<0.05) C18:2 and C18:3 than Purple Guinea, so they were effected for grazing goats to have more CLA than that cut-and-carry goats, there were studied with grazing Guinea grass, Bourapa et al. (2014) study the milk quality of dairy cattle under conditions of group grazing and loose housing on high quality forage intake. Eight crossbred cows (93% Holstein and 7% *Bos indicus*). The first group was allowed free grazing on pasture consisting mainly of Guinea grass using a rotational grazing procedure. The second group was kept in the shaded. The results showed that the milk yields, the percentages of milk fat, protein and SNF, and the content of C18:3 and

CLA were not significantly different between treatments. However, the lactose percentage and the content of C18:2 were significantly different. The outdoor grazing system significantly increased the higher milk content of linoleic acid.

The current study was found that only grazing milking goats both Napier Pak Chong 1 and Leucaena were products Tran-10, Cis-12 CLA, this type of CLA has been reported by many researcher that can decreases adipose tissue lipids in mice (Warren et al. 2003), Tran-10, Cis-12 CLA is the most potent isomer in terms of potential to prevent cell proliferation and induce apoptosis in cancer cells (Cho et al., 2005, 2006; Kim et al., 2002; ;Lee et al., 2006; Ochoa et al., 2004). Trans-10, cis-12 is also associate with decreased body fat and increased lean body mass in overweight and obese humens. (Blankson et al, 2000; Smedman and Vessby, 2001) and also reduce body fat in for healthy exercising humans (Thom et al., 2001).

5.6 Conclusions

Napier Pak Chong 1 grazing milking goats, the average *Butyrivibrio fibrisolvens*, *Fibrobacter succinogenes* and total bacteria were significantly higher ($P<0.05$) than that in Napier Pak Chong 1 cut-and-carry milking goats. Leucaena grazing milking goats, the average *Butyrivibrio fibrisolvens*, *Fibrobacter succinogenes* and total bacteria were significantly higher ($P<0.05$) than that in Leucaena cut-and-carry milking goats.

Grazing Napier Pak Chong 1 milking goats had significantly higher ($P<0.05$) milk yield, milk fat, c9,t11 CLA, t10,c12 CLA and omega-3 than that from cut-and-carry Napier Pak Chong 1 milking goats. Grazing Leucaena milking goats had significantly higher ($P<0.05$) milk yield, milk fat, c9,t11 CLA, t10,c12 CLA and omega-3 than that from cut-and-carry Leucaena milking goats.

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CHAPTER VI

OVERALL CONCLUSION AND IMPLICATION

6.1 Conclusions

The aim of this experiment was to study the effects of forage species and feeding system on CLA content in goat's milk.

The first experiment was carried out to investigate DM, CP, NDF, fat, linoleic (C18:2) and linolenic acid (C18:3) yield, chemical composition, linoleic and linolenic acid contents (% of total fatty acid) and year round yields and also ruminal degradability of 6 forage species, Napier Pak Chong 1 had significantly higher ($P<0.05$) in DM, CP, NDF, fat, linoleic acid and linolenic acid yields, %CP, %linoleic acid and %linolenic acid (% of total fatty acid) than Chinese Pennisetum and Purple Guinea. Leucaena had significantly higher ($P < 0.05$) in DM, CP, NDF, fat, linoleic acid and linolenic acid yields, %CP, %linoleic acid and %linolenic acid (% of total fatty acid) than Hamata and Hedge Lucern.

For grass species, potential and effective DM, CP, NDF, C18:2n6 and C18:3n3 degradabilities of Napier Pak Chong 1 were significantly higher ($P<0.05$) than that of rice straw, but not significantly different from those of Chinese Pennisetum and Purple Guinea. For legume species, potential and effective DM, CP, NDF, C18:2n6 and C18:3n3 degradabilities of Leucaena was significantly higher ($P < 0.05$) than that of rice straw, but not significantly different from those of Hedge Lucern and Hamata.

The second experiment was to investigate the effects of forage species on *B. fibrisolvens, F. succinogenes* and total bacteria population in goat's rumen. For grass experiment, the population of ruminal *B. fibrisolvens, F. succinogenes* and total bacteria of goats were not significantly different among three grass species including Purple Guinea, Chinese Pennisetum and Napier Pak Chong 1. For legume experiment, the population of ruminal *B. fibrisolvens*, *F. succinogenes* and total bacteria of goats were not significantly different among three legume species including Hamata, Hedge Lucern and Leucaena.

The third experiment was to investigate the effects of forage species and feeding system on CLA and omega-3 contents in goat's milk and on *B. fibrisolvens*, *F. succinogenes* and total bacteria population in goat's rumen, for Napier Pak Chong 1 grazing milking goats, the average *B. fibrisolvens*, *F. succinogenes* and total bacteria were significantly higher ($P < 0.05$) than that in Napier Pak Chong 1 cut-and-carry milking goats. While Leucaena grazing milking goats, the average *B. fibrisolvens*, *F. succinogenes* and total bacteria were significantly higher ($P < 0.05$) than that in Leucaena cut-and-carry milking goats.

Grazing Napier Pak Chong 1 milking goats had significantly higher ($P < 0.05$) milk yield and milk fat than that cut-and-carry Napier Pak Chong 1 milking goats. Milk CLA, grazing Napier Pak Chong 1 milking goats had significantly higher ($P < 0.05$) Cis-9, Trans-11 CLA about 0.39% of total fatty acid more than cut-and-carry Napier Pak Chong 1 milking goats (0.23% of total fatty acid). Tran-10, Cis-12 CLA, only grazing Napier Pak Chong 1 milking goats had this CLA 0.15% of total fatty acid. Total CLA, grazing Napier Pak Chong 1 milking goats had significantly higher ($P < 0.05$) total CLA 0.54% of total fatty acid more than cut-and-carry Napier Pak Chong 1 milking goats (0.23% of total fatty acid).

Grazing Leucaena milking goats had significantly higher ($P < 0.05$) milk yield and milk fat than that cut-and-carry Leucaena milking goats. Milk CLA, grazing Leucaena milking goats had significantly higher ($P < 0.05$) Cis-9, Trans-11 CLA 0.44% of total fatty acid more than cut-and-carry Leucaena milking goats (0.25% of total fatty acid). Tran-10, Cis-12 CLA, only grazing Leucaena milking goats had this CLA 0.14% of total fatty acid. Total CLA, grazing Leucaena milking goats had significantly higher ($P < 0.05$) total CLA 0.58% of total fatty acid more than cut-and-carry Leucaena milking goats (0.25% of total fatty acid).

6.2 Implications

For working with grazing animal, product per acre or product per area are important more than product/animal (Mott, 1973). In Napier Pak Chong 1 grazing milking goats might be more efficiency than that Leucaena grazing milking goats in term of product of CLA/area.

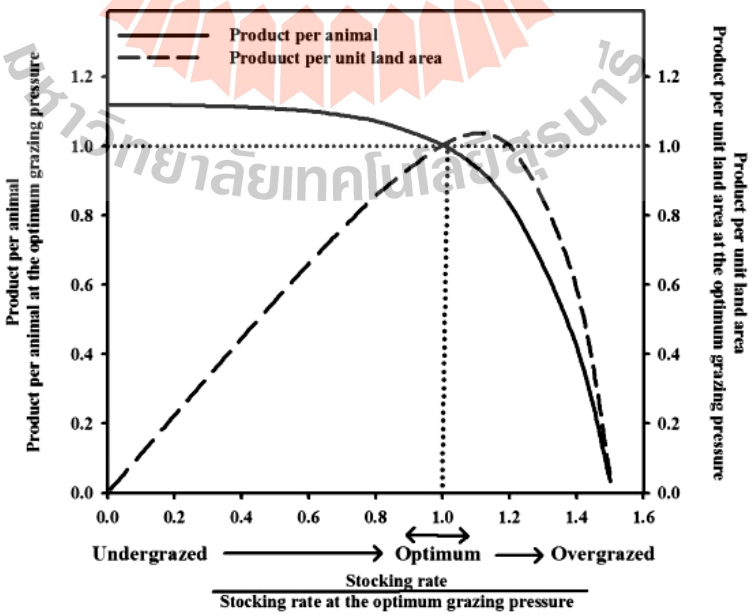

Figure 6.1 Relative between product per animal and product per area (Mott, 1973).

6.3 Reference

Mott, G. O., 1973. Evaluating forage production. In Heath, M. E., Metcalfe, D. S., and Barnes, R. F. (eds.), Forages : **The Science of Grassland Agriculture**., 3rd ed., 126-135. Ames, Iowa State University.

APPENDIX

Table A.1 Meteorological data of the experimental area.

Item	**King's 80th birthday Station**	**Khok Kruat Station**
Rainfall (mm)		
January/2014	0.0	14.3
February/2014	13.6	6.8
March/2014	7.0	7.0
April/2014	65.2	102.7
May/2014	53.8	132.0
June/2014	44.5	39.5
July/2014	114.5	95.2
August/2014	122.7	175.6
September/2014	150.3	195.4
October/2014	25.7	65.9
November/2014	43.0	65.8
December/2014	0.0	0.0
Average/2014	640.3	900.2
Relative humidity (%)	70.0	70.0

Data from Hydrology Irrigation for Lower Northeastern Region, Nakorn Ratchasima, Royal Irrigation Department

Table A.2 Effect of grass species on fatty acid profiles in Experiment I.

Fatty acid (% of Total FA)	Purple Guinea	Chinese Pennisetum	Napier Pak Chong 1	SEM	Pr > F
C4:0	6.49	5.97	5.53	0.228	0.301
C6:0	2.50	2.39	1.61	0.174	0.152
C8:0	1.49	1.14	1.00	0.149	0.431
C10:0	1.93	1.66	1.09	0.120	0.070
C11:0	1.95	2.09	1.35	0.123	0.103
C12:0	2.67	2.92	2.37	0.111	0.209
C13:0	1.24	1.49	1.49	0.124	0.659
C14:0	2.07	2.22	1.80	0.105	0.330
C14:1	2.08	1.28	1.50	0.131	0.106
C15:0	2.90	2.87	1.77	0.106	<0.01
C15:1	2.52	1.45	1.24	0.133	0.017
C16:0	11.03	12.22	12.25	0.351	0.340
C16:1	1.96	1.27	1.78	0.110	0.094
C17:1	1.69	1.57	1.49	0.121	0.794
C18:0	2.27	2.26	1.06	0.106	<0.01
C18:1n9	4.88	3.43	3.50	0.153	0.014
C18:2n6	10.69	10.86	12.60	0.188	0.011
C20:0	2.12	1.98	1.98	0.130	0.892
C18:3n6	3.93	2.06	1.00	0.158	<0.01
C20:1	1.87	1.35	1.17	0.102	0.071
C18:3n3	14.44	28.68	36.52	0.789	<0.01
C21:0	1.75	1.98	1.23	0.114	0.087
C18:4n3	3.51	1.32	1.07	0.135	<0.01
C20:2	3.55	2.12	1.20	0.128	<0.01
C22:0	2.01	1.74	1.05	0.134	0.063
C20:3n6	2.72	1.15	1.05	0.127	<0.01
C22:1n9	0.03	0.03	0.03	0.009	0.984
C20:3n3	1.94	0.20	0.10	0.085	<0.01
C20:4n6	1.72	0.21	0.11	0.076	<0.01
C23:0	0.03	0.10	0.05	0.012	0.120
SFA[1]	42.46	43.02	35.64	1.210	0.045
MUFA[2]	15.04	10.38	10.71	1.659	0.485
PUFA[3]	42.50	46.60	53.65	0.965	<0.01
PUFA/SFA	1.00	1.08	1.51	0.082	0.043

SEM = standard error of means, [1]SFA = Sum of saturated fatty acid from C4:0-C14:0, C15:0, C16:0, C18:0, C20:0, C21:0, C22:0, C23:0, [2]MUFA = Sum of monounsaturated fatty acid from C14:1, C15:1, C16:1, C17:1, C18:1n9, C20:1, C22:1n9, [3]PUFA = Sum of polyunsaturated fatty acid from C18:2n6, C18:3n6, C18:3n3, C18:4n3, C20:2, C20:3n6, C20:3n3, C20:4n6.

Table A.3 Effect of legumes species on fatty acid profiles in Experiment I.

Fatty acid (% of Total FA)	Hamata	Hedge Lucern	Leucaena	SEM	Pr > F
C4:0	3.11	3.88	2.24	0.106	<0.01
C6:0	1.90	1.87	1.11	0.116	0.052
C8:0	2.03	2.53	2.63	0.168	0.359
C10:0	1.81	1.93	1.87	0.113	0.902
C11:0	2.53	1.83	1.46	0.137	0.047
C12:0	2.76	1.59	2.49	0.148	0.041
C13:0	1.70	1.39	1.88	0.106	0.235
C14:0	2.75	2.10	1.82	0.100	0.022
C14:1	2.83	2.43	3.15	0.112	0.102
C15:0	2.15	2.73	2.10	0.103	0.082
C15:1	1.60	1.96	2.78	0.120	0.018
C16:0	11.08	10.68	10.58	0.351	0.831
C16:1	2.33	2.15	2.47	0.186	0.791
C17:1	1.29	1.64	1.45	0.122	0.543
C18:0	2.30	2.51	2.52	0.160	0.826
C18:1n9	3.79	4.14	4.08	0.107	0.416
C18:2n6	10.44	10.75	13.01	0.404	0.040
C20:0	2.31	2.15	1.81	0.132	0.352
C18:3n6	2.68	1.53	1.55	0.147	0.030
C20:1	2.29	2.35	1.02	0.151	0.019
C18:3n3	14.14	15.65	18.56	0.471	0.023
C21:0	2.67	2.57	1.09	0.134	<0.01
C18:4n3	2.26	2.83	2.86	0.121	0.153
C20:2	3.10	3.22	2.45	0.127	0.098
C22:0	3.12	2.30	1.31	0.105	<0.01
C20:3n6	3.47	3.64	3.91	0.127	0.414
C22:1n9	1.07	1.85	1.05	0.213	0.291
C20:3n3	3.37	2.65	3.19	0.105	0.073
C20:4n6	3.06	2.86	2.66	0.111	0.397
C23:0	0.06	0.28	0.90	0.070	<0.01
SFA[1]	42.28	40.35	35.80	1.474	0.026
MUFA[2]	15.20	16.52	16.00	0.468	0.547
PUFA[3]	42.52	43.13	48.19	0.902	0.028
PUFA/SFA	1.01	1.07	1.35	0.087	0.039

SEM = standard error of means, [1]SFA = Sum of saturated fatty acid from C4:0-C14:0, C15:0, C16:0, C18:0, C20:0, C21:0, C22:0, C23:0, [2]MUFA = Sum of monounsaturated fatty acid from C14:1, C15:1, C16:1, C17:1, C18:1n9, C20:1, C22:1n9, [3]PUFA = Sum of polyunsaturated fatty acid from C18:2n6, C18:3n6, C18:3n3, C18:4n3, C20:2, C20:3n6, C20:3n3, C20:4n6.

Table A.4 Chemical composition of rice straw for Experimant I-II.

	Rice Straw
Dry matter	91.27
	---------- % on a dry matter basis ----------
CP	3.84
CF	39.68
NDF	76.48
ADF	52.58
Ash	12.96
Fat	0.77
C18:2n6 (% of total fatty acid)	5.81
C18:3n3 (% of total fatty acid)	2.52

DM : Dry Matter, CP : Crude Protein, CF : Crude Fiber,

NDF : Neutral Detergent Fiber, ADF : Acid Detergent Fiber,

C18:2n6 : Linoleic acid, C18:3n3 : α-linolenic acid.

Table A.5 Effect of cut-and-carry vs grazing system of milking goats fed Napier Pak Chong 1 on fatty acid profile of milk fat (g/kg milk).

	Napier Pak Chong 1	
Fatty acid	**Cut-and-Carry**	**Grazing**
	---------- g/kg milk ----------	
C4:0	2.02	2.09
C6:0	1.48	1.65
C8:0	1.99	1.97
C10:0	3.99	4.28
C12:0	1.07	1.20
C13:0	0.10	0.10
C14:0	1.88	2.06
C14:1	0.18	0.22
C15:0	0.17	0.20
C15:1	0.11	0.18
C16:0	6.40	6.69
C16:1	0.55	0.64
C17:1	0.01	0.01
C18:0	0.94	1.00
C18:1n9t	5.71	6.32
C18:1n9c	6.00	6.68
C18:2n6t	0.55	0.62
C18:2n6c	0.74	1.28
C20:0	0.47	0.46
C18:3n6	0.02	0.02
C20:1	0.01	0.02
C18:3n3	0.26	0.44
c9,t11 CLA	0.08	0.15
t10,c12 CLA	0.00	0.06
C21:0	0.04	0.05
C20:2	0.01	0.02
C22:0	0.05	0.05
C20:3n6	0.01	0.01
C22:1n9	0.01	0.01
C20:3n3	0.01	0.01
C20:4n6	0.01	0.02
C23:0	0.02	0.03
SFA[1]	20.63	21.82
MUFA[2]	12.59	14.06
PUFA[3]	1.69	2.62

SEM = standard error of means, [1]SFA = Sum of saturated fatty acid from C4:0-C14:0, C15:0, C16:0, C18:0, C20:0, C21:0, C22:0, C23:0, [2]MUFA = Sum of monounsaturated fatty acid from C14:1, C15:1, C16:1, C17:1, C18:1n9t, C18:1n9c, C20:1, C22:1n9, [3]PUFA = Sum of polyunsaturated fatty acid from C18:2n6t, C18:2n6c, C18:3n6, C18:3n3, c9, t11 CLA, t10, c12 CLA, C20:2, C20:3n6, C20:3n3, C20:4n6.

Table A.6 Effect of cut-and-carry vs grazing system of milking goats fed Leucaena on fatty acid profile of milk fat (g/kg milk).

	Leucaena	
Fatty acid	**Cut-and-Carry**	**Grazing**
	---------- g/kg milk ----------	
C4:0	1.80	2.02
C6:0	1.74	1.77
C8:0	1.56	1.60
C10:0	3.89	4.27
C12:0	1.23	1.26
C13:0	0.12	0.09
C14:0	1.84	2.05
C14:1	0.17	0.22
C15:0	0.17	0.26
C15:1	0.14	0.25
C16:0	5.98	6.65
C16:1	0.55	0.54
C17:1	0.01	0.01
C18:0	0.88	1.10
C18:1n9t	5.65	6.02
C18:1n9c	5.90	6.32
C18:2n6t	0.56	0.68
C18:2n6c	0.71	1.23
C20:0	0.74	0.82
C18:3n6	0.02	0.02
C20:1	0.01	0.02
C18:3n3	0.22	0.43
c9,t11 CLA	0.09	0.17
t10,c12 CLA	0.00	0.05
C21:0	0.03	0.03
C20:2	0.01	0.02
C22:0	0.04	0.05
C20:3n6	0.01	0.01
C22:1n9	0.01	0.01
C20:3n3	0.01	0.01
C20:4n6	0.01	0.02
C23:0	0.03	0.03
SFA[1]	20.04	22.01
MUFA[2]	12.43	13.37
PUFA[3]	1.63	2.63

SEM = standard error of means, [1]SFA = Sum of saturated fatty acid from C4:0-C14:0, C15:0, C16:0, C18:0, C20:0, C21:0, C22:0, C23:0, [2]MUFA = Sum of monounsaturated fatty acid from C14:1, C15:1, C16:1, C17:1, C18:1n9t, C18:1n9c, C20:1, C22:1n9, [3]PUFA = Sum of polyunsaturated fatty acid from C18:2n6t, C18:2n6c, C18:3n6, C18:3n3, c9,t11 CLA, t10,c12 CLA, C20:2, C20:3n6, C20:3n3, C20:4n6.

BIOGRAPHY

Mr. Saranpong Thongruang was born on January 1st, 1976 in Nakorn Sawan Province and moved to live in Ubon Ratchathani Province. He graduated Bachelor of Science in Agriculture (Animal Science), Department of Animal Science, Faculty of Agriculture, Kasetsart University in 1998. He received Master of Science in Agriculture (Animal Science), Department of Animal Science, Faculty of Agriculture, Kasetsart University in 2003. He working at Faculty of Animal Science and Agricultural Technology, Silpakorn University since 2003 until now, he continued to study Doctor of Philosophy in Animal Production Technology, School of Animal Production Technology, Institute of Agricultural Technology, Suranaree University of Technology.