THE EFFECTS OF SUNFLOWER OIL AND NITRATE LEVELS ON METHANE PRODUCTION, NUTRIENT DIGESTIBILITY AND GROWTH PERFORMANCE IN MEAT GOATS FED WITH DIFFERENT ROUGHAGE QUALITY

Jiravan Khotsakdee

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

Suranaree University of Technology

Academic Year 2015

ผลของระดับของน้ำมันทานตะวันและไนเตรท ต่อการผลิตแก๊สมีเทน การย่อยได้ของโภชนะ และสมรรถภาพการเจริญเติบโต ของแพะเนื้อที่ได้รับอาหารหยาบที่มีคุณภาพต่างกัน

นางสาวจิระวัลย์ โคตรศักดี

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรดุษฎีบัณฑิต
สาขาวิชาเทคโนโลยีการผลิตสัตว์
มหาวิทยาลัยเทคโนโลยีสุรนารี
ปีการศึกษา 2558

THE EFFECTS OF SUNFLOWER OIL AND NITRATE LEVELS ON METHANE PRODUCTION, NUTRIENT DIGESTIBILITY AND GROWTH PERFORMANCE IN THE MEAT GOATS FED WITH DIFFERENT ROUGHAGE QUALITY

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

Thesis Examining Committee

W. Molee

(Dr. Wittawat Molee)
Chairperson

Pramote Paengkoum

(Assoc. Prof. Dr. Pramote Paengkoum)
Member (Thesis Advisor)

Chalermpon Yuangklang

(Asst. Prof. Dr. Chalermpon Yuangklang)
Member

(Assoc. Prof. Dr. Surintorn Boonanuntanasarn)
Member

(Prof. Dr. Christopher Simon McSweeney)
Member

(Asst. Prof. Dr. Amonrat Molee)
Member

Siwaporn Paengkoum

(Dr. Siwaporn Paengkoum)
Member

(Prof. Dr. Sukit Limpijumnong)
Vice Rector for Academic Affairs and Innovation

(Prof. Dr. Neung Teaumroong)
Dean of Institute of Agricultural Technology

จิระวัลย์ โคตรศักดี : ผลของระดับน้ำมันทานตะวันและไนเตรท ต่อการผลิตแก๊สมีเทน การย่อยได้ของโภชนะ และสมรรถภาพการเจริญเติบโต ของแพะเนื้อที่ได้รับอาหารหยาบที่มีคุณภาพต่างกัน (THE EFFECTS OF SUNFLOWER OIL AND NITRATE LEVELS ON METHANE PRODUCTION, NUTRIENT DIGESTIBILITY AND GROWTH PERFORMANCE IN MEAT GOATS FED WITH DIFFERENT ROUGHAGE QUALITY) อาจารย์ที่ปรึกษา : รองศาสตราจารย์ ดร.ปราโมทย์ แพงคำ, 125 หน้า.

วิทยานิพนธ์นี้มีวัตถุประสงค์เพื่อศึกษาผลของระดับน้ำมันทานตะวันและไนเตรท ต่อการผลิตแก๊สมีเทน การย่อยได้ของโภชนะ และประสิทธิภาพการเจริญเติบโต ของแพะเนื้อที่ได้รับอาหารหยาบที่มีคุณภาพต่างกัน การศึกษาครั้งนี้ประกอบด้วย 3 การทดลอง ดังนี้

การทดลองที่ 1 แบ่งออกเป็น 2 งานทดลองย่อย เพื่อศึกษาผลของการเสริมน้ำมันทานตะวันร่วมกับไนเตรท ต่อการผลิตแก๊ส การย่อยได้ของอินทรียวัตถุโดยใช้เทคนิคการวัดแก๊ส วางแผนการทดลองแบบสุ่มสมบูรณ์ (complete randomized design; CRD) จัดสิ่งทดลองแบบแฟคทอเรียล มี 2 ปัจจัย คือ ระดับของน้ำมันทานตะวัน (0 3 และ 6 เปอร์เซ็นต์ในอาหาร) และระดับของไนเตรท (0 1 2 และ 3 เปอร์เซ็นต์ในอาหาร) งานทดลองที่ 1.1 ฟางข้าว และงานทดลองที่ 1.2 ใช้หญ้าแพงโกล่าแห้ง เป็นแหล่งอาหารหยาบ ทั้ง 2 งานทดลองใช้อาหารข้นเหมือนกัน ผลการทดลองพบว่า การเสริมน้ำมันทานตะวัน 6 เปอร์เซ็นต์ ร่วมกับไนเตรท 2 เปอร์เซ็นต์ มีผลต่อการลดผลผลิตมีเทน แต่พบว่าที่ระดับ 3 เปอร์เซ็นต์ไนเตรทเพิ่มการย่อยได้ของอินทรียวัตถุเพิ่มขึ้นในขณะที่ความเข้มข้นของกรดไขมันที่ระเหยได้เพิ่มขึ้น เมื่อเสริมน้ำมันทานตะวันที่ระดับ 3 เปอร์เซ็นต์ ร่วมกับไนเตรท 2 เปอร์เซ็นต์

การทดลองที่ 2 แบ่งออกเป็น 2 งานทดลองย่อยตามคุณภาพอาหารหยาบ เพื่อศึกษาผลของคุณภาพของอาหารหยาบต่อการกินได้ การย่อยได้ของโภชนะ ผลผลิตสุดท้ายของกระบวนการหมัก การใช้ประโยชน์ของแอมโมเนียไนโตรเจนและจำนวนของจุลินทรีย์ในรูเมน วางแผนการทดลองแบบ 4×4 ลาตินสแควร์ จัดทรีทเมนต์ แบบ 2×2 แฟคทอเรียล มี 2 ปัจจัย คือ ระดับของน้ำมันทานตะวัน 2 ระดับ (3 และ 6 เปอร์เซ็นต์ในอาหาร) และระดับของไนเตรท 2 ระดับ (2 และ 3 เปอร์เซ็นต์ในอาหาร) ซึ่งคัดเลือกทรีทเมนต์ที่ดีที่สุดจากงานทดลองที่ 1.1 และ 1.2 ผลการทดลองพบว่า ระดับของการเสริมน้ำมันทานตะวันที่เพิ่มขึ้นมีผลต่อการเพิ่มค่าความสมดุลย์ของไนโตรเจน แต่ลดจำนวนของจุลินทรีย์ทั้งหมดและจุลินทรีย์ในกลุ่ม *Prevotella bryantii* และ *Prevotella ruminicola* ลดลง ระดับไนเตรทที่เพิ่มขึ้นทำให้ความเข้มข้นของกรดอะซิติค และสัดส่วนของกรดอะซิติคต่อโพพิออนิคเพิ่มขึ้น แต่ไม่มีผลต่อค่าการกินได้ การย่อยได้ของโภชนะ ผลผลิต

สุดท้ายของกระบวนการหมัก และค่าของแอมโมเนียไนโตรเจน ขณะเดียวกันระดับของไนเตรทมีผลทำให้จำนวนแบคทีเรียในรูเมนลดลง

การทดลองที่ 3 เพื่อศึกษาระดับการเสริมไนเตรทร่วมกับคุณภาพของอาหารหยาบต่อประสิทธิภาพการเจริญเติบโต และการสะสมกรดไขมันที่จำเป็นในเนื้อ ทำการทดลองในแพะเนื้อลูกผสม วางแผนการทดลองแบบสุ่มในบล็อคสมบูรณ์ (randomized complete block design; RCBD) จัดสิ่งทดลองแบบ 2×2 แฟคทอเรียล มี 2 ปัจจัย คือ ระดับของโพแทสเซียมไนเตรท (2 และ 3 เปอร์เซ็นต์ในอาหาร) และคุณภาพของอาหารหยาบ (ต่ำและสูง) จากผลการทดลองพบว่า เมื่อพิจารณาจากคุณภาพของอาหารหยาบมีผลต่อการกินได้ ประสิทธิภาพการเจริญเติบโต เปอร์เซ็นต์-ซาก สีเนื้อ องค์ประกอบโปรตีนในเนื้อ และกรดไขมันที่จำเป็นในเนื้อ พบว่าอาหารหยาบคุณภาพสูงเพิ่มปริมาณการกินได้ สมรรถภาพการเจริญเติบโต เปอร์เซ็นต์ซาก สีของเนื้อ องค์ประกอบโปรตีน และกรดไขมันมันในเนื้อ นอกจากนี้พบว่าระดับของไนเตรทเพิ่มขึ้นสามารถเพิ่มเปอร์เซ็นต์ซาก และพบว่าการให้อาหารหยาบคุณภาพสูงร่วมกับไนเตรท 3 เปอร์เซ็นต์ เพิ่มกรดไขมันที่ไม่อิ่มตัวเชิงซ้อน (Polyunsaturated fatty acids; PUFA) และกรดคอนจูเกเตดไลโนลีอิก (Conjugated Linoleic Acids; CLA) ในเนื้อ

สาขาวิชาเทคโนโลยีการผลิตสัตว์
ปีการศึกษา 2558

ลายมือชื่อนักศึกษา _____
ลายมือชื่ออาจารย์ที่ปรึกษา _____
ลายมือชื่ออาจารย์ที่ปรึกษาร่วม _____
ลายมือชื่ออาจารย์ที่ปรึกษาร่วม _____
ลายมือชื่ออาจารย์ที่ปรึกษาร่วม _____

JIRAVAN KHOTSAKDEE : THE EFFECTS OF SUNFLOWER OIL AND NITRATE LEVELS ON METHANE PRODUCTION, NUTRIENT DIGESTIBILITY AND GROWTH PERFORMANCE IN MEAT GOATS FED WITH DIFFERENT ROUGHAGE QUALITY. THESIS ADVISOR : ASSOC. PROF. PRAMOTE PAENGKOUM, Ph.D., 125 PP.

SUNFLOWER OIL/NITRATE/METHANE /QUALITY ROUGHAGE

The aims of this thesis were to investigate the effects of sunflower oil and nitrate levels on methane production, nutrient digestibility and growth performance in meat goats fed with different roughage quality. This research was divided into 3 experiments as follows : The first experiment was divided into 2 sub-experiments. The experiment was carried out to study the effects of sunflower oil and nitrate levels *in vitro* through the use of a gas production technique. This experiment was designed as a completely randomized 3×4 factorial arrangement. Factor 1 had three levels of sunflower oil (0%, 3%, and 6% of the total diet) and Factor 2 had four levels of nitrate (0%, 1%, 2%, and 3% of the total diet). In experiment 1.1, rice straw was used as a roughage source and pangola grass hay was used as a source of roughage in experiment 1.2. Both experiments (1.1 and 1.2), made use of the same diet concentrate. The results showed that the 6% sunflower oil diet with 2% of nitrate resulted in decreased methane production, but the diet with 3% nitrate increased organic matter digestibility, while volatile fatty acids were increased when supplemented with 3% sunflower oil and 2% nitrate.

The second experiment was conducted to investigate the effect of roughage quality on nutrient digestibility, rumen fermentation and rumen microbes. This experiment was divided into 2 sub-experiments according to roughage quality. The

experiment was designed in a 4×4 Latin square with a 2×2 factorial arrangement. Factor 1 had two levels of sunflower oil (3% and 6% of the total diet) and Factor 2 had two levels of nitrate (2% and 3% of the total diet) selected on the basis of the best results from experiments 1.1 and 1.2. The result showed that the N-balance increased, but the total bacteria count and population rumen microbes (*Prevotella bryantii* and *Prevotella ruminocola*) decreased with sunflower oil. Providing increased levels of nitrate supplement alone increased the concentration of acetate and acetate:propionate ratio, but did not have a negative influence on feed intake, nutrient digestibility, rumen fermentation and NH_3-N. It also decreased rumen microbes. The third experiment was to study the level of nitrate supplementation in combination with the quality of roughage on growth performance and essential fatty acid accumulation in goat meat. The experiment was designed in a 2×2 factorial arrangement in a randomized complete block design (RCBD). Factor 1 was levels of nitrate (2% and 3%) and Factor 2 was quality of roughage (low and high). The results showed that high-quality roughage increased feed intake, growth performance, percentage of carcass, meat color, protein content and essential fatty acids in meat. In addition, carcass percentage increased with increasing levels of nitrate. The combination of high-quality roughage and 3% nitrate provided optimal increases in PUFA and CLA in meat.

School of Animal Production Technology
Academic Year 2015

Student's Signature Jiravan Khotsakdee
Advisor's Signature Prasanote Kee
Co-advisor's Signature
Co-advisor's Signature
Co-advisor's Signature

ACKNOWLEDGEMENT

First of all, I would like to express my sincerest appreciation and heartfelt gratitude to the following persons:

My thesis advisor Associate Professor Dr. Pramote Paengkoum, for his supervision, kindness, support, and friendship. His kind encouragement, guidance, and instruction are invaluable things for me forever. I am extremely grateful to my research guide, Assistant Professor Dr. Chalermpon Yuangklang, my co-supervisor. I am very glad to have been able to discuss with him. I got the insight of how to do the scientific thinking and I will cherish the way of his thinking. This thesis could not have been completed without his constructive and support. Associate Professor Dr. Surintorn Boonanuntanasarn, my co-supervisor. I am very glad to have been able to discuss with her. I got an insight of how to do the scientific thinking. This thesis could not have been completed without her constructive and support. Professor Dr. Christopher Simon McSweeney, my co-supervisor. I am very glad to have been able to discuss with his. I got the insight of how to do the scientific thinking. This thesis could not have been completed without his constructive and support and assistance during my stay in Brisbane, Australia. In addition, I am grateful for the teachers of School of Animal production Technology and all their help. Most importantly, I would like to give the biggest thank to my family for their love, support, suggestion and given me the opportunity to pursue my graduate studies.

Jiravan Khotsakdee

CONTENTS

Page

CONTENTS (Continued)

Page

CONTENTS (Continued)

Page

CONTENTS (Continued)

Page

CONTENTS (Continued)

Page

CONTENTS (Continued)

Page

CONTENTS (Continued)

Page

LIST OF TABLES

Table **Page**

LIST OF TABLES (Continued)

Table **Page**

LIST OF TABLES (Continued)

Table **Page**

LIST OF TABLES (Continued)

Table **Page**

LIST OF FIGURES

Figure **Page**

LIST OF ABBREVIATIONS

ADF	=	Acid detergent fiber
ADICP	=	Acid detergent insoluble crude protein
ADIN	=	Acid detergent insoluble N
ADL	=	Acid detergent lignin
C4 : 0	=	Butyric acid
C6 : 0	=	Caproic acid
C8 : 0	=	Caprylic acid
C10 : 0	=	Capric acid
C11 : 0	=	Cis-10-Pentadecenoic acid
C12 : 0	=	Lauric acid
C13 : 0	=	Tridecanoic acid
C14 : 0	=	Myristic acid
C14 : 1	=	Myristoleic acid
C15 : 0	=	Pentadecanoic acid
C16 : 0	=	Palmitic acid
C16 : 1	=	Palmitoleic acid
C17 : 1	=	Heptadecenoic Acid
C18 : 0	=	Stearic acid
C18 : 1n9t	=	Elaidic acid
C18 : 1n9c	=	Oleic acid

LIST OF ABBREVIATIONS (Continued)

C18 : 2n6t	=	Linolelaidic acid
C18 : 2n6c	=	Linoleic acid
C18 : 3n3	=	α -Linoleic acid
C20 : 0	=	Arachidic acid
C20 : 1	=	Gondoic acid
C22 : 0	=	Behenic acid
C20 : 3n-6	=	dihomo-γ-linolenic acid
C20 : 4n-6	=	arachidonic acid
C22 : 6n-3	=	docosahexaenoic acid
c	=	cis
cfu	=	Colony-forming unit
CP	=	Crude protein
DDM	=	Digestible dry matter
DM	=	Dry matter
DMD	=	Dry matter digestibility
DMI	=	Dry matter intake
DOM	=	Digestible organic matter
FA	=	Fatty acid or fatty acids
FAME	=	Fatty acid methyl ester
GC	=	Gas chromatography
GHG	=	Greenhouse gases

LIST OF ABBREVIATIONS (Continued)

IVDOM	=	In vitro digestible organic matter
IVNDFD	=	In vitro neutral detergent fiber digestibility
IVTD	=	In vitro true digestibility
MCP	=	Microbial crude protein
N	=	Nitrogen
NA	=	Nitrogen absorbed nitrogen
NB	=	Nitrogen balance (g/day)
NI	=	Nitrogen intake
NPN	=	Non-protein nitrogen
NDF	=	Neutral detergent fiber
NE	=	Net energy
NFC	=	Non-fiber carbohydrate
NPN	=	Non protein nitrogen
NRC	=	National research council
NFC	=	Non-fiber carbohydrates
OM	=	Organic matter
OMD	=	Organic matter digestibility
OMI	=	Organic matter intake
SO	=	Sunflower oil
t	=	*trans*
ω-3	=	Omega-3 or n-3
ω-6	=	Omega-6 or n-6

CHAPTER I

INTRODUCTION

1.1 Rationale of the study

It has been forecasted that the word population of ruminants produces as much as 86 million metric tons (MT) methane per year (McMichael et al., 2007). In addition, methane expresses a loss of feed energy to the animal and a significant source of greenhouse gas, policies to decrease methane emissions have been proposed (Johnson and Johnson, 1995). Rumen *methanogenesis* results in the loss of 6-10% of gross energy intake (GEI) or 8-14% of the digestible energy intake of ruminant (Johnson and Johnson, 1993; McAllister et al., 1996). Enteric methane production in ruminants can be decreased in 3 ways : by removing methanogens from the rumen, by reducing H_2 production, or by providing an alternative H_2 sink (Joblin, 1999). Ellis et al. (2008) concluded that methanogens usually compete with other micro-organisms for utilization of H_2. McAllister and Newbold (2008) stated that the decreasing of an alternative electron acceptor has energetically more desirable than production of methane from CO_2, to reduce enteric methane production. Gonzalez-Avalos and Ruiz- Suarez (2001) demonstrated that diet are the most important factor, measuring determining difference in methane emission from manure. However, for most dietary strategies, designed to alleviate enteric methane.

Nitrate (NO_3^-), a sample inorganic salt with a high redox potential (E_h), has also been shown to be effective in lowering methane production by ruminal microbiota both *in vitro* (Bozic et al., 2009; Sar et al., 2005; Zhou et al., 2001) and *in vivo* (Lewis, 1950; mMarais et al., 1988; Sar et al., 2005; van Zijderveld et al., 2011). Which, nitrate is an electron receptor which has been reported that nitrate reduced the emission of methane (Takahashi and Young,1999; van Zijderveld et al., 2010; Nolan et al., 2010; Van Zijderveld et al., 2011). Moreover, the rumen is place for the nitrate reduction of nitrate to ammonia, which is an important nitrogen source for ruminal microbes.

Conjugated linoleic acid (CLA), a natural derivative of the essential fatty acid linoleic acid (LA), has gained increasing attention in recent years due to its potential health effects. Several studies realized in animal models and have shown antitumor, antiobese, antiatherogenic, antidiabetic, immunomodulatory and osteosynthetic activities (Bhattacharya et al., 2006; Benjamin and Spener, 2009). Conjugated linoleic acid derived from ruminant animals (Fritsche et al., 1999; Adamczale et al., 2008). The presence of CLA of ruminants is due to the biotransformation of polyunsaturated fatty acids by the rumen micro- organisms, in a process called biohydrogenation and to the conversion of vaccenic acid.

Supplemental oils particularly unsaturated fats such as sunflower oil successfully reduced methane production through the competition of utilization of H^+ in the process of biohydrogenation in the rumen. Sunflower oil wish in polyunsaturated unsaturated fatty acid can reduce methane production by reducing rumen ciliated protozoa (Ivan et al., 2001; McGinn et al., 2004), an alternative metabolic hydrogen acceptor (Johnson and Johnson, 1995). There have been shown

that methane production was inhibited by unsaturated fatty acids in experiments *in vitro* and *in vivo* with cows and sheep (Van Nevel and Demeyer, 1981; Jenkins and Palmquist, 1982). Dietary fats have been identified as efficient means of lowering ruminal *methanogenesis* (Jouany, 1994). The resulting in the lower of H^+ for methane production (Czerkawski et al., 1966; Demeyer et al., 1969) and the CLA isomer apportion can escape the rumen and can be further hydrogenated to vaccenic acids (Kepler et al., 1966).

Moreover, Diet is most important factor, which influences the number and relative proportion of different species of microbes in the rumen. The effect of supplementation of good quality roughage has a major important on methane production, being highest with low forage quality. Past studies with ruminant animal have demonstrated that digestible feeds yield lower methane emission when compared to poor quality and enhancing animal performance. Pasture improvement importantly method if small number of animal are introduced. The objective of this study aim to investigate the effect of sunflower oil and nitrate levels on methane production, nutrients digestibility and growth performance in meat goats fed with different roughage quality.

1.2 Research hypothesis

1) The utilization of sunflower oil with nitrate effect of sunflower oil with nitrate would be inhibited the activity of methanogens leading to decrease methane production and improve rumen fermentation and eventually improve growth performance of meat goats fed with different roughage quality.

2) Research objectives

1) To quantify the appropriate levels of sunflower oil and nitrate supplementation in meat goats fed with different roughage quality.

2) To study the effect of diet on nutrient digestibility, methane production, N-balance, bacteria population in meat goats fed with different roughage quality.

3) To study the effect of diet on growth performance and fatty acids deposition in meat of goats.

1.3 Scope and limitation of this study

1) This study was focused on the effect of sunflower oil and nitrate supplementation on methane production, feed intake, rumen microorganisms, nutrients digestibility, growth performance and fatty acid deposition in meat goats fed with different roughage quality.

1.4 Expected results

1) It is expects to know about the optimal level of sunflower oil and suitable nitrate supplementation on methane production, and rumen fermentation when fed different sunflower oil level with nitrate in meat goats fed with low or high quality roughage.

2) It is expects to know about the effect of optimal level of sunflower oil and suitable nitrate supplementation on rumen fermentation, nitrogen utilization, rumen microorganisms and nutrient digestibility in meat goats fed with low or high quality roughages.

3) It is expects to know about the effect of optimal level of sunflower oil and suitable nitrate supplementation on growth performance, carcass quality and fatty acid deposition in meat goats fed with low or high quality roughages.

1.6 References

Benjamin, S., and Spener, F. (2009). Conjugated linoleic acids as functional food: an insight into their health benefits. **Nutrition, Metabolism and Cardiovascular Diseases**. 6: 36.

Bhattacharya, A., Banu, J., Rahman, M., Causey, J., and Fernandes, G. (2006). Biological effects of conjugated linoleic acids in health and disease. **Journal of Nutritional Biochemistry**. 17: 789-810.

Bozic, A.K., Anderson, R.C., Carstens, G.E., Ricke, S.C., Callaway, T.R., and Yokoyama, M.T. (2009). Effects of the methane-inhibitors nitrate, nitroethane, lauric acid, Lauricidin and the Hawaiian marine algae chaetoceros on ruminal fermentation *in vitro*. **Bioresource Technology**. 100: 4017-4025.

Fritsche, J., Rickert, R., Steinhart, H., Yurawecz, M.P., Mossaba, M.M., Sehat, N., Roach, J.A.G., Kramer, J.K.G., and Ku, Y. (1999). Conjugated linoleic acid (CLA) isomers: formation, analysis, amounts in foods and dietary intake. **European Journal of Lipid Science and Technology**. 101: 272-276.

González–Avalos, E., and Ruiz–Suárez, L.G. (2001). Methane emission factors from cattle manure in México. **Bioresource Technology**. 80: 63-71.

Ivan, M., Mir, P.S., Koenig, K.M., Rode, L.M., Neill, L., Entz, T., and Mir, Z. (2001). Effects of dietary sunflower seed oil on rumen protozoa population and tissue

concentration of conjugated linoleic acid in sheep. **Small Ruminant Research**. 41: 215-227.

Jenkins, T.C., and Palmquist, D.L. (1982). Effect of added fat and calcium on *in vitro* formation of insoluble fatty acid soaps and cell wall digestibility. **Journal of Animal Science**. 55: 957-963.

Joblin, K.N. (1999). Ruminal acetogens and their potential to lower ruminant methane emissions. **Australian Journal of Agricultural Research**. 50: 1307-1313.

Johnson, K. A., and Johnson, D.E. (1995). Methane emissions from cattle. **Journal of Animal Science**. 73: 2483-2492.

Jouany, J.P. (1994). Methods of manipulating the microbial metabolism in the rumen. **Annales De Zootechnie**. 43: 49-62.

Kepler, C.R., W.P. Tucker and Tove, S.B. (1966). Intermediates and products of the biohydrogenation of linoleic acid by *Butyrivibrio fibrisolvens*. **Journal of Biological Chemistry**. 241: 1350-1354.

McAllister, T.A., Okine, E.K., Mathison, G.W., and Cheng, K.J. (1996). Dietary environmental and microbiological aspects of methane production in ruminants. **Canadian Journal of Animal Science**. 76: 231-243.

McAllister, T.A., and Newbold, C.J. (2008). Redirecting rumen fermentation to reduce *methanogenesis*. **Australian Journal of Experimental Agriculture**. 48: 7-13.

McGinn, S.M., Beauchemin, K.A., Coates, T., and Colombatto, D. (2004). Methane emissions from beef cattle: Effects of monensin, sunflower oil, enzymes, yeast, and fumaric acid. **Journal of Animal Science**. 82: 3346-3356.

McMichael, A.J., Powles, J.W., Butler, C.D., and Uauy, R. (2007). Food, livestock production, energy, climate change, and health. **The Lancet Global Health**. 370: 1253-1263.

Marais, J.P., Therion, J.J., Mackie, R.I., Kistner, A., and Dennison, C. (1988). Effect of nitrate and its reduction products on the growth and activity of the rumen microbial population. **British Journal of Nutrition**. 59: 301-313

Nolan, J.V., Hegarty, R.S., Hegarty, J., Godwin I.R., and Woodgate, R. (2010). Effects of dietary nitrate on fermentation, methane production and digesta kinetics in sheep. **Animal Production Science**. 50: 801-806.

Patra, A.K. (2012). Enteric methane mitigation technologies for ruminant livestock: A synthesis of current research and future directions. **Environmental Monitoring and Assessment**. 184: 1929-1952.

Sar, C., Mwenya, B., Santoso, B., Takaura, K., Morikawa, R., Isogai, N., Asakura, Y., Toride, Y., and Takahashi, J. (2005). Manipulation of rumen methanogenesis by a combination of nitrate with beta 1-4 galctooligosaccharide or nisin in sheep. **Animal Feed Science and Technology**. 115: 129-142.

Takahashi, J., and Young, B.A. (1999). The modulation of nitrate-enhanced hypothermia by sulphur compounds in cold-exposed sheep. **Animal Feed Science and Technology**. 39: 347-355.

van Zijderveld, S. M., Gerrits, W.J.J., Apajalahti, J.A., Newbold, J.R., Dijkstra, J., Leng, R.A., and Perdok, H.B. (2010). Nitrate and sulfate: Effective alternative hydrogen sinks for mitigation of ruminal methane production in sheep. **Journal of Dairy Science**. 93: 5856-5866.

van Zijderveld, S.M., Gerrits, W.J.J., Dijkstra, J., Newbold, J.R., Hulshof, R.B.A., and Perdok, H.B. (2011). Persistency of methane mitigation by dietary nitrate supplementation in dairy cows. **Journal of Dairy Science**. 94: 4028-4038.

CHAPTER II

LITERATURE REVIEW

2.1 Introduction

Goat population in Southeast Asia was estimated to be 29.1 million and increased by 7.57% annually to 26.9 million in 2010 with 0.45 million heads were in Thailand (FAO, 2015). Goat production is an important component of livestock rearing in the developing countries. The developing countries, a goat makes a very valuable contribution such as meat and milk. The population of goat keeps increasing by an annual growth rate of 1.3% due to the increased price, expanded market (Figure 2.1) (McMillin and Brock, 2005), Consequently, damage to the environment is inevitable no control, especially in situations where feeds are scarce.

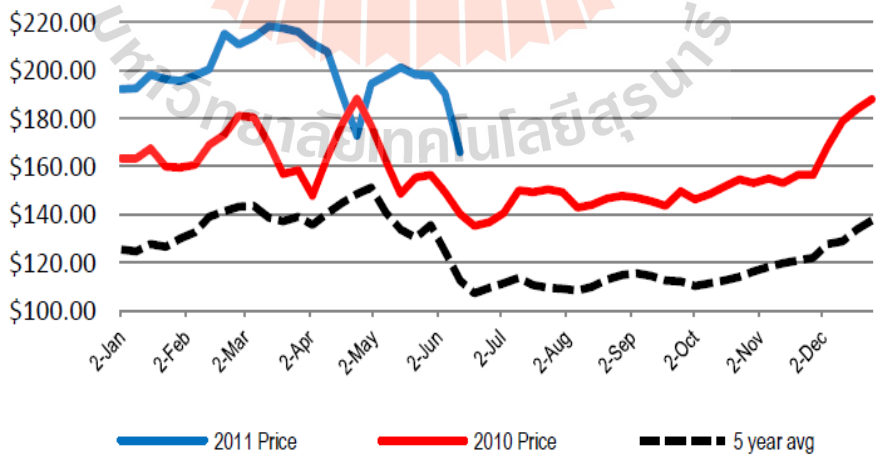

Figure 2.1 Average monthly prices and goats sold through Producers Auction.

Source : http://www.desertvikingranch.com

The ruminant animal is important to smallholder farmers in developing countries to provide meat, milk and livestock farming (Preston and Leng, 2009).

2.2 Nutritional characteristics of ruminants and goats

Ruminant animals have evolved the ability to utilize agricultural by- products are a source of nutrition (Hofman, 1989). Goats consume both kinds of grass and brush material and are therefore considered intermediate feeders, more specifically intermediate browsers (Pande et al., 2002). Concentrate ingredients are commonly used in ruminant feeding systems to supply both protein and energy to the animal.

In goat meat, the growth rate is very important because farmers earn money from the meat production. To maximize the growth rate, feeding plays a crucial role. A goat needs to get sufficient energy for their growth. However, where browse is not available, goats can feed on grasses and crop residues such as cereal straws, but rice straw has poor digestibility and low nutritive value.

2.3 Methane emissions

Methane is emitted by natural sources such as from natural gas systems and the raising of livestock. Domestic livestock such as cattle, buffalo, sheep and goats produce large amounts of methane as part of their normal digestive process. CH_4 is produced, because humans raise these animals for food, the emissions are considered human-related.

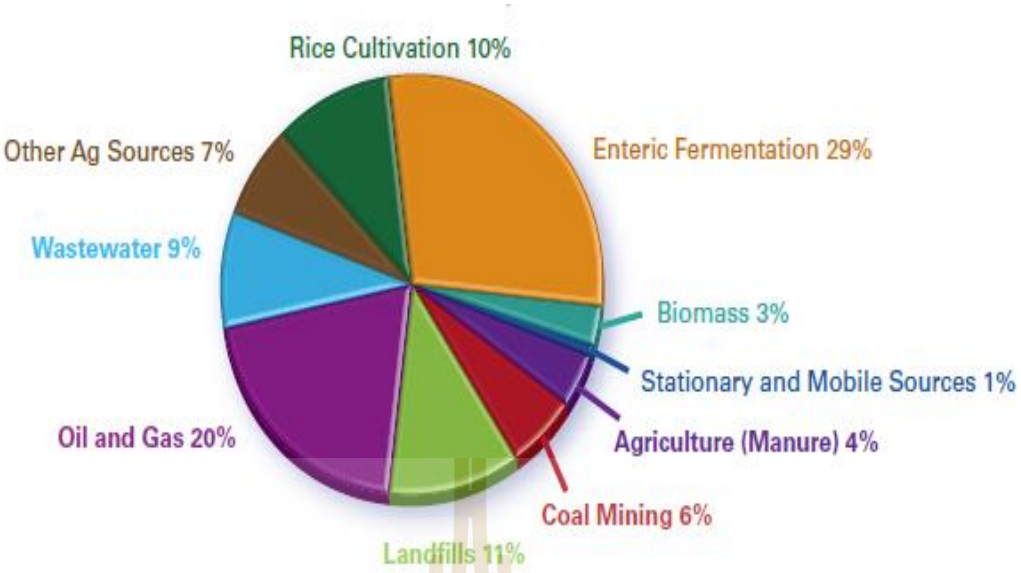

Figure 2.2 Estimated global anthropogenic methane emissions by source.

Source : http://www.epa.gov.

2.3.1 Fermentation reaction in the rumen involving H_2 production and H_2 sinks

The rumen is characterized via microbial population density and diversity and complexity of micro-ecological interactions. Interest in the rumen methanogens has resulted from ruminants typically lose 2-15% of dry matter intake as methane (Moss et al., 2000). Fermentation of the carbohydrate emission from roughage, is an oxidation process under anaerobic conditions and giving reduced co-factors like NADH. Some physical associations between fermentative species and H2 sink may facilitate interspecies transfer in the rumen. In the rumen, the formation of methane is the major way of hydrogen elimination through the following reaction :

$$CO_2 + 4\,H_2 \longrightarrow CH_4 + 2\,H_2O$$

The ratio of acetate plus butyrate divide propionate (C2 + C4)/C3, which accounts for acetate and butyrate both of which are involved in the H_2 production, and propionate, which is involved in H_2 utilization, improved the relationship slightly (r^2 = 0.778).

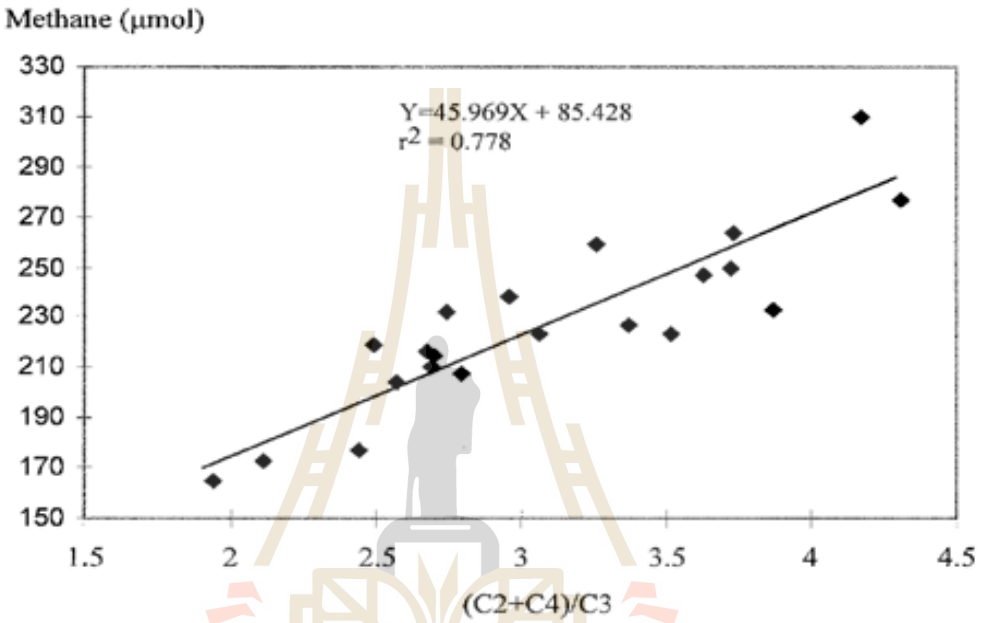

Figure 2.3 Relationship between methane and (C2 + C4)/C3 ratio.

Source : Demeyer (1990).

This means that the microbial ecosystem related in propionate formation differs with the dietary circumstances. The cellulolytic bacteria is the major propionate producers through the succinate pathway in roughage diets, while lactate is the main intermediate in the conversion of roughage to propionate. Unlike cellulolytic bacteria and methanogens, lactic bacteria are known to be strong of low pH, making them able to use H_2 and be competitive with methanogens even in unfavorable pH conditions.

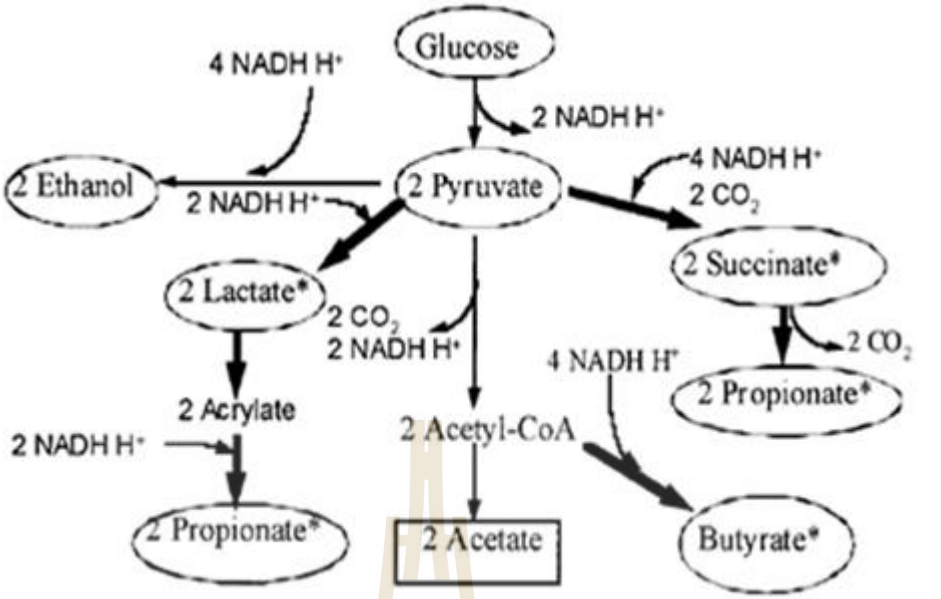

Figure 2.4 Metabolism of NADH H^+ and the electron sink products in *anaerobiosis*.

Source : Moss et al. (2000).

The syntheses of acetate and butyrate in the rumen enhance the CH_4 production in the rumen. Although, the presence of others electron acceptors than CO_2 has an effect on the presence and activity of H_2 producers and utilisers (Morgavi et al., 2010) (Figure 2.5). Another function that is associated with the production of H_2, also reported by Morgavi et al. (2010) is the degradation of fibrous plant material. If the transfer between H_2 producers and H_2 interspecies is affected, the build-up of H_2 in the milieu inhibits the reoxidation of co-enzymes involved in redox reactions within bacterial cells, ultimately depressing the fermentation processes. In the productive system of ruminants, nutritionists challenge is to develop strategies to mitigate methane production (Martin et al., 2008) that increase daily gain (Perdok and Newbold, 2009).

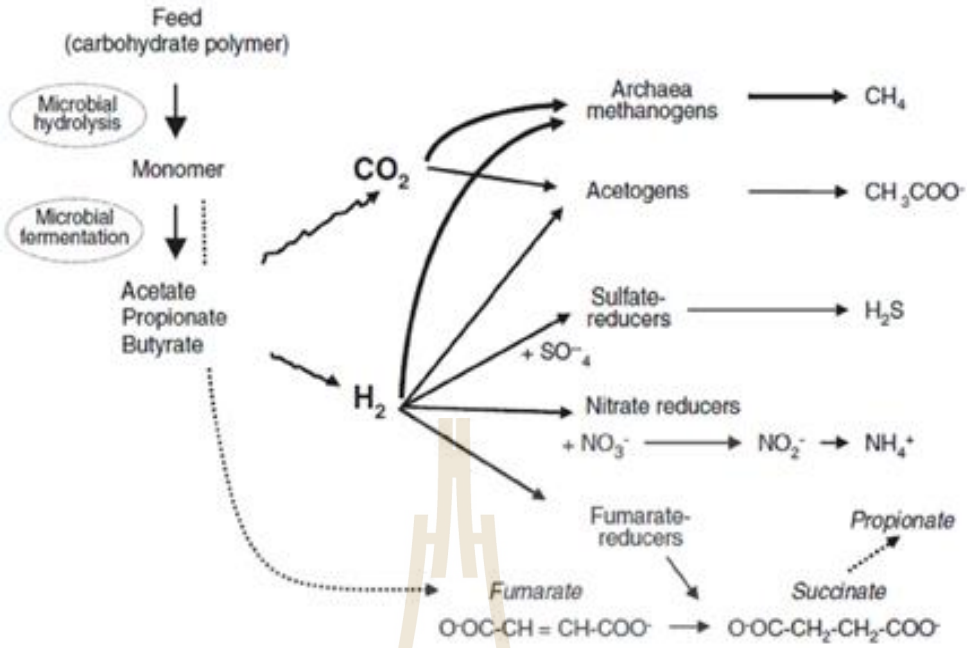

Figure 2.5 Schematic microbial fermentation of feed polysaccharides and H_2 reduction pathways in the rumen.

Source : Morgavi et al. (2010).

2.4 Control of methane emission by the livestock

Methane production by livestock represents 2% of total methane production (Moss et al., 2000). Various options such as chemical feed additives and manipulation of feed and feeding can be taken to reduce methane emission in livestock (Tamminga et al., 2007). Modulating enteric fermentation in livestock is one of the latest modus operandi for control of *methanogenesis.* Mitigate enteric methane production considerable effort is being devoted to strategies that will reduce CH_4 production by ruminal microorganisms. In ruminants their role has not yet been quantified, but they are used in rumen manipulation along with nitrate had the potential to reduce methane production. The propionate production by stimulating *Selenomonas Succinomonas*

with simultaneous inhibition of acetate producers such as *Rumininococcus, Butyrivibrio* (Mwenya et al., 2004).

2.5 Using nitrate as a non-protein nitrogen source for ruminants

Leng (1990) reported that factors affect the efficiency of utilization of poor quality roughage by ruminants for productive useful including the availability of nutrients to support an efficient microbial growth and extent of digestion in the rumen. The strategy for improving production by providing optimum conditions for microbial growth and then by supplementation to procure dietary nutrients to complement and balance the products of digestion to requirement (FAO/IAEA, 1997). Nitrate as a sole fermentable N source in a diet could altogether inhibit enteric methane production by ruminants. Availability of rumen ammonia is often a primary shortage in diets fed to ruminants in tropical countries. Additionally, nitrate-reducing microbes contest with methanogens for H_2, and have a competitive advantage (Jones, 1972). The diagram of nitrate metabolism in the rumen is shown in Figure 2.6 Both assimilatory nitrite reduction, direct to ammonia was shown to germinate in rumen contents (Jones, 1972; Kaspar and Tiedje, 1981). In incubations with ruminal digested in vitro, the assimilatory nitrate reduction was predominant and emerged from nitrite addition (Kaspar and Tiedje, 1981). Nitrate is a potent inhibitor of *methanogenesis* in systems from fermentative digestion in the rumen to under fermentation in an extensive range of systems from anaerobic biodigestors to sediments (Hungate, 1965; Allison et al., 1981; Akunna et al., 1994).

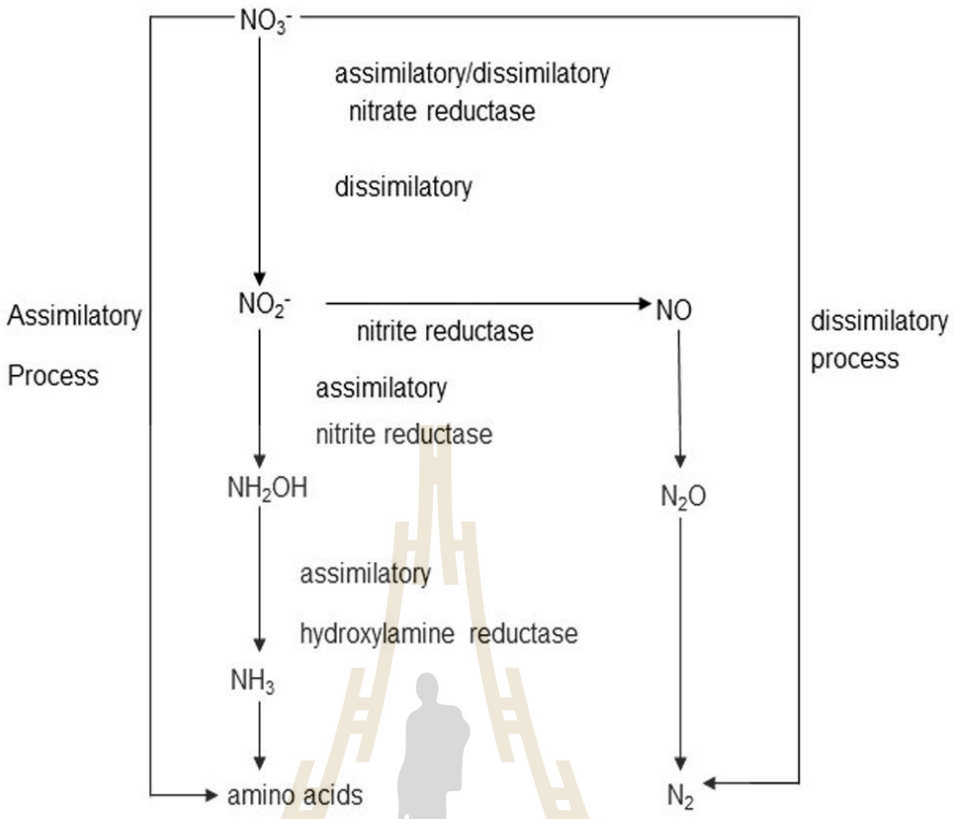

Figure 2.6 The assimilatory and dissimilatory routes of nitrate/nitrite metabolism.

Source : Yang et al. (2016).

It appears that respiratory conversion of nitrate to ammonia by anaerobic organisms is competitive as an H_2 sink consuming 8 electrons in the process and out competing methanogens for electrons in convention with the free energy convert in the reactions which are -598 kJ for the reduction of nitrate to ammonium and -131 kJ for the reduction of carbon dioxide to methane (Allison and Reddy, 1984; Allison et al., 1981). The dependence of *methanogenesis* and nitrate reduction on electron sources is described by the following two stoichiometries :

$$4H_2+HCO_3^-+H^+ \rightarrow CH_4+3H_2O;\ \Delta Go' = -175\ kJ/reaction$$

(Conrad and Wetter, 1990)

$NO_3^- + 2H^+ + 4H_2 \rightarrow NH_4^+ + 3H_2O$; $\Delta Go' = -598$ kJ/reaction

(Allison and Reddy, 1984)

Methanogens in the Archaea are characterized by their ability to produce methane under highly anoxic conditions (Guo et al., 2005). Reports from *in vitro* (Anderson and Rasmussen, 2000; Guo et al., 2009) and *in vivo* (Takahashi and Young, 1991) also reported that addition of nitrate dramatically inhibited ruminal *methanogenesis* and grow up microbial crude protein (MCP) production. Hydrogen is a major substrate for ruminal *methanogenesis*, and also for nitrate reduction.

2.6 Effect of Unsaturated fatty acids in ruminant

The effect of unsaturated fatty acids in ruminant diets depress protozoa numbers and the use of lipids as a defaunating agent has been suggested. Lipids have also been shown to inhibit *methanogenesis* even in the absence of rumen protozoa, probably due to the toxicity of long chain fatty acids to methanogenic bacteria. These longer chains FA have the capacity to hold more H_2 atoms and thus may be more able to influence the H_2 balance in the rumen when laturge quantities are included in the diet compared to short chain FA (Ellis et al., 2008). Jouany et al. (2008) showed that utilization of polyunsaturated fatty acids, to decrease rumen methanogen may be a practical abatement in ruminant production.

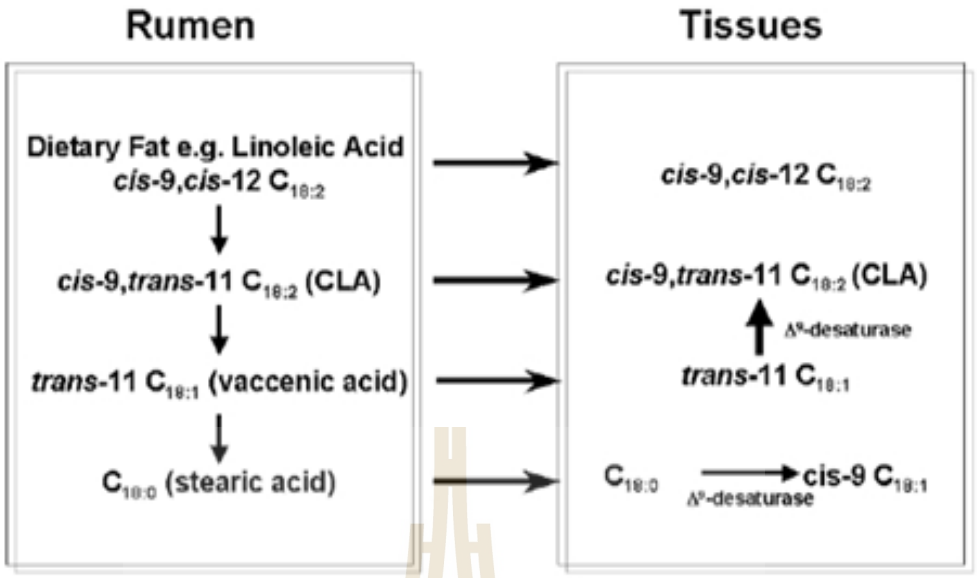

Figure 2.7 Biohydrogenation of *cis*- 9, *trans*-11

Source : Griinari and Bauman (1999).

Fat is an important energy component and increased the energy density of the ruminant diet (Bauman et al., 2003). The type of fat in the ration is to affect the composition of body fat (Bas and Morand Fehr, 2000) deposition in ruminants. Kott et al. (2003) reported that the sunflower oil with a high concentration of linoleic or oleic fatty acids increasing concentration of CLA. The unsaturated fatty acids are H_2 sinks and are biohydrogenation in the rumen. During biohydrogenation, isomerization is a first critical step, which leads the formation of conjugated linoleic acids (CLA). On the other hand, use oil supplementation ruminant diet to increasing the content of PUFA in tissues (Yu et al., 2008). Dietary supplementation with linoleic acids significantly increased CLA contents in muscle and fat tissues (Mir et al., 2000). In addition, dietary fats have been identified decreasing *methanogenesis* (Jouany, 1994). Sunflower oil rich in unsaturated fatty acid had effect in ciliated protozoa, reduce

methane production (Ivan et al., 2001; McGinn et al., 2004), and an alternative hydrogen acceptor (Johnson and Johnson, 1995).

2.6.1 Fatty acid composition in meat and adipose tissue

It is well established that the FA composition of muscle lipids has an important impact on meat quality. However, ruminant nutritionist shows that different nutritional condition can change muscle lipid FA composition, PUFA level, and the ω3 : ω6 PUFA ratio (Banskalieva et al., 2000). The adipose tissue seems to be the major site of endogenous synthesis of CLA in growing animals. The amount of triglycerides stored within the adipocyte results from the equilibrium between *de novo* FA synthesis, FA uptake, FA esterification, triglyceride hydrolysis (lipolysis), and esterification of FA produced by lipolysis (Figure 2.8). Because *de novo* synthesis of FA is very low in ruminant liver (Hood et al., 1980), adipose tissue plays a major role in this function.

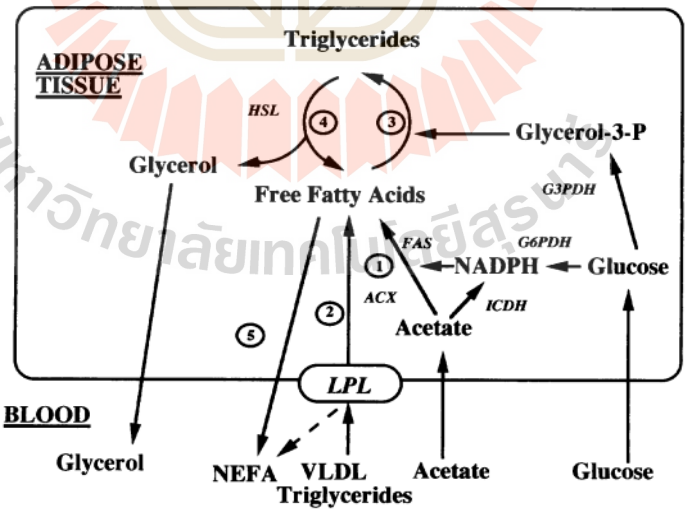

Figure 2.8 Metabolic pathways in ruminant adipose tissue.

1 = *De novo* fatty acid (FA) synthesis; 2 = hydrolysis and uptake of circulating triglycerides; 3, (re)esterifi&on of FA; 4 = lipolysis; and 5 = lipomobilization, 4 - 3.

Very low density lipoprotein (VLDL); acetyl-coenzyme A carboxylase (AQD; glucose-dphosphate dehydrogenase (G6PDH); hormone-sensitive lipase (HSL); NADP-isocitrate dehydrogenase (ICDH); lipoprotein lipase (LPL), glycerol-3-phosphate dehydrogenase (G3PDH).

Source : Chilliard (1993).

2.7 References

Allison, M.J., Reddy, C.A., and Cook, H.M. (1981). The effects of nitrate and nitrite on VFA and CH_4 production by rumen microbes. **Journal Animal Science**. 53: 283.

Allison, M.J., and Reddy, C.A. (1984). Adaptations of gastrointestinal bacteria in response to changes in dietary oxalate and nitrate. **Proceedings of the 3rd International Symposium on Microbial Ecology**. American Society Microbiology. Washington DC.

Anderson, R.C., Rasmussen, M.A., Jensen, N.S., and Allison, M.J. (2000). *Denitrobacterium detoxificans gen*. nov., sp. nov., a ruminal bacterium that respires on nitrocompounds. **International Journal of Systematic and Evolutionary Microbiology**. 50: 633-638.

Akunna, J.C., Bizeau, C., and Moletta, R. (1993). Nitrate and nitrite reductions with anaerobic sludge using various carbon sources: glucose, glycerol, acetic acid, lactic acid and methanol. **Water Research**. 27: 1303-1312.

Banskalieva, V., Sahlu, T., and Goetsch, A.L. (2000). Fatty acid composition of goat muscle fat depots. A review. In: **Small Ruminant Research**. 37: 255-268.

Bas, P., and Morand Fehr, P. (2000). Effect of nutritional factors on fatty acid composition of lamb fat deposits. **Livestock Production Science**. 64: 61.

Bauman, D.E., Corl, B.A., and Peterson, D.G. (2003). The biology of conjugated linoleic acid in ruminants. **Advances in Conjugated Linoleic Acid Research**. 2: 146-173.

Chilliard, Y. (1993). Dietary fat and adipose tissue metabolism in ruminants, pigs and rodents: a review. **Journal of Dairy Science**. 76: 3897-3931

Ellis, J.L., Kebreab, E., Odongo, N.E., Mcbride, B.W., Okine, E.K., and France, J. (2007). Prediction of methane Production from dairy and beef cattle. **Journal of Dairy Science**. 90: 3456-3467.

FAO. (2015). **FAOSTAT** data. Food and Agriculture Organization, Rome http://faostat.external. fao.org.

Griinari, J.M., and Bauman, D.E. (1999). Biosynthesis of conjugated linoleic acid and its incorporation into meat and milk in ruminants. **Advances in Conjugated Linoleic Acid Research**. 1: 180-200.

Guo, W.S., Schaefer, D.M., Guo, X.X., Ren, L.P., and Meng, Q.X. (2009). Use of nitrate-nitrogen as a sole dietary nitrogen source to inhibit ruminal *methanogenesis* and to improve microbial Nitrogen synthesis *in vitro*. **Asian Australasian Journal of Animal Science**. 22: 542-549.

Guo, Y.Q., Hu, W.L., and Liu, J.X. (2005). Methanogens and manipulation of methane production in the rumen. **Wei Sheng Wu Xue Bao**. 45: 145-148

Hungate, R.E. (1966). **The rumen and its microbes**. Academic Press, New York, USA.

Hood, R. L., Cook, L.J., Mills, S.C., and Scott, T.W. (1980). Effect of feeding protected lipids on fatty acid synthesis in ovine tissues. **Lipids**. 15: 644.

Hofman, R.R. (1989) Evolutionary steps of ecophysiological adaptation and diversification of ruminants: a comparative view of their digestive system. **Oecologia**. 78: 443-457

Jones, G.A. (1972). Dissimilatory metabolism of nitrate by the rumen microbiota. **Canadian Journal of Microbiology**. 18: 1783-1787.

Johnson, K.A., and Johnson, D.E. (1995). Methane emissions from cattle. **Journal of Animal Science**. 73: 2483-2492.

Jouany, J.P. (1994). Methods of manipulating the microbial metabolism in the rumen. **Annales De Zootechnie**. 43: 49-62.

Kaspar, H. F., and Tiedje. J.M. (1981). Dissimilatory reduction of nitrate and nitrite in the bovine rumen: nitrous oxide production and effect of acetylene. **Applied and Environmental Microbiology**. 41: 705-709.

Kepler, C.R., Tucker, W.P., Tove, S.B., Martin, G.S., Lunt, D.K., Britain, K.G., and Smith, S.B. (1999). Intermediates and products of the biohydrogenation of linoleic acid by *Butyrivibrio fibrisolvens*. **Journal of Biological Chemistry**. 241: 1350-1354.

Kott, R.W., Hatfield, P.G., Bergman, J.W., Flynn, C.R., Van Wagoner, H., and Boles, J.A. (2003). Feedlot performance, carcass composition, and muscle and fat, CLA concentrations of lambs fed diets supplemented with safflower seeds. **Small Ruminant Research**. 49: 11-17.

Leng, R.A. (1990). Factors affecting the utilization of poor-quality forages by ruminants particularly under tropical conditions. **Nutrition Research Reviews**. 3: 277-303

Martin, S.A., Streeter M.N., Nisbet D.J., Hill, G.M., and Willamns, S.E. (1999). Effects of DL-malate on ruminal metabolism and performance of cattle fed a high-concentrate diet. **Journal of Animal Science**. 77: 1008-1015.

McGinn, S.M., Beauchemin, K.A., Coates, T., and Colombatto, D. (2004). Methane emissions from beef cattle: effect of monensin, sunflower oil, Enzymes, yeast and fumaric acid. **Journal of Animal Science**. 82: 3346-3356.

McMillin, K.W., and Brock, A.P. (2005). Production practices and processing for value-added goat meat. **Journal of Animal Science**. 83: 57-68.

Mir, Z., Rushfeldt, M.L., Mir, P.S., Paterson, L.J., and Weselake, R.J. (2000). Effect of dietary Supplementation with either conjugated linoleic acid (CLA) or linoleic acid rich oil on the CLA Content of lamb tissues. **Small Ruminant Research**. 36: 25-31.

Morgavi, D.P., Forano, E., Martin, C., and Newbold, C.J. (2010). Microbial ecosystem and *methanogenesis* in ruminants. **Journal of Animal Sciences**. 4: 1024-1036.

Moss, A., Jouany, J.P., and Newbold, J. (2000). Methane production by ruminants: its contribution to global warming. **Annales de zootechnie**. 49: 231-253.

Mwenya, B., Santoso, B., Sar, C., Gamo, Y., Kobayashi, T., Arai, I., and Takahashi, J. (2004). Effects of including β1-4 galactooligosaccharides, lactic acid bacteria or yeast culture on *methanogenesis* as well as energy and nitrogen metabolism in sheep. **Animal Feed Science and Technology**. 115: 313-326.

Pande, R.S., Kemp, P.D., and Hodgson, J. (2002). Preference of goats and sheep for browse species under field conditions. **New Zealand Journal of Agricultural Research**. 45: 97-102.

Perdok, H., and Newbold, C.J. (2010). **Reducing the carbon footprint of beef production**, Part 2. Provimi Research and Innovation Centre, Brussels. Belgium.

Preston, T.R., and Leng, R.A (2009). **Matching ruminant production systems with available resources in the tropics and sub- tropics**. Penambul Press: Armidale, Australia.

Takahashi, J., and Young, B.A. (1991). Prophylactic effect of L-cysteine on nitrate-induced alteration in respiratory exchange and metabolic rate in sheep. **Animal Feed Science and Technology**. 35: 105-113.

Tamminga, S., Bannink, A., Dijkstra, J., and Zom, R. (2007). **Feeding strategies to reduce methane loss in cattle**. Animal Science. Group of Wageningen UR, Lelystad, the Netherlands.

Yang, G., Zhou, R., Tang, T., and Shi, S. (2008). Simple and efficient isolation of high-quality total RNA from *Hibiscus tiliaceus*, a mangrove associate and its relatives. **Preparative Biochemistry and Biotechnology**. 38: 257-264.

Yu, L.L., Wang, R.L., Zhang, Y.Z., Kleemann, D.O., Zhu, X.P., and Jia, Z.H. (2008). Effects of selenium supplementation on polyunsaturated fatty acid concentrations and antioxidant status in plasma and liver of lambs fed linseed oil or sunflower oil diets. **Animal Feed Science and Technology**. 140: 39-51.

http:// www. desertvikingranch.com (Goat Market Prices)

http:// www.epa.gov (Estimated Global Anthropogenic Methane Emissions by source)

CHAPTER III

EXPERIMENT I

THE EFFECTS OF SUNFLOWER OIL AND NITRATE LEVELS *IN VITRO* ON METHANE PRODUCTION AND RUMEN FERMENTATION IN GOATS FED WITH DIFFERENT ROUGHAGE QUALITY

3.1 Abstract

This study was aimed to determine the effect of sunflower oil level and potassium nitrate supplementation on methane production and rumen fermentation and nutrient digestibility in meat goats fed with pangola grass hay (Experiment 1.1) and rice straw (Experiment 1.2) by using *in vitro* gas production technique. There were two factors; Factor A was sunflower oil levels (0%, 3%, 6 % basal DM of total diet) and Factor B nitrate levels (0%, 1%, 2%, and 3% basal DM of total diet).

Experiment 1.1, the result shows that the potential, efficiency digestibility (ED), ammonia nitrogen (NH_3-N) and proportion of acetate had greater increases at 3% of sunflower oil but, there were significant organic matter digestibility (OMD) and metabolizable energy (ME) higher at 6% of sunflower oil. Total bacteria was decreases when increasing sunflower oil and nitrate levels but were did not effect in total protozoa and propionate and decreased proportion of butyrate. The methane production was decreased when increased at 6% of sunflower oil and 3% of nitrate.

Experiment 1.2, the results showed that the potential, ED, OMD and ME had greater increased (P<0.001) at 6% of sunflower oil. Total bacteria, total protozoa, propionate and acetate: propionate ratio were not effected by level of sunflower oil. However, total volatile fatty acids and ammonia nitrogen were significantly increased (P<0.05) with increasing level of nitrate. But there was significant when increasing levels of nitrate increase total volatile fatty acids and ammonia nitrogen. The methane production were effected decreased when supplemented with 6% of sunflower oil and 3% of nitrate in the diets.

Key Words : Gas production technique, Digestibility, Sunflower oil, Nitrate.

3.2 Introductions

Greenhouse gas (GHG) emissions have become an increasingly important focus worldwide due to their effects on global warming and climate change (IPCC, 2007). Most of the methane from ruminant livestock originates from microbial fermentation of carbohydrates in the rumen, which also accounts for a substantial gross energy loss of feeds depending upon the types of diets. Concerning ruminants, methane is formed during the fermentation of the feed in the rumen and the amount is dependent on the quality and quantity of the diet. The loss of ingested energy as eructated methane in cattle is around 6% (Johnson et al., 1995). There are many strategies to reduce methane mitigation and improve the efficiency energy utilization in ruminant. If these feeds are supplemented, they need to supply more nitrogen for microbial activity in the rumen. Non protein nitrogen such as urea or nitrogen is well accepted to use as N source for microbes growth.

Pangola grass (*Digitaria eriantha Steud*.) is one of the recent examples of grasses that have been successfully of the highest quality tropical grasses. It has been shown to improve performances in body weight gain, feed conversion ratio, carcass yield, meat quality. However, in Tropical county using rice straw for ruminants is still essential. (Wanapat et al., 2009; Su et al., 2012). During the long dry period, although these feeds contain a low protein and slowly degradable fiber occurs in low voluntary intake and digestibility (Leng et al., 2008). In addition, nitrate suppresses methane production by acting as a hydrogen sink as well as directly inhibiting the methanogens (Patra and Yu, 2013) and also a potential nitrogen source of non-protein nitrogen for ruminants. Several studies have reported that nitrate administration decreased *methanogenesis in vivo* (Takahashi and Young, 1991; Sar et al., 2005; van Zijderveld et al., 2010; Hulshof et al., 2012). In the rumen, nitrate is converted to ammonia, which provides N for microbial protein synthesis. Additionally, the long chain fatty acid hexadecatrienoic acid inhibited *in vitro* methane production by 97% (Ungerfeld et al., 2005). Sunflower oil rich in 12% saturated fatty acid and 88 % unsaturated fatty acids (Grant and Kubik, 1990), there had affected to populations of methanogens and protozoa may have an effect on methanogen in the rumen.

3.3 Objectives

The objectives of this study were to examine the effect of sunflower oil and nitrate levels on methane production, rumen fermentation and nutrient digestibility in meat goats fed with different roughage quality.

3.4 Material and methods

3.4.1 Experimental design and treatments

This study we divide by the quality of roughage (Experiment 1.1 we used pengola grass hay and Experiment 1.2, rice straw). This experiment was carried out using a syringe gas production technique. The experiment was according to a 3 × 4 factorial arrangements in a complete randomized design (CRD). Factor A was the levels of sunflower oil (0%, 3% and 6% of DM in diet) and factor B was the level of potassium nitrate (0%, 1%, 2% and 3% of DM in diet), with combination the treatments included :

T1= Sunflower oil 0% + 0% Nitrate of DM in diet,

T2 = Sunflower oil 0% + 1% Nitrate of DM in diet,

T3= Sunflower oil 0% + 2% Nitrate of DM in diet,

T4 = Sunflower oil 0% + 3% Nitrate of DM in diet,

T5 = Sunflower oil 3% + 0% Nitrate of DM in diet,

T6= Sunflower oil 3% + 1% Nitrate of DM in diet,

T7 = Sunflower oil 3% + 2% Nitrate of DM in diet,

T8 = Sunflower oil 3% + 3% Nitrate of DM in diet,

T9 = Sunflower oil 6% + 0% Nitrate of DM in diet,

T10 = Sunflower oil 6% + 1% Nitrate of DM in diet,

T11 = Sunflower oil 6% + 2% Nitrate of DM in diet, and

T12 = Sunflower oil 6% + 3% Nitrate of DM in diet.

3.4.2 Substrates, added oils, and rumen inoculum

Roughage and concentrate were ground in a Retsch mill (SR200 model, Retsch, Haan, Germany) to pass a 1-mm mesh prior to analyzing for chemical

compositions and *in vitro* gas production measurements. The incubation substrate consisted of roughages (Pangola grass hay and rice straw) and concentrate were mixed at a ratio of 40 : 60 (w/w, on DM basis). Sunflower oil was bought from Makro store, Nakhon Ratchasima, Thailand. Chemical characteristics of feeds and oils used in this study are presented in Table 3.1.

3.4.3 Medium preparation

The medium solution was prepared according to Menke and Steingass (1988) with some minor a volumetric flask, followed by respective addition of 0.125 ml micro mineral solution (prepared by diluting 13.2 g $CaCl_2.2H_2O$, 10.0 g $MnCl_2.4H_2O$, 1.0 g $CoCl_2.6H_2O$, and 8.0 g $FeCl_2.6H_2O$ in deionized water to a final volume of 100 ml), 250 ml buffer solution (prepared by diluting 4.0 g NH_4HCO_3 and 25.0 g $NaHCO_3$ in deionized water to a final volume of 1 L), 250 ml macro mineral solution (prepared by diluting 5.7 g Na_2HPO_4, 6.0 g KH_2PO_4, and 0.6 g $MgSO_4.7H_2O$ in deionized water to a final volume of 1 L), 1.25 ml 0.1% resazurin solution (prepared by dissolving 0.1 g resazurin in deionized water to a final volume of 100 ml), and deionized water to a final volume of 1000 ml. The solution was then placed in a water bath (39°C) and gassed CO_2 for 45 min. While still flushing with CO_2, the exact amounts of 0.313 g L-cysteine hydrochloride and 0.313 g sodium sulphide were weighed and added directly to the medium. The medium solution was continued to flush with CO_2 for another approximate 30 minutes or until the solution turns gray to clear.

3.4.4 *In vitro* fermentation

Substrates were weighed to 200 mg of DM into 100 ml glass syringes. Three blank syringes for gas production were added the substrate. Under continuous

CO_2 flushing, the filtrated rumen fluid was mixed (1 : 4, v/v) with pre-warmed (39°C) medium and then introduced (50 ml of rumen fluid and medium mixture) into gastight glass syringes. The lower end of syringes was closed afterward, and the syringes were incubated in a water bath at 39°C for 96 h. The gas volume was recorded after 0, 3, 6, 8, 12, 24, 48, 72, 84, and 96 h of incubation.

3.4.5 Sampling, measurements and chemical analysis

Rumen fluid was collected at 0, 3, and 6 h post incubation. Incubation was stopped by placing the syringes into ice-cold water, and the pH of syringe contents was immediately measured. One milliliter of rumen content was sampled and mixed with 9 ml of 10% formalin solution. Total protozoa were directly counted using hemocytometer following the methods of Galyen (1989). The samples for NH_3–N and VFA analyses were acidified with 1 M sulfuric acid (10/1, v/v), centrifuged at 14,000 × g for 15 min, and the supernatant was then stored at –20°C. At 96 h post inoculation. Organic matter content was calculated as the difference from ash, determined according to the AOAC (1995). Crude protein was determined by Kjeldahl method of AOAC (1995). The ether extract was determined using petroleum ether in a Soxtec System of AOAC (1995). Neutral detergent fiber and ADF were determined using the methods described by Van Soest et al. (1991), adapted for Fiber Analyzer. The NDF analysis used sodium sulfite in the neutral detergent solution. Both NDF and ADF are expressed inclusive of residual ash. All chemical compositions were expressed on DM basis. The rumen NH_3–N was determined by Kjeldahl method (AOAC, 1995). The volatile fatty acids (VFA) were analyzed using gas chromatography (Hewlett Packard GC system HP6890 A; Hewlett Packard, Avondale, PA, USA). Gas chromatography was equipped with a 30 m × 0.32

mm × 0.15 μm film fused silica capillary column (HP Innowax, AB 002, Agilent, USA). Injector and detector temperatures were 250°C. The column temperature was respectively set as follow: kept at 80°C for 5 min, increased at 10°C/min to 170°C, increased at 30°C/min to 250°C and finally held at 250°C for 5 min.

3.4.6 Calculations

Methane concentration was calculated from individual net molar of VFA with the equation proposed by Moss et al. (2000) as follow :

$$CH_4 \text{ (mmol)} = (0.5 \times \text{acetate}) - (0.25 \times \text{propionate}) + (0.5 \times \text{butyrate})$$

The *in vitro* digestible organic matter (IVDOM) was calculated from the gas produced and chemical composition according to the equation of Menke et al. (1988) :

$$\text{IVDOM (g/kg DM)} = (14.88 + 0.889 \times Gp + 0.45 \times CP + 0.0651 \times XA)$$

where CP is the crude protein (% of DM), XA is the ash (% of DM), and Gp is the net gas production (ml) from 200 mg (DM of the sample) after 24 h incubation.

$$\text{ME (MJ/kg DM)} = 7.81 + 0.07559 \times GP - 0.00384 \times \text{ash} + 0.00565 \times CP + 0.01898 \times \text{Crude fat} - 0.00831 \times \text{ADFom},$$

where GP is *in vitro* gas production at 24 h (mL/200 mg DM) and ash, CP, crude fat and ADFom are expressed in g/kg DM.

Data were fitted to an exponential model given by McDonald (1981) :

$$y = a + b(1 - e^{(-ct)})$$

where a = the gas production from the immediately soluble fraction,

b = the gas production from the insoluble fraction,

c = the gas production rate constant for the insoluble fraction,

t = incubation time,

a + b = the potential extent of gas production, and

y = gas produced at time “t”

3.4.7 Calculations statistical analysis

Data analyses were conducted using the mixed model procedure of SAS (SAS Institute Inc., Cary, NC). Data from Exp. 1.1 and 1.2 were analyzed separately as a completely randomized design with levels of sunflower oil and levels of potassium nitrate and their interaction included in the model as fixed effects. Within an experiment, the run was considered a random effect. When the interaction between levels of sunflower oil and levels of potassium nitrate was significant ($P<0.05$), contrasts and orthogonal polynomial contrasts were performed to determine linear, quadratic and cubic responses to levels of potassium nitrate within levels of sunflower oil. When the main effect of levels was significant ($P<0.05$), contrasts and orthogonal polynomial contrasts were performed to determine overall linear and quadratic responses to sunflower oil. Significance was declared at ($P<0.05$).

3.5 Experimental locations

The experiment was conducted at Suranaree University of Technology’s Goat Farm, The Center for Scientific and Technological Equipment Building 10, Suranaree University of Technology.

3.6 Experimental period

The duration of the present experiment was from August to December 2013.

3.7 Results

3.7.1 Chemical compositions of experimental feeds and diets

The dietary ingredients and chemical compositions of the individual feeds and experimental diets used in the current study are presented in Table 3.1. The concentrate was used as the main source of protein (14.63%).

Table 3.1 Chemical composition of experimental diet (dry matter basis).

Item	Experimental feed		
	Concentrate[3/]	Panggola hay	Rice straw
Dry matter	93.91	85.21	91.82
	..... % Dry matter based.....		
Ash	7.00	8.46	12.31
Crude protein	14.63	7.35	3.35
Ether extract	4.07	1.92	1.05
Crude fiber	17.13	32.08	39.79
Neutral detergent fiber	42.59	73.46	76.31
Acid detergent fiber	26.3	42.15	52.34
Acid detergent lignin	10.95	4.00	6.34
TDN (%)[1/]	60.23	50.94	46.14
NFC[2/]	31.71	8.81	6.98

[1/]Total digestible nutrients, TDN1X (%) = tdNFC + tdCP + (tdFA x 2.25) + tdNDF – 7 (NRC, 2001); [2/]Non Fiber Carbohydrate, NFC = 100 – (CP – NDF – EE – ash); [3/]Contained (as DM basis) : 32% cassava distillers dried meal, 20% soybean meal, 17.5% corn distillers dried grains with solubles, 10% rice bran, 10% wheat bran, 8% molasses, and 2.5% mineral and vitamin mix. Mineral and vitamin mix : provided per

kg of concentrate including vitamin A, 5,000 IU; vitamin D3, 2,200 IU; vitamin E, 15 IU; Ca, 8.5 g; P, 6 g; K, 9.5 g; Mg, 2.4 g; Na, 2.1 g; Cl, 3.4 g; S, 3.2 g; Co, 0.16 mg; Cu, 100 mg; I, 1.3 mg; Mn, 64 mg; Zn, 64 mg; Fe, 64 mg; Se, 0.45 mg.

3.7.2 *In vitro* gas production characteristics

In Experiment 1.1, the effect sunflower oil (SO) and nitrate (NO_3) had affected on gas production characteristics (Table 3.2). There were differences in the asymptotic (a) was decreased linearly effect when increase levels of SO and (b) gas production was increased when increased level of SO and NO3, but the rate constant did not effect by SO. The potential extent of gas production (a + b) with greater in the group with 2% of SO and had effect in the combination of SO × NO3. After 24 and 96 h, significant when increasing a level of SO. Also, there were significant in ED, OMD and ME ($P<0.001$). In Experiment 1.2, there was the cumulative gas volume at each sampling time was affected by the level of SO. There was linearly effect on b, c, G24, ED, OMD, and ME. But there was 2% of SO better on the potential extent of gas production (a + b). The levels of NO_3 did not effect on all parameter, except for the rate constant (Table 3.6).

3.7.3 Bacteria and ammonia nitrogen (NH_3-N)

In Experiment 1.1, there were levels of SO × NO_3 did not effect on a number of protozoa and total bacteria number, except for at 3 h of total bacteria and at 6 h after incubated was decreased when increasing the level of SO and at 6 h of bacteria had effect by NO_3 (Table 3.3). In Experiment 1.2, there was significant in ammonia nitrogen when increase levels of NO_3 increased the concentration of ammonia nitrogen at 0 and 6 h after incubated (Table 3.7).

3.7.4 Volatile fatty acids (VFA)

In Experiment 1.1, the proportion of acetate had linearly effect at 6 h after incubated by levels of SO and had cubic effect at 3h by levels of NO_3. The concentration of propionic decreased when increasing the level of SO at 3 h. Moreover, there was significant in butyrate at 0 h by an interaction between SO and NO_3. The total volatile fatty acids decreased when increasing level of SO but there found that increasing levels of potassium was increased concentration of TVFA. The ratio of acetate : propionate did not effect by potassium nitrate (Table 3.4). In Experiment 1.2, the proportion of acetate had effect by SO and NO_3 and the level of SO no effect on the proportion of propionate but had effect when added 2% of NO_3 was increased baster other levels. The concentration of butyrate was higher in 3% of SO at 3 h after incubated. The potassium nitrate had the effect on acetate at 3h and at 0h of propionate trend to decrease when increased level of NO_3. The ratio of acetate: propionate did not effect by SO but there had effect by potassium nitrate at 3 h when increased level of NO_3 (Table 3.9).

3.7.5 Methane production

In Experiment 1.1, the effect of sunflower oil and potassium nitrate on methane production had quadratically effect at 0 and 3 h and had linearly effect at 3 and 6 h. Moreover, the interaction between SO × NO_3 had effect methane production (Table 3.5). In Experiment 1.2, the methane production had affected when increased levels of SO trend to decrease at 0 and 6 h but at 3 h trend increased the proportion of methane after incubated. However, there found that at 3 h decrease when increased level of potassium nitrate (Table 3.9). For the methane measurements (expressed per unit of OM incubated) at 24 h had linearly effect was suppressed

methane with the addition of sunflower oil at 6% with potassium nitrate at 3% of DM diets (Table 3.10).

Table 3.2 Effect of sunflower oil and nitrate supplementation *in vitro* on gas production characteristics, total gas production (ml/g DM), efficiency digestibility, ED (%), organic matter digestibility, OMD (%) and metabolizable energy and ME (MJ/kg DM) (Exp. 1.1).

Items	0% of Sunflower oil				3% of Sunflower oil				6% of Sunflower oil				SEM	P-value		
	0% NO_3	1% NO_3	2% NO_3	3% NO_3	0% NO_3	1% NO_3	2% NO_3	3% NO_3	0% NO_3	1% NO_3	2% NO_3	3% NO_3		SO	NO_3	SO × NO_3
a (ml)	8.06	8.78	10.49	10.50	10.25	3.15	5.59	12.71	3.10	2.45	3.43	5.68	0.28	***	NS	NS
B (ml)	21.11	21.72	31.86	31.61	63.24	67.85	64.52	60.36	61.38	62.81	65.33	71.52	1.53	***	NS	NS
c (h^{-1})	0.13^{a}	0.13^{a}	0.05^{d}	0.05^{d}	0.08^{c}	0.09^{b}	0.09^{b}	0.07^{c}	0.09^{b}	0.09^{b}	0.13^{a}	0.10^{b}	0.002	NS	**	**
a + b (ml)	29.17	30.50	42.35	42.10	73.49	70.99	70.11	73.07	64.47	65.27	68.76	77.19	1.41	***	NS	NS
G24 (ml/g DM)	27.40	27.90	27.40	27.90	55.00	49.30	54.40	48.00	60.00	51.60	54.40	53.20	1.03	***	NS	NS
G96 (ml/g DM)	29.17	30.50	42.09	42.91	73.45	70.98	70.08	72.89	64.46	65.26	68.76	77.19	1.40	***	NS	NS
ED (%)	26.32	27.62	33.29	33.44	60.69	58.57	57.75	59.36	53.13	53.72	60.18	65.22	1.15	***	NS	NS
OMD (%)	50.74^{e}	56.77^{d}	67.04^{c}	68.86^{c}	50.32^{d}	51.54^{d}	72.64^{b}	75.09^{a}	72.65^{b}	73.56^{b}	75.34^{a}	77.21^{a}	0.84	***	NS	***
ME (MJ/kg DM)	6.52^{d}	7.45^{c}	9.03^{b}	9.31^{b}	6.45^{d}	6.64^{d}	9.90^{b}	10.28^{a}	9.90^{b}	10.04^{a}	10.31^{a}	10.60^{a}	0.13	***	NS	***

$^{a, b, c, d, e}$ Mean within the same row for the main effects of sunflower oil and potassium nitrate having different letters are different at P<0.05. *P<0.05; **P<0.01; ***P<0.001; NS = not significantly different (P>0.05); SEM = standard error of the mean; Sunflower oil; SO, Potassium nitrate; NO_3.

Table 3.2 Effect of sunflower oil and nitrate supplementation *in vitro* on gas production characteristics, total gas production (ml/g DM), efficiency digestibility, ED (%), organic matter digestibility, OMD (%) and metabolizable energy, ME (MJ/kg DM) (Exp. 1.1) (Continued).

Items	Levels of sunflower oil (% of diets)			SEM	Contrast	Level of potassium nitrate (% of diets)				SEM	Contrast
	0	3	6			0	1	2	3		
a (ml)	9.46^{A}	7.93^{B}	3.67^{C}	0.20	L	7.14	4.79	6.50	9.63	0.14	NS
b (ml)	26.58^{C}	63.99^{B}	65.26^{A}	1.50	L	48.58^{D}	50.79^{C}	53.90^{B}	54.50^{A}	0.20	C
c (h^{-1})	0.09	0.08	0.10	0.006	NS	0.10^{A}	0.10^{A}	0.09^{B}	0.07^{C}	0.009	C
a + b (ml)	36.03^{C}	71.92^{A}	68.92^{B}	1.35	Q	55.71	55.59	60.41	64.12	0.30	NS
G24 (ml/g DM)	27.65^{C}	51.68^{B}	54.80^{A}	1.01	Q	47.47	42.93	45.40	43.03	0.16	NS
G96 (ml/g DM)	36.17^{C}	71.85^{A}	68.92^{B}	1.35	Q	55.69	55.58	60.31	64.33	0.30	NS
ED (%)	30.17^{C}	59.09^{A}	58.06^{B}	1.12	Q	46.71	46.64	50.41	52.67	0.21	NS
OMD (%)	60.85^{C}	62.40^{B}	74.69^{A}	0.52	Q	57.90	60.62	71.67	73.72	0.57	NS
ME (MJ/kg DM)	8.08^{B}	8.32^{B}	10.21^{A}	0.08	Q	7.62	8.04	9.75	10.06	0.09	NS

$^{A, B, C, D}$ Mean within the same row for the main effects of levels of sunflower oil and potassium nitrate having different letters are different at P<0.05. *P<0.05; **P<0.01; ***P<0.001; NS = not significantly different (P>0.05); SEM = standard error of the mean; L= linear; Q = quadratic; C = cubic.

Table 3.3 Effect of sunflower oil and nitrate levels *in vitro* on protozoa, bacteria, and NH_3-N (Exp. 1.1).

Items	0% of Sunflower oil				3% of Sunflower oil				6% of Sunflower oil				SEM	P-value		
	0% NO_3	1% NO_3	2% NO_3	3% NO_3	0% NO_3	1% NO_3	2% NO_3	3% NO_3	0% NO_3	1% NO_3	2% NO_3	3% NO_3		SO	NO_3	SO × NO_3
Protozoa count, 10^5 cells/ml; after incubation																
0 h	3.05	3.08	3.19	3.14	2.90	3.23	3.05	3.08	2.99	2.90	3.23	3.05	0.204	NS	NS	NS
3 h	2.99	3.02	2.99	2.99	2.75	3.08	3.10	3.26	3.08	3.10	3.25	3.20	0.364	NS	NS	NS
6 h	2.6	2.75	2.60	2.75	2.75	2.75	2.75	2.75	2.60	2.60	2.90	2.75	0.521	NS	NS	NS
Bacteria, 10^8 cells/ml; after incubation																
0 h	4.80^a	4.73^b	4.73^b	4.81^a	4.84^a	4.76^b	4.71^b	4.68^c	4.56^c	4.59^c	4.80^a	4.73^b	0.490	NS	NS	*
3 h	4.85	4.78	4.81	4.80	4.85	4.78	4.82	4.73	4.72	4.62	4.52	4.74	0.415	***	NS	NS
6 h	4.82^a	4.51^c	4.80^a	4.73^b	4.79^b	4.84^a	4.76^b	4.710^b	4.74^b	4.56^c	4.59^c	4.80^a	0.487	NS	**	**
Ammonia nitrogen, NH_3-N; mg%; after incubation																
0 h	12.58	20.39	19.90	15.01	11.69	16.80	15.01	11.69	16.80	13.70	19.90	19.90	0.611	NS	NS	NS
3 h	12.81	14.82	13.37	14.26	13.37	21.02	16.13	12.81	14.82	13.37	13.93	13.70	0.464	NS	NS	NS
6 h	10.34	13.03	12.02	11.69	19.49	19.01	14.12	10.79	12.81	11.35	13.03	12.02	0.342	*	NS	NS

$^{a, b, c, d}$ Mean within the same row for the main effects of levels of sunflower oil and potassium nitrate having different letters are different at P<0.05. *P<0.05; **P<0.01; ***P<0.001; NS = not significantly different (P>0.05); SEM = standard error of the mean; NO_3 = Potassium nitrate.

Table 3.3 Effect of sunflower oil and nitrate levels *in vitro* on protozoa, bacteria, and NH_3-N (Exp. 1.1) (Continued).

Items	Levels of sunflower oil (% of diets)			SEM	Contrast	Level of potassium nitrate (% of diets)				SEM	Contrast
	0	3	6			0	1	2	3		
Protozoa count, 10^5 cells/ml; after incubation											
0 h	3.12	3.07	3.04	0.03	NS	2.98	3.07	3.16	3.09	0.05	NS
3 h	3.00	3.05	3.16	0.06	NS	2.94	3.07	3.11	3.15	0.07	NS
6 h	2.68	2.75	2.71	0.03	NS	2.65	2.70	2.75	2.75	0.03	NS
Bacteria, 10^8 cells/ml; after incubation											
0 h	4.77	4.75	4.67	0.04	NS	4.73	4.69	4.75	4.74	0.02	NS
3 h	4.81^A	4.80^A	4.65^B	0.06	L	4.81	4.73	4.72	4.76	0.03	NS
6 h	4.72	4.78	4.67	0.04	NS	4.78^A	4.64^C	4.72^B	4.75^A	0.005	Q
Ammonia nitrogen, NH_3-N; mg%; after incubation											
0 h	16.97	13.80	17.58	0.14	NS	13.69	16.96	18.27	15.53	0.14	NS
3 h	13.82	15.83	13.96	0.08	NS	13.67	16.40	14.48	13.59	0.09	NS
6 h	11.77^C	15.85^A	12.30^B	0.15	Q	14.21	14.46	13.06	11.50	0.10	NS

$^{A, B, C, D}$ Mean within the same row for the main effects of levels of sunflower oil and potassium nitrate having different letters are different at P<0.05. *P<0.05; **P<0.01; ***P<0.001; NS = not significantly different (P>0.05); SEM = standard error of the mean; L = linear; Q = quadratic; C = cubic.

Table 3.4 Effect of sunflower oil and nitrate levels *in vitro* on volatile fatty acid (VFA) concentrations (Exp. 1.1).

Items	0% of Sunflower oil				3% of Sunflower oil				6% of Sunflower oil				SEM	P-value		
	0% NO_3	1% NO_3	2% NO_3	3% NO_3	0% NO_3	1% NO_3	2% NO_3	3% NO_3	0% NO_3	1% NO_3	2% NO_3	3% NO_3		SO	NO_3	SO × NO_3
Acetate (C2) (mol/100 mol)																
0 h	69.73	69.27	67.31	67.60	68.17	68.24	70.62	71.56	66.78	66.62	67.36	69.22	2.34	NS	NS	NS
3 h	69.68	70.68	64.92	72.10	68.67	72.43	70.95	70.92	67.94	72.98	68.51	69.68	2.19	NS	**	NS
6 h	65.07^{d}	75.59^{a}	72.94^{b}	73.36^{b}	51.13^{f}	56.03^{e}	66.98^{d}	70.23^{c}	75.22^{a}	66.3^{d}	51.71^{f}	56.84^{e}	2.26	*	NS	*
Propionate (C3) (mol/100mol)																
0 h	24.65^{b}	19.75^{d}	22.07^{c}	23.49^{b}	23.98^{b}	27.49^{a}	25.41^{b}	19.86^{d}	23.46^{b}	28.13^{a}	29.24^{a}	25.82^{b}	2.05	NS	NS	*
3 h	19.30^{d}	24.10^{c}	31.67^{a}	22.02^{c}	17.92^{e}	16.23^{e}	17.23^{e}	21.08^{d}	27.62^{b}	18.6^{d}	19.64^{d}	19.30^{d}	2.16	***	NS	**
6 h	22.82	21.71	22.8	21.34	20.36	20.92	20.92	22.39	21.87	20.52	20.52	21.21	2.26	NS	NS	NS
Butyrate (C4) (mol/100mol)																
0 h	5.63^{d}	10.99^{a}	10.64^{a}	8.92^{b}	7.85^{c}	4.27^{d}	3.98^{e}	8.58^{b}	9.77^{b}	5.25^{d}	3.40^{e}	4.96^{d}	2.29	NS	NS	*
3 h	11.03	5.22	3.42	5.89	13.42	11.35	11.83	8.01	4.45	8.42	11.85	11.03	2.25	NS	NS	NS
6 h	8.47^{c}	7.13^{d}	11.15^{b}	15.28^{a}	3.21^{f}	4.61^{e}	7.80^{d}	8.93^{c}	15.65^{a}	9.27^{c}	3.25^{f}	4.74^{e}	2.23	NS	NS	*

$^{a, b, c, d, e, f}$ Mean within the same row for the main effects of levels of sunflower oil and potassium nitrate having different letters are different at P<0.05. *P<0.05; **P<0.01; ***P<0.001; NS = not significantly different (P>0.05); SEM = standard error of the mean; NO_3 = Potassium nitrate.

Table 3.4 Effect of sunflower oil and nitrate levels *in vitro* on proportion volatile fatty acid (VFA) (Exp. 1.1) (Continued).

Items	Levels of sunflower oil (% of diets)			SEM	Contrast	Level of potassium nitrate (% of diets)				SEM	Contrast
	0	3	6			0	1	2	3		
Acetate (C2) (mol/100 mol)											
0 h	75.75^{B}	81.65^{A}	80.95^{A}	0.22	L	76.85	80.25	81.17	79.53	0.13	NS
3 h	80.29^{A}	77.35^{B}	77.02^{B}	0.12	Q	76.86^{B}	78.80^{A}	78.27^{A}	78.94^{A}	0.07	L
6 h	83.57^{A}	79.18^{C}	81.09^{B}	0.15	Q	81.41	80.71	81.07	81.92	0.04	NS
Propionate (C3) (mol/100mol)											
0 h	3.10	2.95	2.56	0.02	NS	2.84	2.84	2.72	3.07	0.01	NS
3 h	2.98	3.98	3.44	0.03	NS	3.34	3.80	3.25	3.47	0.02	NS
6 h	3.25	2.90	2.98	0.01	NS	2.95	3.13	2.99	3.09	0.01	NS
Butyrate (C4) (mol/100mol)											
0 h	33.14^{A}	30.84^{B}	30.01^{B}	0.11	Q	31.98	31.16	30.83	31.34	0.04	NS
3 h	30.18^{C}	34.62^{A}	32.09^{B}	0.15	Q	33.80^{B}	35.27^{A}	32.11^{B}	28.00^{C}	0.23	C
6 h	33.84^{A}	24.79^{C}	28.75^{B}	0.31	L	31.04	31.23	27.57	26.67	0.17	NS

$^{A, B, C}$ Mean within the same row for the main effects of levels of sunflower oil and potassium nitrate having different letters are different at P<0.05. *P<0.05; **P<0.01; ***P<0.001; NS = not significantly different (P>0.05); SEM = standard error of the mean; L = linear; Q = quadratic; C = cubic.

Table 3.5 Effect of sunflower oil and nitrate levels *in vitro* on acetate : propionate ratio, total volatile fatty acid (mM/L**)** and methane production (Exp. 1.1).

Items	0% of Sunflower oil				3% of Sunflower oil				6% of Sunflower oil				SEM	P-value		
	0% NO_3	1% NO_3	2% NO_3	3% NO_3	0% NO_3	1% NO_3	2% NO_3	3% NO_3	0% NO_3	1% NO_3	2% NO_3	3% NO_3		SO	NO_3	SO × NO_3
Total volatile fatty acid (mM/L)																
0 h	70.00	75.93^{d}	77.55^{c}	79.51^{b}	83.03^{a}	82.49^{a}	83.68^{a}	77.40^{c}	77.51^{c}	82.32^{a}	82.29^{a}	81.68^{b}	2.43	**	NS	**
3 h	77.71^{c}	83.81^{a}	80.21^{b}	79.44^{b}	75.92^{d}	76.67^{c}	77.06^{c}	79.74^{b}	76.95^{c}	75.93^{d}	77.55^{c}	77.65^{c}	2.57	***	*	**
6 h	80.17^{c}	81.83^{c}	84.88^{b}	87.38^{a}	76.67^{e}	78.22^{d}	81.65^{c}	80.17^{c}	87.38^{a}	82.08^{c}	76.67^{e}	78.22^{d}	2.61	**	NS	**
Acetate : Propionate ratio																
0 h	2.83	3.61	3.05	2.89	2.84	2.55	2.80	3.62	2.85	2.37	2.32	2.70	2.64	NS	NS	NS
3 h	3.65^{b}	2.94^{d}	2.05^{e}	3.29^{c}	3.84^{b}	4.47^{a}	4.12^{a}	3.47^{c}	2.54^{d}	3.98^{b}	3.58^{b}	3.65^{b}	2.53	**	NS	*
6 h	2.85^{c}	3.49^{a}	3.21^{b}	3.44^{a}	2.55^{c}	2.67^{c}	3.21^{b}	3.15^{b}	3.44^{a}	3.24^{b}	2.55^{c}	2.67^{c}	2.54	NS	NS	*
Methane (mol/mol VFA)[1/]																
0 h	31.52^{c}	35.19^{a}	33.46^{b}	32.39^{c}	32.02^{c}	29.38^{e}	30.95^{d}	31.00^{c}	32.41^{c}	28.90^{e}	28.07^{e}	30.64^{d}	2.60	***	NS	***
3 h	35.53^{b}	31.93^{c}	26.25^{e}	27.00^{e}	36.57^{b}	37.83^{a}	37.08^{a}	27.00^{e}	29.29^{d}	36.05^{b}	33.00^{c}	30.00^{d}	2.53	***	**	***
6 h	31.07^{d}	35.93^{b}	36.35^{b}	32.00^{c}	22.08^{f}	25.09^{e}	24.00^{e}	28.00^{d}	39.97^{a}	32.66^{c}	22.35^{f}	20.00^{f}	2.60	**	NS	**
24 h	10.00^{a}	8.50^{b}	8.75^{b}	8.90^{b}	7.89^{c}	7.70^{c}	7.25^{c}	7.15^{c}	7.75^{c}	6.20^{d}	6.22^{d}	6.13^{d}	0.10	**	**	**

$^{a, b, c, d, f}$ Mean within the same row for the main effects of levels of sunflower oil and potassium nitrate having different letters are different at P<0.05. *P<0.05; **P<0.01; ***P<0.001; NS = not significantly different (P>0.05); SEM = standard error of the mean; NO_3 = Potassium nitrate; Calculation : Moss et al. (2000).

Table 3.5 Effect of sunflower oil and nitrate levels *in vitro* on acetate : propionate ratio, total volatile fatty acid (mM/L**)** and methane production (Exp. 1.1) (Continued).

Items	Levels of sunflower oil (% of diets)			SEM	Contrast	Level of potassium nitrate (% of diets)				SEM	Contrast
	0	3	6			0	1	2	3		
Total volatile fatty acid (mM/L)											
0 h	75.75^{C}	81.65^{A}	80.95^{B}	0.22	L	76.85	80.25	81.17	79.53	0.13	NS
3 h	80.29^{A}	77.35^{B}	77.02^{B}	0.12	Q	76.86^{B}	78.80^{A}	78.27^{A}	78.94^{A}	0.07	L
6 h	83.57^{A}	79.18^{C}	81.09^{B}	0.15	Q	81.41	80.71	81.07	81.92	0.04	NS
Acetate : Propionate ratio											
0 h	3.10	2.95	2.56	0.02	NS	2.84	2.84	2.72	3.07	0.01	NS
3 h	2.98	3.98	3.44	0.03	NS	3.34	3.80	3.25	3.47	0.02	NS
6 h	3.25	2.90	2.98	0.01	NS	2.95	3.13	2.99	3.09	0.01	NS
Methane (mol/mol VFA)[1/]											
0 h	33.14^{A}	30.84^{B}	30.01^{B}	0.11	Q	31.98	31.16	30.83	31.34	0.04	NS
3 h	30.18^{C}	34.62^{A}	32.09^{B}	0.15	Q	33.80^{B}	35.27^{A}	32.11^{C}	28.00^{D}	0.23	C
6 h	33.84^{A}	24.79^{C}	28.75^{B}	0.31	L	31.04	31.23	27.57	26.67	0.17	NS
24 h	9.04^{A}	7.50^{B}	6.58^{C}	0.08	L	8.55^{A}	7.47^{B}	7.41^{B}	7.39^{B}	0.04	L

$^{A, B, C, D}$ Mean within the same row for the main effects of levels of sunflower oil and potassium nitrate having different letters are different at P<0.05. *P<0.05; **P<0.01; ***P<0.001; NS = not significantly different (P>0.05); SEM = standard error of the mean; L= linear; Q = quadratic; C = cubic; Calculation : Moss et al. (2000).

Table 3.6 Effect of sunflower oil and nitrate levels *in vitro* on gas production characteristics, total gas production (ml/g DM), efficiency digestibility, ED (%), organic matter digestibility, OMD (%) and metabolizable energy, and ME (MJ/kg DM) (Exp. 1.2).

Items	0% of Sunflower oil				3% of Sunflower oil				6% of Sunflower oil				SEM	P-value		
	0% NO_3	1% NO_3	2% NO_3	3% NO_3	0% NO_3	1% NO_3	2% NO_3	3% NO_3	0% NO_3	1% NO_3	2% NO_3	3% NO_3		SO	NO_3	SO × NO_3
a (ml)	8.06	8.78	10.49	10.49	10.25	3.14	5.59	12.71	2.45	3.09	3.42	5.68	1.32	**	NS	NS
B (ml)	21.10	21.71	31.86	31.61	63.23	67.84	64.52	60.36	62.81	61.38	65.33	71.52	1.93	***	NS	NS
c (h^{-1})	0.13^{a}	0.13^{a}	0.05^{d}	0.05^{d}	0.08^{c}	0.09^{b}	0.09^{b}	0.07^{c}	0.09^{b}	0.09^{b}	0.13^{a}	0.10^{b}	1.94	**	***	***
a + b (ml)	29.17	30.50	42.35	42.10	73.48	70.99	70.11	73.07	65.27	64.47	68.75	77.19	2.60	***	NS	NS
G24 (ml/g DM)	28.19	29.56	32.84	34.24	63.93	63.02	61.45	61.23	57.81	57.06	65.9	70.62	2.56	***	NS	NS
G96 (ml/g DM)	29.17	30.50	42.09	42.90	73.44	70.97	70.07	72.88	65.25	64.45	68.75	77.19	2.60	***	NS	NS
ED (%)	26.32	27.62	33.29	33.44	60.69	58.57	57.75	59.36	53.72	53.13	60.18	65.22	2.64	***	NS	NS
OMD (%)	51.37^{f}	52.58^{f}	80.45^{c}	80.65^{c}	55.46^{e}	56.7^{e}	76.78^{d}	84.56^{b}	77.44^{d}	82.03^{b}	82.83^{b}	88.71^{a}	2.58	***	NS	***
ME (MJ/kg DM)	6.61^{d}	6.80^{d}	11.11^{a}	11.14^{a}	7.24^{c}	7.44^{c}	10.54^{b}	11.74^{a}	10.64^{b}	11.35^{a}	11.47^{a}	12.38^{a}	2.54	***	NS	***

$^{a, b, c, d, e, f}$ Mean within the same row for the main effects of levels of sunflower oil and potassium nitrate having different letters are different at P<0.05. *P<0.05; **P<0.01; ***P<0.001; NS = not significantly different (P>0.05); SEM = standard error of the mean; NO_3 = Potassium nitrate.

Table 3.6 Effect of sunflower oil and nitrate levels *in vitro* on gas production characteristics, total gas production (ml/g DM), efficiency digestibility, ED (%), organic matter digestibility, OMD (%) and metabolizable energy, and ME (MJ/kg DM) (Exp. 1.2) (Continued).

Items	Levels of sunflower oil (% of diets)			SEM	Contrast	Level of potassium nitrate (% of diets)				SEM	Contrast
	0	3	6			0	1	2	3		
a (ml)	9.46^{A}	7.92^{B}	3.66^{C}	0.20	L	6.92	5.00	6.50	9.63	0.14	NS
B (ml)	26.57^{C}	63.99^{B}	65.26^{A}	1.50	Q	49.05^{D}	50.31^{C}	53.90^{B}	54.50^{A}	0.19	Q
c (h^{-1})	0.09^{B}	0.08^{C}	0.10^{A}	0.00	Q	0.10	0.10	0.09	0.07	0.00	NS
a + b (ml)	36.03^{C}	71.91^{A}	68.92^{B}	1.35	Q	55.97	55.32	60.40	64.12	0.30	NS
G24 (ml/g DM)	31.21^{B}	62.41^{A}	62.85^{A}	1.23	Q	49.98	49.88	53.40	55.36	0.19	NS
G96 (ml/g DM)	36.17^{C}	71.84^{A}	68.91^{B}	1.35	Q	55.95	55.31	60.30	64.32	0.30	NS
ED (%)	30.17^{C}	59.09^{A}	58.06^{B}	1.12	Q	46.91	46.44	50.41	52.67	0.21	NS
OMD (%)	66.26^{C}	68.38^{B}	82.75^{A}	0.61	Q	61.42	63.77	80.02	84.64	0.84	NS
ME (MJ/kg DM)	8.92^{C}	9.24^{B}	11.46^{A}	0.09	L	8.16	8.53	11.04	11.75	0.13	NS

$^{A, B, C, D}$ Mean within the same row for the main effects of levels of sunflower oil and potassium nitrate having different letters are different at P<0.05. *P<0.05; **P<0.01; ***P<0.001; NS = not significantly different (P>0.05); SEM = standard error of the mean; L = linear; Q = quadratic; C = cubic.

Table 3.7 Effect of sunflower oil and nitrate levels *in vitro* on protozoa, bacteria, and NH_3-N (Exp. 1.2).

Items	0% of Sunflower oil				3% of Sunflower oil				6% of Sunflower oil				SEM	P-value		
	0% NO_3	1% NO_3	2% NO_3	3% NO_3	0% NO_3	1% NO_3	2% NO_3	3% NO_3	0% NO_3	1% NO_3	2% NO_3	3% NO_3		SO	NO_3	SO × NO_3
Protozoa count, 10^5 cells/ml; after incubation																
0 h	2.99	3.02	2.95	2.99	2.75	3.07	3.10	3.26	3.07	3.10	3.25	3.20	0.168	NS	NS	NS
3 h	3.05	3.07	3.19	3.14	2.90	3.22	3.05	3.07	2.99	2.29	3.22	3.05	0.406	NS	NS	NS
6 h	2.60	2.75	2.60	2.60	2.75	2.75	2.75	2.75	2.60	2.60	2.90	2.75	0.516	NS	NS	NS
Bacteria, 10^8 cells/ml; after incubation																
0 h	4.80	4.73	4.73	4.80	4.84	4.76	4.70	4.67	4.56	4.59	4.80	4.73	0.572	NS	NS	NS
3 h	4.84	4.78	4.81	4.75	4.84	4.87	4.82	4.73	4.72	4.61	4.56	4.74	0.562	NS	NS	NS
6 h	4.52	4.50	4.80	4.73	4.79	4.84	4.76	4.70	4.74	4.56	4.53	4.80	0.575	NS	NS	NS
Ammonia nitrogen, NH_3-N; mg%; after incubation																
0 h	3.00^{f}	10.43^{e}	17.15^{b}	19.75^{a}	19.19^{a}	17.60^{b}	19.75^{a}	19.19^{a}	17.83^{b}	12.34^{d}	10.21^{e}	15.14^{c}	0.617	NS	***	***
3 h	3.00	16.60	18.61	17.72	16.60	20.20	16.93	18.34	19.98	18.86	16.17	15.16	0.556	NS	NS	NS
6 h	6.00	12.00	12.67	16.17	16.77	16.17	20.65	14.72	9.67	11.02	9.34	12.25	0.535	NS	**	NS

$^{a, b, c, d, e, f}$ Mean within the same row for the main effects of levels of sunflower oil and potassium nitrate having different letters are different at P<0.05. *P<0.05; **P<0.01; ***P<0.001; ns = not significantly different (P>0.05); SEM = standard error of the mean; NO_3 = Potassium nitrate.

Table 3.7 Effect of sunflower oil and nitrate levels *in vitro* on protozoa, bacteria, and NH_3-N (Exp. 1.2) (Continued).

Items	Levels of sunflower oil (% of diets)			SEM	Contrast	Level of potassium nitrate (% of diets)				SEM	Contrast
	0	3	6			0	1	2	3		
Protozoa count, 10^5 cells/ml; after incubation											
0 h	2.99	3.05	3.16	0.06	NS	2.94	3.06	3.10	3.15	0.07	NS
3 h	3.11	3.06	2.89	0.08	NS	2.98	2.86	3.15	3.09	0.09	NS
6 h	2.64	2.75	2.71	0.04	NS	2.65	2.70	2.75	2.70	0.03	NS
Bacteria, 10^8 cells/ml; after incubation											
0 h	4.77	4.74	4.67	0.03	NS	4.73	4.69	4.74	4.73	0.02	NS
3 h	4.80	4.82	4.66	0.06	NS	4.80	4.75	4.73	4.74	0.02	NS
6 h	4.64	4.77	4.66	0.05	NS	4.68	4.63	4.70	4.74	0.03	NS
Ammonia nitrogen, NH_3-N; mg%; after incubation											
0 h	11.83	18.93	13.88	0.25	NS	12.34^{D}	13.46^{C}	15.70^{B}	18.03^{A}	0.18	Q
3 h	13.98	18.02	17.54	0.15	NS	13.19	18.55	17.24	17.07	0.17	NS
6 h	11.71	17.08	10.57	0.24	NS	10.81^{C}	13.06^{B}	14.22^{A}	14.38^{A}	0.12	C

$^{A, B, C, D}$ Mean within the same row for the main effects of levels of sunflower oil and potassium nitrate having different letters are different at P<0.05. *P<0.05; **P<0.01; ***P<0.001; NS = not significantly different (P>0.05); SEM = standard error of the mean; L= linear; Q = quadratic; C = cubic.

Table 3.8 Effect of sunflower oil and nitrate levels *in vitro* on proportion of volatile fatty acid (VFA) (Exp. 1.2).

Items	0% of Sunflower oil				3% of Sunflower oil				6% of Sunflower oil				SEM	P-value		
	0% NO_3	1% NO_3	2% NO_3	3% NO_3	0% NO_3	1% NO_3	2% NO_3	3% NO_3	0% NO_3	1% NO_3	2% NO_3	3% NO_3		SO	NO_3	SO × NO_3
Acetate (C2) (mol/100 mol)																
0 h	74.94	68.83	73.46	77.72	73.35	74.97	76.83	78.24	77.09	76.36	72.02	74.75	0.21	NS	NS	NS
3 h	69.24^{d}	76.36^{a}	72.02^{b}	73.59^{b}	69.75^{c}	71.96^{b}	70.49^{c}	68.78^{d}	74.43^{a}	72.50^{b}	71.22^{c}	69.24^{c}	0.19	***	***	***
6 h	58.63^{b}	60.43^{a}	59.72^{a}	58.29^{b}	46.53^{e}	51.45^{d}	58.86^{b}	58.63^{b}	59.39^{a}	56.25^{c}	45.52^{f}	48.52^{e}	0.44	**	NS	**
Propionate (C3) (mol/100mol)																
0 h	19.19^{b}	19.93^{b}	15.67^{c}	13.24^{d}	18.58^{b}	20.50^{b}	18.94^{b}	13.05^{d}	13.04^{d}	18.20^{b}	24.25^{a}	20.04^{b}	0.28	NS	*	**
3 h	19.48^{c}	18.20^{d}	24.25^{a}	20.24^{b}	16.58^{e}	16.45^{e}	17.43^{e}	22.91^{b}	20.84^{c}	18.81^{d}	16.67^{e}	19.48^{c}	0.20	NS	NS	*
6 h	13.64	14.12	15.31	17.21	14.52	13.57	15.14	13.64	15.9	15.84	15.31	16.2	0.09	NS	NS	NS
Butyrate (C4) (mol/100mol)																
0 h	5.88^{e}	11.25^{a}	10.88^{a}	9.05^{b}	8.08^{d}	4.53^{f}	4.23^{f}	8.72^{d}	9.88^{b}	5.45^{e}	3.73^{f}	5.22^{e}	0.22	***	NS	***
3 h	11.28^{b}	5.45^{d}	3.73^{e}	6.17^{d}	13.68^{a}	11.60^{b}	12.08^{b}	8.31^{c}	4.73^{e}	8.70^{c}	12.12^{a}	11.28^{b}	0.27	***	NS	***
6 h	7.19^{b}	5.69^{c}	8.92^{b}	12.41^{a}	2.95^{e}	4.07^{d}	6.79^{c}	7.19^{b}	12.41^{a}	7.87^{b}	2.95^{e}	4.07^{d}	0.26	NS	NS	**

$^{a, b, c, d, e, f}$ Mean within the same row for the main effects of levels of sunflower oil and potassium nitrate having different letters are different at P<0.05. *P<0.05; **P<0.01; ***P<0.001; NS = not significantly different (P>0.05); SEM = standard error of the mean; NO_3 = potassium nitrate.

Table 3.8 Effect of sunflower oil and nitrate levels *in vitro* on proportion of volatile fatty acid (VFA) (Exp. 1.2) (Continued).

Items	Levels of sunflower oil (% of diets)			SEM	Contrast	Level of potassium nitrate (% of diets)				SEM	Contrast
	0	3	6			0	1	2	3		
Acetate (C2) (mol/100 mol)											
0 h	73.74	75.85	75.06	0.07	NS	75.13	73.39	74.10	76.90	0.11	NS
3 h	72.80^{A}	70.25^{C}	71.85^{B}	0.09	Q	71.14^{B}	73.61^{A}	71.24^{C}	70.54^{C}	0.10	C
6 h	59.27^{A}	53.87^{B}	52.42^{B}	0.25	L	54.85	56.04	54.70	55.15	0.04	NS
Propionate (C3) (mol/100 mol)											
0 h	17.01	17.77	18.88	0.06	NS	16.94^{B}	19.54^{A}	19.62^{A}	15.44^{C}	0.15	Q
3 h	20.54	18.34	18.95	0.08	NS	18.97	17.82	19.45	20.88	0.09	NS
6 h	15.07	14.22	15.81	0.05	NS	14.69	14.51	15.25	15.68	0.04	NS
Butyrate (C4) (mol/100 mol)											
0 h	9.27^{A}	6.39^{B}	6.07^{B}	0.12	L	7.95	7.08	6.28	7.66	0.05	NS
3 h	6.66^{C}	11.42^{A}	9.21^{B}	0.16	Q	9.90	8.58	9.31	8.59	0.05	NS
6 h	8.55	5.25	6.83	0.11	NS	7.52	5.88	6.22	7.89	0.07	NS

$^{A, B, C, D}$ Mean within the same row for the main effects of levels of sunflower oil and potassium nitrate having different letters are different at $P<0.05$. *$P<0.05$; **$P<0.01$; ***$P<0.001$; ns = not significantly different ($P>0.05$); SEM = standard error of the mean; L = linear; Q = quadratic; C = cubic.

Table 3.9 Effect of sunflower oil and nitrate levels *in vitro* on acetate : propionate ratio, total volatile fatty acid (mM/L**)** and methane production (Exp.1.2).

Items	0% of Sunflower oil				3% of Sunflower oil				6% of Sunflower oil				SEM	P-value		
	0% NO_3	1% NO_3	2% NO_3	3% NO_3	0% NO_3	1% NO_3	2% NO_3	3% NO_3	0% NO_3	1% NO_3	2% NO_3	3% NO_3		SO	NO_3	SO × NO_3
Total volatile fatty acid (mM/L)																
0h	81.68^{c}	75.93^{f}	77.55^{e}	79.51^{d}	83.03^{a}	82.49^{b}	83.68^{a}	77.40^{e}	77.51^{e}	82.32^{b}	82.29^{b}	81.68^{c}	0.21	*	NS	**
3h	77.71^{d}	83.81^{a}	80.21^{b}	79.44^{c}	75.92^{f}	76.67^{e}	77.06^{e}	79.74^{c}	76.95^{e}	75.93^{f}	77.55^{d}	77.65^{d}	0.18	***	*	**
6h	79.45^{c}	80.23^{c}	83.95^{b}	87.91^{a}	64.00^{e}	69.08^{d}	80.79^{c}	79.45^{c}	87.70^{a}	79.96^{c}	63.78^{e}	68.78^{d}	0.68	*	NS	*
Acetic acid : Propionic acid ratio																
0h	3.94	3.55	4.69	6.20	4.01	3.67	4.20	6.29	5.92	4.23	3.00	3.73	0.09	NS	NS	NS
3h	3.59^{b}	4.23^{a}	3.00^{c}	3.64^{c}	4.22^{a}	4.38^{a}	4.05^{b}	3.02^{d}	3.59^{c}	3.91^{b}	4.28^{a}	3.59^{c}	0.04	NS	*	*
6h	4.31	4.28	3.99	3.39	3.25	3.79	3.90	4.31	3.76	3.55	3.07	3.05	0.04	NS	NS	NS
Methane (mol/mol VFA)[1/]																
0h	35.61^{d}	35.06^{e}	38.25^{a}	40.08^{a}	36.07^{c}	34.63^{f}	35.80^{d}	40.22^{a}	40.23^{a}	36.36^{c}	31.81^{g}	34.98^{f}	0.21	***	***	***
3h	35.39^{c}	36.36^{b}	31.81^{f}	34.82^{d}	37.57^{a}	37.67^{a}	36.93^{a}	32.82^{e}	34.37^{d}	35.90^{c}	37.50^{a}	35.39^{b}	0.15	***	*	***
6h	29.50^{c}	29.53^{c}	30.49^{b}	31.05^{a}	21.11^{f}	24.37^{e}	29.04^{c}	29.50^{c}	31.93^{a}	28.10^{d}	20.41^{g}	22.25^{f}	0.33	*	NS	**
24 h	15.00^{a}	14.13^{b}	14.10^{b}	13.00^{c}	12.20^{c}	11.50^{d}	10.35^{d}	9.26^{e}	9.20^{e}	8.75^{f}	8.01^{f}	7.35^{f}	0.21	**	**	**

[a, b, c, d, e, f, g] Mean within the same row for the main effects of levels of sunflower oil and potassium nitrate having different letters are different at P<0.05. *P<0.05; **P<0.01; ***P<0.001; ns = not significantly different (P>0.05); SEM = standard error of the mean; NO_3 = potassium nitrate; [1/]Calculation: Moss et al. (2000).

Table 3.9 Effect of sunflower oil and nitrate levels *in vitro* on acetate : propionate ratio, total volatile fatty acid (mM/L**)** and methane production (Exp. 1.2) (Continued).

Items	Levels of sunflower oil (% of diets)			SEM	Contrast	Level of potassium nitrate (% of diets)				SEM	Contrast
	0	3	6			0	1	2	3		
Total volatile fatty acid (mM/L)											
0 h	78.67^{B}	81.65^{A}	80.95^{A}	0.11	L	80.74	80.25	81.17	79.53	0.05	NS
3 h	80.29^{A}	77.35^{B}	77.02^{B}	0.12	Q	76.86^{B}	78.80^{B}	78.27^{C}	78.94^{A}	0.07	L
6 h	82.89^{A}	73.33^{B}	75.06^{B}	0.35	L	77.05	76.42	76.17	78.71	0.08	NS
Acetate : Propionate ratio											
0 h	4.60	4.54	4.22	0.01	NS	4.62^{B}	3.82^{D}	3.96^{C}	5.41^{A}	0.05	Q
3 h	3.62	3.92	3.84	0.01	NS	3.80^{B}	4.17^{A}	3.78^{B}	3.42^{C}	0.02	Q
6 h	3.99	3.81	3.36	0.02	NS	3.77	3.87	3.65	3.58	0.01	NS
Methane (mol/mol VFA)[1/]											
0 h	37.25^{A}	36.68^{B}	35.85^{C}	0.05	Q	37.30	35.35	35.29	38.43	0.11	NS
3 h	34.60^{C}	36.25^{A}	35.79^{B}	0.06	Q	35.78^{A}	36.64^{B}	35.41^{C}	34.34^{C}	0.07	C
6 h	30.14^{A}	26.01^{B}	25.67^{C}	0.17	Q	27.51	27.33	26.65	27.60	0.03	NS
24 h	14.06^{A}	10.83^{B}	8.33^{C}	0.20	L	12.13^{A}	11.46^{B}	10.82^{C}	9.87^{D}	0.069	L

$^{A, B, C, D}$ Mean within the same row for the main effects of sunflower oil and potassium nitrate having different letters are different at P<0.05. *P<0.05; **P<0.01; ***P<0.001; NS = not significantly different (P>0.05); SEM = standard error of the mean; L = linear; Q = quadratic; C = cubic; [1/]Calculation : Moss et al. (2000).

3.8 Discussion

The present experiment was aimed to study the effect of sunflower oil and nitrate levels on methane production and rumen fermentation. The results showed that rumen fermentation characteristic had effect by dietary treatment the volume of gas production has positive correlation in levels of sunflower oil and potassium nitrate. Menke and Steingass (1988) also reported that physical and chemical traits of diets are effective on gas production. The potential extent of gas production was higher at 3% of sunflower oil in diets, but with inclusion of 6% a little decrease may be because of sunflower oil had effected to substrate availability and possible toxicity for bacteria agree with Jordan et al. (2006) reported that bacteria is sensitive to the high level of oil. It is possible due to act as a toxin for proliferation and activity of bacteria.

The results of this study, 6% of sunflower oil with 3% of nitrate can increase efficiency of digestibility, organic matter digestibility and metabolizable energy. Beauchemin et al. (2009) reported that sunflower oil could be increased energy content for ruminant. In this study, rate constant was low and total protozoa number did not affect when increased nitrate level. Also, Jenkins and Palmquist (1984) suggested that fats in from of triglycerides did not negative effects on rumen microbial activity and increased microbial nitrogen flow to the lower gut. Results from this research, the levels sunflower oil increased proportion of acetate and decreased propionate Farra and Satter (1971) observed a shift in the VFA profile from propionate to acetate when fed with nitrate dietary. The butyrate concentration was also significantly reduced (Allison and Reddy, 1984).

The concentrations of VFA were observed in the present study trend to decrease by sunflower oil level. But, there was increased when potassium nitrate.

Sunflower oil could be expected to induce the relative proportions of gluconeogenic and acetogenic fermentation end products. In addition, the results of present study methane production tendency decreased, due to oil supplement changes in ruminal bio-hydrogenation for electron acceptor. Fievez et al. (2003) suggested that it is not only the total amount of fat, but also its composition that exerts biologically important influences on rumen fermentation. Machmuller et al. (2000) also reported that supplemented sunflower oil in ruminant diets can prevent to methane production due to considerable negative effect on rumen pH. Because of unsaturated fatty acids can modify rumen fermentation.

3.9 Conclusions

In conclusion, the rumen fermentation characteristic had affected at 6% of sunflower oil increases efficiency digestibility, organic matter digestibility, and metabolizable energy but potassium nitrate was lower the rate constant. The protozoa number did not affect. However, the level of nitrate decreased total bacteria and increased concentration of ammonia nitrogen. For the concentration volatile fatty acid had positive at 3% of sunflower oil but at 6% was tendency decreased a little bit when to compare with 3%. However, the ratio of acetate: propionate did not effect by dietary treatment. Methane production had effect at 6% of sunflower oil and 3% of potassium nitrate can be decreased by 51% in low-quality roughage and38 % in high-quality roughage.

3.10 References

Allison, M. J. and Reddy, C. A. (1984). Adaptations of gastrointestinal bacteria in response to changes in dietary oxalate and nitrate. In: **Proceedings of the Third International Symposium on Microbial Ecology**. American Society for Microbiology. Washington, DC. pp. 248-256.

AOAC. (1995). **Official Methods of Analysis**, 16th ed., Association of Official Analytical Chemists, Washington, DC.

Beauchemin, K.A., McGinn, S.M., Benchaar, C., and Holtshausen, L. (2009). Crushed sunflower, flax, or canola seeds in lactating dairy cow diets: effects on methane production, rumen fermentation, and milk production. **Journal of Dairy Science**. 92: 2118-2127.

Farra, P.A., and Satter, L.D. (1971). Manipulation of the ruminal fermentation. III. Effect of nitrate on ruminal volatile fatty acid production and milk composition. **Journal of Dairy Science**. 54: 1018-1024.

Fievez, V., Dohme, F., Danneels, M., Raes, K., and Demeyer, D. (2003). Fish oils as potent rumen methane inhibitors and associated effects on rumen fermentation *in vitro* and *in vivo*. **Animal Feed Science and Technology**. 104: 41-58.

Grant, R., and Kubik, D. (1990). **Supplemental fat for high producing dairy cows**. Institute of Agriculture and Natural Resources, University of Nebraska-Lincoln. USA.

Galyean, M. (1989). **Laboratory Procedure in Animal Nutrition Research**. Department of Animal and Life Science. New Mexico states University, USA.

Hulshof, R.B.A., Berndt, A., Gerrits, W.J.J., Dijkstra, J., van Zijderveld, S. M.,

Newbold, J.R., and Perdok, H.B. (2012). Dietary nitrate supplementation reduces methane emission in beef cattle fed sugarcane based diets. **Journal of Animal Science**. 90: 2317-2323.

Jenkins, T. C. and Palmquist, D.L. (1984). Effects of fatty acids or calcium soaps on rumen and total nutrient digestibility of dairy rations. **Journal of Dairy Science**. 67: 978.

Johnson, K.A., and Johnson, D.E. (1995). Methane emissions from cattle. **Journal of Animal Science**. 73: 2483-2492.

Jordan, E., Kenny, D., Hawkins, M., Malone, R., Lovett, D.K., and O'Mara, F.P. (2006). Effect of refined soy oil or whole soybeans on intake, methane output, and performance of young bulls. **Journal of Animal Science**. 84: 2418-2425.

Kolver, E.S., Aspin, P.W., Jarvis, G.N., Elborough, K.M., and Roche, J.R. (2004) Fumarate reduces methane production from pasture fermented in continuous culture. **Proceedings of the New Zealand Society of Animal Production**. 64: 155-159.

Leng, R.A. (2008). The potential of feeding nitrate to reduce enteric methane production in ruminants. A report to the department of climate change. **Commonwealth Government of Australia**. Canberra, Australia.

Machmuller, A., Ossowski, D.A., and Kreuzer, M. (2000). Comparative evaluation of the effects of coconut oil, oilseeds and crystalline fat on methane release, digestion and energy balance in lambs. **Animal Feed Science and Technology**. 85: 41-60.

McDonald, I. (1981). A revised model for the estimation of protein degradability in the rumen. **Journal of Agriculture Science**. 96: 251-252.

Menke, K.H., and Steingass, H. (1988). Estimation of the energetic feed value obtained from chemical analysis and in vitro gas production using rumen fluid. **Animal Science and Reproduction Nutrition Development**. 28: 7-55.

Moss, A. R., Jouany, J.P., and Newbold, J. (2000). Methane production by ruminants: Its contribution to global warming. **Annales De Zootechnie**. 49: 231-253.

Patra, A. K. and Yu, Z. (2013). Effective reduction of enteric methane production by a combination of nitrate and saponin without adverse effect on feed degradability, fermentation, or bacterial and archaeal communities of the rumen. **Bioresource Technol**. 148: 352-360.

Sar, C., Mwenya, B., Pen, B., Takaura, K., Morikawa, R., Tsujimoto, A., Kuwaki, K., Isogai, N., Shinzato, I., Asakura, Y., Toride, Y., and Takahashi, J. (2005). Effect of ruminal administration of Escherichia coli wild type or a genetically modified strain with enhanced high nitrite reductase activity on methane emission and nitrate toxicity in nitrate-infused sheep. **British Journal of Nutrition**. 94: 691-697.

Su, Y., Zhao, G., Wei, Z., Yan, C., and Liu, S. (2012) Mutation of cellulose synthase gene improves the nutritive value of rice straw. **Asian - Australasian Journal of Animal Science**. 25: 800-805.

Takahashi, J. and Young, B.A. (1991). Prophylactic effect of L - cysteine on nitrate-induced alterations in respiratory exchange and metabolic rate in sheep. **Animal Feed Science and Technology**. 35: 105-113

Van Soest, P. J., Robertson, J. B., and Lewis, B. A. (1991). Methods for dietary fiber, neutral detergent fiber and non-starch polysaccharide in relation to animal nutrition. **Journal of Dairy Science**. 74: 3585-3597.

Van Zijderveld, S.M., Gerrits, W.J.J., Apajalahti, J.A., Newbold, J.R., Dijkstra, J., Leng, R.A., and Perdok, H.B. (2010). Nitrate and sulfate: Effective alternative hydrogen sinks for mitigation of ruminal methane production in sheep. **Journal of Dairy Science**. 93: 5856-5866.

Ungerfeld, E.M., and Kohn, R.A. (2006) .**The role of thermodynamics in the control of rumen fermentation**. In 'Ruminant physiology: digestion, metabolism and impact of nutrition on gene expression, immunology and stress. pp. 55-85.

Wanapat, M., and Cherdthong, A. (2009). Use of real-time PCR technique in studying rumen cellulolytic population as affected by level of roughage in swamp buffalo. **Current Microbiology**. 58: 294-299.

https://www.epa.gov/sites/production/files/2015-07/documents/emission-factors_2014.pdf (IPCC).

CHAPTER IV

EXPERIMENT II

THE EFFECT OF SUNFLOWER OIL AND NITRATE LEVELS ON RUMEN FERMENTATION, NITROGEN UTILIZATION, NUTRIENT DIGESTIBILITY AND RUMEN MICROORGANISMS IN MEAT GOATS FED WITH DIFFERENT ROUGHAGE

4.1 Abstract

Two experiments were conducted to evaluate the effect of sunflower oil and nitrate levels on rumen fermentation, nitrogen utilization, rumen microorganisms and nutrient digestibility in meat goats with difference quality roughage. Eight male ruminal fistulated, Thai native × Anglo-Nubian, goats were assigned in 2 × 2 factorial arrangements in a 4 × 4 Latin square design to receive 4 dietary treatments. Dietary treatments were two levels of nitrate and two levels of sunflower oil.

The results Exp. 2.1, showed that feed intake (g/day, g/$BW^{0.75}$/day, % g/BW/day), nutrient intake and nutrient digestibility and the total volatile fatty acids and the N-balance concentration and pH did not influence by in the interaction. The ratio of acetate : propionate ratio, methane production, and blood urea nitrogen were increased in goats fed supplement with nitrate alone. Besides, there was the highest effect on methane and NH_3-N concentration at preliminary feeding and 6 hours post

feeding. There was not effected on the number of protozoa, but there was effected on the number of bacteria. Moreover, there was significant reduced in *Prevotella byantii* and *Prevotella ruminicola* population by sunflower oil.

The result of Exp. 2.2, showed the nutrient digestibility, and N-balance did not effect by the interaction between sunflower oil and nitrate. But, sunflower oil increased N-balance. Moreover, there was significant increase in acetate and acetate: propionate could when increasing levels of nitrate. The populations microbial did not differ ($P>0.05$) by the interaction sunflower oil with nitrate.

Keywords : Sunflower oil, Nitrate, Rumen fermentation, Microorganisms and Digestibility

4.2 Introduction

In the rumen, methane (CH_4) is produced by methanogens that utilized primarily CO_2 and H_2. Methane expresses losses of up to 15% of gross energy intake. (Johnson and Johnson, 1995; Van Nevel and Demeyer, 1996). One strategy for reducing ruminal methane production is to provide alternative electron acceptors that more effectively consume reducing equivalents produced during fermentation so as to redirect electron flow away from the reduction of carbon dioxide to methane (Anderson and Rasmussen, 1998; Sar et al., 2004). The electron-accepting reactions alternative to methanogenesis includes fumarate and nitrate reduction pathways. Several studies have reported that nitrate administration decreased methanogenesis *in vivo* (Takahashi and Young, 1991; Sar et al., 2005; van Zijderveld et al., 2010; Hulshof et al., 2012). In the rumen, nitrate is converted to ammonia, which provides N for microbial protein synthesis. Additionally, the medium chain fatty acid inhibits

ruminal *methanogenesis* by as much as 89% *in vitro* (Dohme et al., 2001; Soliva et al., 2003) and by up to 76% *in vivo* (Machmuller et al., 2002), and the long chain fatty acid counteracts *in vitro* methane production by 97% (Ungerfeld et al., 2005). Medium and long chain fatty acids are beleived to inhibit the growth of Gram-positive and methanogenic bacteria via absorption and disruption of cell membranes (Galbraith and Miller, 1973; Soliva et al., 2003). Sunflower oil is a premising source of fat that can be fed as a supplement, which contains 12% saturated fatty acid and 88% unsaturated fatty acid (Grant and Kubik, 1990). It has been reported that methanogens are attached to the cell surface of protozoa and utilize H_2 produced by protozoa for *methanogenesis* (Hillman et al., 1988; Ushida and Jouany, 1996). The objectives of this study were to examine the effect of sunflower oil and nitrate levels on rumen fermentation, nitrogen utilization, rumen microorganisms and nutrient digestibility in meat goats fed with rice straw and pangola grass hay.

4.3 Materials and methods

4.3.1 Feed and animals

Experiment 4.1, eight male ruminal fistulated crossbred (Thai native × Anglo-Nubian) goats (approximately 28.8 ± 2.0 kg average body weights) were used as randomly assigned as 2 × 2 factorial arrangements in 4 × 4 Latin square design to receive four dietary treatments. Dietary treatment was used two level of sunflower oil and two level of potassium nitrate. The experimental treatments are follows as : Treatment1 : Sunflower oil at 3% with nitrate compound at 2% in concentrate; Treatment 2 : Sunflower oil at 3% with nitrate compound at 3% in concentrate; Treatment 3 : Sunflower oil at 6% with nitrate compound at 2% in concentrate; Treatment 4 : Sunflower oil at 6% with nitrate compound at 3% in concentrate. The

roughage for the goats in experiment 2.1 was raised by rice straw and pangola grass hay for experiment 2.2. The length each period of was 28 days, which the first 7 days used as an adjustment period to the experimental diets. During each period, animals were received concentrate at 1.5% of BW and were fed ad libitum of roughage. Additionally, all goats were housed individually in good ventilation and shed in individual feeding and watering arrangements. All goats were provided by clean drinking water at all time. They were dewormed at the beginning by Ivomectin® injection and intramuscular injected with vitamin AD_3E. The goats were weighed at the beginning and the end of each experimental period, individual daily DM intake was recorded. Experiment 2.2, the experimental procedure was the same as described for Exp. 2.1.

4.3.2 Chemical analysis

Each subsample was dried to determine DM content, then grounded to pass through a 1 mm 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 (1990). Neutral detergent fiber (NDF) and acid detergent fiber (ADF) determined by the methods described by Goering and van Soest (1970).

4.3.3 Feed sampling

Concentrates and roughages were sampled daily during the collection period and were composed by period prior to analysis. During the last seven days of each period, feed samples were collected every day and divided into two parts, the first part being analyzed for DM, while the second part kept and pooled at the end of each period for chemical analysis. Samples were dried at 60°C and ground (1 mm screen using Cyclotech Mill, Tecator, Sweden).

4.3.4 Fecal sampling

Fecal samples were collected and weighed during the last 7 days of each period. The fecal samples were collected about 5% of total fresh weight and divided into two parts, the first part being analyzed for DM, the second part kept for chemical analysis at the end of each period.

4.3.5 Urinary sampling

Total urine was collected on the same day with feces collected by using plastic container within a drop of concentrate sulfuric acid (10%) to avoid nitrogen losing. The urinary samples were collected about 10% of volume and kept in the refrigerator and pooled at the end of the period to analyzed for NH_3-N by the hypochlorite – phenol procedure (Beecher and Whitton, 1978) for determining nitrogen balance.

4.3.6 Blood sampling

A blood sample (about 10 ml) was collected at 0, 3, and 6 h post-feeding of each animal, and separated by centrifugation at 5000 × g for 10 minutes (Table Top Centrifuge PLC-02, U.S.A.) and stored at -20°C until analysis of blood urea nitrogen (BUN) according to the method of Crocker (1967).

4.3.7 Rumen fluid sampling

Rumen fluid samples were collected at 0, 3, and 6 h post feeding. Approximately 200 ml of rumen fluid were taken from the middle part of the rumen at each time at the end of each period. The samples were divided into 5 portions : The first portion was used for pH. Rumen fluids were immediately measured for pH by pH meter (HANNA instrument HI 8424 microcomputer, Singapore). Rumen fluid samples were then filtered through four layers of cheesecloth. The second portion was used for ammonia nitrogen (NH_3-N) analysis where 5 ml of H_2SO_4 solution (1M) for

preserving reagent and then was added to 50 ml of rumen fluid and then it was stored at -20°C for subsequent analyses of ruminal ammonia - N (Bremner and Keeney, 1965). The third portion was used for volatile fatty acid (VFAs) the mixture was centrifuged at 13,000 × g for 15 minutes and the supernatant was stored at -20 °C prior to volatile fatty acid (VFAs) analyses using gas chromatography (GC) analysis (Erwin, Marco, and Emery, 1961). With that, all samples were kept at -20°C until further analysis. The fourth portion was used for total count bacteria 1 ml of the samples were measured and truly with a pipette into the tubes containing 9 ml 10% formalin (v : v; 9 : 1) as a preserving reagent and then were closed tightly with screw caps for checking the counts of ruminal protozoa and bacteria counts using the hematocytometer according to Hungate (1966). The fifth portion of rumen fluid and digest were used for DNA extraction of real-time PCR.

4.3.8 Metabolism trial

The metabolism trial of 7 days collection was conducted for nutrient utilization in goats. 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. Body weight of the animals was recorded before and after the metabolism trials. Measurement data of feed offer and residue were obtained.

4.3.9 Rumen microbial population analysis

The detailed procedures of the RBB+C method were described in Table 1. Cell lysis was 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 were very active in the rumen and gastrointestinal samples (Yu and Morrison, 2004). After bead beating, most of the impurities and the SDS were removed by precipitation with ammonium

acetate, and then the nucleic acids were recovered by precipitation with isopropanol. Genomic DNA could then be purified via sequential digestions with RNase and proteinase K, followed by the use of QIAamp columns. The concentration of the plasmid was determined with Nano Drop 1000 (Thermo Scientific, German). Tenfold dilution series ranging from 1 to 109 copies were prepared for each target. The target DNA was quantified by using serial ten-fold dilutions from 103 to 109 DNA copies of the previously quantified DNA standards. Real-time PCR amplification and detection were performed in a Roche Light Cycler® Nano. All primer set sequences (forward and reverse primers) are shown in Table 1.

4.3.10 Statistical analysis

Data were statistically analyzed according to 2 × 2 factorial arrangements in 4 × 4 Latin square design using the PROC GLM procedure (SAS, 1996). The statically model included terms for sunflower oil level and nitrate of level of total diet. Significant differences ($P<0.05$) among treatments were determined using Duncan's News Multiple Range Test according to Steel and Torrie (1980).

4.3.11 Experimental site

The experiment was conducted on the farm of Suranaree University of Technology. The chemical analysis was performed at the Center for Scientific and Technological Equipment (CSTE) of Suranaree University of Technology.

4.3.12 Duration

The duration of the present experiment was from September, 2013 to May, 2014.

Table 4.1 Targeted primers used for real-time PCR.

Target		Sequence 5'-… - 3'	Tm	PCR product	References
General bacterial	Fw	CGGCAACGAGCGCAACCC*	60	130	Denman and McSweeney, 2006
	Rev	CCATTGTAGCACGTGTGTAGCC			
General anaerobic fungi	Fw	GAGGAAGTAAAAGTCGTAACAAGGTTTC	60	120	Denman and McSweeney, 2006
	Rev	CAAATTCACAAAGGGTAGGATGATT			
Total bacteria	Fw	CCTACGGGAGGCAGCAG	60	194	Mosoni et al., 2007
	Rev	ATTACCGCGGCTGCTGG			
F. succinogenes	Fw	GTTCGGAATTACTGGGCGTAAA	60	121	Denman and McSweeney, 2006
	Rev	CGCCTGCCCCTGAACTATC			
R. flavefaciens	Fw	CGAACGGAGATAATTTGAGTTTACTTAGG	60	132	Denman and McSweeney, 2006
	Rev	CGGTCTCTGTATGTTATGAGGTATTACC			
Ruminococcus albus	Ra1281f	CCC TAA AAG CAG TCT TAG TTCG	60	175	Koike and Kobayashi, 2001
	Ra1439r	CCTCCTTGCGGTTAGAACA			
Prevotella bryantii	Fw	AGTCGAGCGGTAAGATTG	68	540	Tajima et al., 2001
	Rev	CAAAGCGTTTCTCTCACT			
Prevotella ruminicola	Fw	GGTTATCTTGAGTGAGTT′	53	485	Tajima et al., 2001
	Rev	CTGATGGCAACTAAAGAA			
Selenomonas ruminantium	Fw	TGCTAATACCGAATGTTG	57	513	Tajima et al., 2001
	Rev	TCCTGCACTCAAGAAAGA			
Archaea, mcrA	Fw	TTC GGT GGA TCD CAR AGR GC	56°C	470	Denman et al., 2007
	Rev	GBA RGT CGW AWC CGT AGA ATC C			

4.4 Results

4.4.1 Composition of experimental diets

The ingredients and chemical compositions of concentrate and chemical composition, of roughage and concentrate has shown in Table 4.2.

Table 4.2 The ingredients and chemical composition of concentrate and roughage of experimental feed and diets.

Item	Concentrate	Rice straw	Pangola grass hay
Ingredient, % dry matter			
Cassava distillers dried meal	32.0	-	-
Soybean meal	20.0	-	-
Corn distillers dried grains	17.5	-	-
Rice bran	10.0	-	-
Wheat bran	10.0	-	-
Molasses	8.0	-	-
Mineral and vitamin mixture[1]	2.5	-	-
Chemical composition			
Dry matter, %	92.2	91.8	87.5
% of dry matter			
Ash	7.0	12.3	8.4
Crude protein	14.6	3.3	7.3
Ether extract	4.0	1.0	1.9
Crude fiber	17.1	39.7	32.0
Neutral detergent fiber	42.5	76.3	73.4
Acid detergent fiber	26.3	52.3	35.9
Acid detergent lignin	10.9	6.3	4.0
TDN (%)[2/]	60.2	46.1	50.9
NFC[3/]	31.7	6.9	8.8

[1/]Mineral and vitamin mix : provided per kg of concentrate including vitamin A, 5,000 IU; vitamin D3, 2,200 IU; vitamin E, 15 IU; Ca, 8.5 g; P, 6 g; K, 9.5 g; Mg, 2.4 g; Na, 2.1 g; Cl, 3.4 g; S, 3.2 g; Co, 0.16 mg; Cu, 100 mg; I, 1.3 mg; Mn, 64 mg; Zn, 64 mg; Fe, 64 mg; Se, 0.45 mg.

[2/]Total digestible nutrients, TDN = tdNFC + tdCP + (tdFA x 2.25) + tdNDF – 7 (NRC, 2001).

[3/]Non Fiber Carbohydrate, NFC = 100 - (CP – NDF – EE - ash).

4.4.2 Feed intake and nutrient intake and nutrient digestibility

The concentration, roughage, and total intakes of meat goats were showed that in Table 4.3, concentrate, roughage, and total dry matter intake express as g/day increased as increasing the level of sunflower oil and nitrate. There was no effect (P>0.05) on dry matter intake (g/day, %BW, g/kgBW$^{0.75}$) by the interaction between sunflower oil. In this experiment, it was found that level of sunflower oil increase nutrient intake (P<0.001) and there was the significant difference in apparent digestibility. There was did not effect by nitrate and the interaction between sunflower oil and nitrate.

Table 4.3 Effect of sunflower oil and nitrate on feed intake (Exp. 2.1).

Item	S3		S6		SEM	P-value		
	N2	N3	N2	N3		S	N	S × N
Concentrate DMI, g/day								
g/day	349.13	384.60	417.36	437.49	2.01	NS	NS	NS
%BW	1.50	1.50	1.50	1.50	0.01	NS	NS	NS
g/kgBW$^{0.75}$	34.89	35.89	36.00	36.37	1.53	NS	NS	NS

Table 4.3 Effect of sunflower oil and nitrate on feed intake (Exp. 2.1) (Continued).

Item	S3		S6		SEM	P-value		
	N2	N3	N2	N3		S	N	S × N
Roughage DMI, g/day								
g/day	443.09	486.15	524.90	551.83	1.41	***	***	NS
%BW	1.51	1.57	1.58	1.59	0.09	NS	NS	NS
g/kgBW$^{0.75}$	35.16	37.01	38.03	38.53	0.08	***	NS	NS
Total DMI, g/day								
g/day	792.22	870.75	942.28	989.33	52.88	***	***	NS
%BW	3.01	3.07	3.08	3.09	0.04	NS	NS	NS
g/kgBW$^{0.75}$	70.05	72.90	74.03	74.90	0.26	**	NS	NS

S = Sunflower oil; N = Nitrate; BW = Body weight, S = sunflower oil; N = Nitrate; S × N = interaction between sunflower oil and nitrate; SEM = standard error of the mean; *P<0.05; **P<0.01; ***P<0.001; NS = not significantly different (P>0.05).

Table 4.4 Effect of sunflower oil and nitrate on nutrient intake and nutrient digestibility (Exp. 2.1).

Item	S3		S6		SME	P-value		
	N2	N3	N2	N3		S	N	S × N
Nutrient intake, g/day								
OM	647.33	711.53	770.00	808.43	10.86	***	**	NS
CP	59.26	62.22	70.69	74.15	1.20	***	**	NS
EE	15.05	16.56	17.95	18.83	0.30	***	***	NS
NDF	443.24	486.89	526.52	553.04	6.77	***	***	NS
ADF	294.97	323.99	350.33	367.99	4.44	***	***	NS

Table 4.4 Effect of sunflower oil and nitrate on nutrient intake and nutrient digestibility (Exp. 2.1) (Continued).

	S3		S6			P-value		
Item	**N2**	**N3**	**N2**	**N3**	**SME**	**S**	**N**	**S × N**
Apparent digestibility , %								
DM	76.07	75.52	77.52	79.35	0.70	**	NS	NS
OM	77.27	77.02	78.38	81.00	0.78	*	NS	NS
CP	58.57	58.74	61.98	63.60	1.52	**	NS	NS
EE	74.04	72.17	6560	71.66	1.41	**	NS	NS
NDF	65.23	63.80	67.29	70.38	1.07	**	NS	NS
ADF	59.60	58.55	60.87	65.50	1.399	*	NS	NS

DM = Dry matter; OM = Organic matter; CP = Crude protein; EE = Ether extract; NDF = Neutral detergent fiber; ADF = Acid detergent fiber; S = Sunflower oil; N = Nitrate; S × N = interaction between Sunflower oil and Nitrate; SEM = standard error of the mean; *P<0.05; **P<0.01; ***P<0.001; NS = not significantly different (P>0.05).

4.4.3 Rumen fermentation parameter

The effect of sunflower oil and nitrate levels on total volatile fatty acid (TVFA), the concentration of volatile fatty acids and acetate : propionate ratio have been shown in Table 4.5. The interaction between sunflower oil and nitrate did not effect on total volatile fatty acid (TVFA), a concentration of volatile fatty acids and acetate : propionate ratio. However, there was significant difference on total VFA, increasing concentration of TVFA can be in an increase level of sunflower oil increased. Moreover, acetic acids and acetate : propionate ratio were increased and tend reduce propionate when increased level of nitrate in diets. Methane production

calculate from concentration VFA did not affect with sunflower oil and an interaction between sunflower oil and nitrate, but there was the significant difference by added nitrate alone. Ammonia nitrogen did not effect, except, there was an interaction between sunflower oil and nitrate at 3h after morning feeding. There was significant difference in the rumen pH by sunflower oil added in diets. The blood urea nitrogen did not affect in sunflower oil and the interaction with between sunflower oil and nitrate then there was significant in blood urea nitrogen at 0h and 6h after morning feeding tends to decrease with 3% of sunflower oil and an increased with 6% of sunflower oil in diets.

Table 4.5 Effect of sunflower oil and nitrate on total volatile fatty acid (TVFA) and acetate : propionate ratio (Exp. 2.1).

Items	S3		S6		SEM	P-value		
	N2	N3	N2	N3		S	N	S × N
Total volatile fatty acids (mM/L)								
0 h	92.97	90.02	96.33	95.74	0.86	**	NS	NS
3 h	81.83	78.73	84.76	83.98	0.88	*	NS	NS
6 h	86.56	84.33	88.57	89.00	0.88	NS	NS	NS
Volatile fatty acids proportion (mol/ 100 mol)								
Acetate (C2)								
0 h	66.89	71.18	67.13	70.21	0.70	NS	*	NS
3 h	64.84	69.94	65.54	69.18	0.83	NS	**	NS
6 h	68.09	72.17	69.31	71.84	0.73	NS	*	NS
Propionate (C3)								
0 h	26.64	20.38	25.24	25.53	0.74	NS	**	NS
3 h	28.41	21.31	26.89	25.00	0.85	NS	**	NS
6 h	25.77	18.85	24.69	22.56	0.82	NS	**	NS

Table 4.5 Effect of sunflower oil and nitrate on total volatile fatty acid (TVFA) and acetate : propionate ratio (Exp. 2.1) (Continued).

Items	S3		S6		SEM	P-value		
	N2	N3	N2	N3		S	N	S × N
Volatile fatty acids proportion (mol/ 100 mol)								
Butyrate (C4)								
0 h	6.47	8.44	7.63	4.26	0.43	NS	NS	*
3 h	6.75	8.75	7.57	5.82	0.49	NS	NS	NS
6 h	6.14	8.98	6.00	5.60	0.50	NS	NS	NS
Acetate : Propionate ratio (C2 : C3)								
0 h	2.57	3.72	2.65	2.75	0.14	NS	*	NS
3 h	2.34	3.54	2.43	2.76	0.15	NS	**	NS
6 h	2.72	4.19	2.80	3.18	0.19	NS	**	NS

S= Sunflower oil; N = Nitrate; S × N = interaction between sunflower oil and nitrate; SEM = standard error of the mean; *P<0.05; **P<0.01; ***P<0.001; NS = not significantly different (P>0.05).

Table 4.6 Effect of sunflower oil and nitrate on methane production, ammonia nitrogen, pH and blood urea nitrogen (Exp. 2.1).

Items	S3		S6		SEM	P-value		
	N2	N3	N2	N3		S	N	S × N
Methane (CH_4, mol/100 mol)[1/]								
0 h	25.15	27.77	26.65	28.29	0.48	NS	**	NS
3 h	20.95	23.57	22.43	24.06	0.48	NS	*	NS
6 h	24.09	26.75	25.52	27.22	0.49	NS	*	NS
Ammonia-N (NH_3-N, mg%)								
0 h	11.02	10.61	10.99	13.56	0.658	NS	NS	NS
3 h	19.42	18.86	18.71	19.02	0.208	NS	NS	NS
6 h	12.99	9.84	9.81	13.86	0.628	NS	NS	***

Table 4.6 Effect of sunflower oil and nitrate on methane production, ammonia nitrogen, pH and blood urea nitrogen (Exp. 2.1) (Continued).

Items	S3		S6		SEM	P-value		
	N2	N3	N2	N3		S	N	S × N
pH								
0 h	7.54	7.53	7.42	7.37	0.02	**	NS	NS
3 h	7.21	7.22	7.42	7.37	0.03	**	NS	NS
6 h	6.76	6.70	6.54	6.51	0.06	**	NS	NS
Blood urea nitrogen (BUN, mg%)								
0 h	16.43	18.18	17.00	18.05	0.48	NS	*	NS
3 h	26.85	28.03	28.04	27.83	0.57	NS	NS	NS
6 h	23.24	26.51	23.90	26.82	0.53	NS	**	NS

S = Sunflower oil; N = Nitrate; S × N = interaction between Sunflower oil and Nitrate; SEM = standard error of the mean; *P<0.05; **P<0.01; ***P<0.001; NS = not significantly different (P>0.05). [1/]CH_4 = (0.45 × acetate) − (0.275 × propionate) + (0.4 × butyrate) according to Moss et al. (2000).

Table 4.7 Effect of sunflower oil and nitrate on N-balance (Exp. 2.1).

Items	S3		S6		SEM	P-value		
	N2	N3	N2	N3		S	N	S × N
N intake (g)	10.53	11.59	12.56	13.18	0.22	***	**	NS
N excretion (g)								
Fecal	4.35	4.76	4.73	4.77	0.17	NS	NS	NS
Urine	2.44	2.99	3.27	3.35	0.51	NS	NS	NS
N absorption (g)	6.18	6.83	7.84	8.40	0.29	***	NS	NS
N retention (g)	5.99	6.28	7.06	6.93	0.19	***	NS	NS
N absorption (%)	58.57	58.74	61.98	63.60	1.53	NS	NS	NS
N retention (%)	32.26	35.54	36.77	38.84	3.81	NS	NS	NS

S = Sunflower oil; N = Nitrate; S × N = interaction between sunflower oil and nitrate; SEM = standard error of the mean; *P<0.05; **P<0.01; ***P<0.001; NS = not significantly different (P>0.05).

4.4.4 Nitrogen balance

There was no effect of experimental treatments on N intake in this experiment in term of the interaction. However, there a significant difference on N excretion, N absorption and N retention (P<0.01) after feeding. Also, it showed that N intake was increased when increased sunflower oil or potassium nitrate levels (P<0.01).

Table 4.8 Effect of level of sunflower oil and nitrate on the population of microbial in rumen fluid of direct count methods (Exp. 2.1).

Items	S3		S6		SEM	P-value		
	N2	N3	N2	N3		S	N	S × N
Protozoa count (10^6 cells/ml)								
0 h	2.16	2.19	2.08	2.20	0.01	NS	NS	NS
3 h	2.44	2.13	2.42	2.10	0.07	**	**	NS
6 h	2.19	2.20	2.16	2.21	0.04	NS	NS	NS
mean	2.29	2.18	2.25	2.20	0.03	NS	*	NS
Total Bacteria count (10^{11} cells/ml)								
0 h	5.56	5.50	5.87	7.06	0.29	NS	NS	NS
3 h	5.18	2.93	4.93	2.62	1.01	**	NS	**
6 h	2.12	1.31	2.68	1.06	0.30	**	NS	*
mean	4.31	3.25	4.50	3.58	0.37	*	NS	**

S = Sunflower oil; N = Nitrate; S × N = interaction between sunflower oil and nitrate; SEM = standard error of the mean; *P<0.05; **P<0.01; ***P<0.001; NS = not significantly different (P>0.05).

4.4.5 Ruminal microbe population

The effect of level of sunflower oil and nitrate levels on the population of microbial in rumen have been showed Table 4.9 and Table 4.10. The interaction between sunflower oil and nitrate did not effect on protozoa in rumen fluid of direct count methods, there was affected by bacteria and rumen microbial population using real-time PCR.

Table 4.9 Effect of sunflower oil and nitrate on population of rumen microbial population using real-time PCR (Exp. 2.1).

Items	S3		S6		SEM	P-value		
	N2	N3	N2	N3		S	N	S × N
Quantity real-time PCR, copies/ml of rumen content								
Total Protozoa, $\times 10^{6}$	6.93	7.22	6.14	7.13	0.51	NS	NS	NS
Total bacteria, $\times 10^{11}$	2.60	2.52	2.75	2.73	0.07	NS	NS	NS
Total Fungi, $\times 10^{8}$	5.57	5.83	5.76	4.83	0.44	NS	NS	NS
F. succinogenes, $\times 10^{8}$	5.86	7.19	5.84	6.12	0.09	NS	NS	NS
R. flavefaciens, $\times 10^{9}$	2.54	2.34	3.01	2.78	0.14	NS	NS	NS
R. albus, $\times 10^{8}$	2.33	2.02	2.42	2.07	0.07	NS	NS	NS
P. bryantii, $\times 10^{4}$	9.26	8.98	7.58	6.91	0.28	**	NS	NS
P. ruminicola, $\times 10^{7}$	9.38	9.11	7.80	7.17	0.25	**	NS	NS
S. ruminantium, $\times 10^{4}$	3.44	4.57	3.41	3.86	0.11	NS	NS	NS
Archae mcrA, $\times 10^{3}$	2.36	1.80	2.37	1.98	0.08	NS	NS	NS

S = Sunflower oil; N = Nitrate; S × N = interaction between Sunflower oil and Nitrate; SEM = standard error of the mean; *P<0.05; **P<0.01; ***P<0.001; NS = not significantly different (P>0.05).

Experiment 2

4.4.6 Feed intake and nutrient intake and nutrient digestibility

The intake (g/day, g/BW$^{0.75}$/day, % g/BW/day) of concentrate and roughage and the total intake were high for the goats raised with 3% nitrate of 6% of sunflower oil, and then there were significant difference when increase sunflower oil of level in experiment diets (P<0.01 for g/day and P<0.05 for g/BW$^{0.75}$/day and % g/BW/day).

Table 4.10 Effect of sunflower oil and nitrate on feed intake (Exp. 2.2).

Items	S3		S6		SEM	P-value		
	N2	N3	N2	N3		S	N	S × N
Concentrate DMI								
g/day	387.66	417.75	444.49	457.86	5.57	***	**	NS
%BW	1.15	1.15	1.16	1.15	0.01	NS	NS	NS
g/kg BW$^{0.75}$	27.64	28.17	28.74	28.74	28.85	***	NS	NS
Roughage DMI								
g/day	557.94	603.45	662.45	690.76	0.17	***	NS	NS
%BW	1.65	1.66	1.72	1.73	0.02	**	NS	NS
g/kg BW$^{0.75}$	39.84	40.69	42.84	43.52	0.44	***	NS	NS
Total DMI								
g/day	945.61	1021.20	1106.95	1148.62	13.06	***	NS	NS
%BW	2.80	2.80	2.87	2.88	0.02	NS	NS	NS
g/kg BW$^{0.75}$	67.48	68.87	71.57	72.37	0.49	***	NS	NS

S = Sunflower oil; N = Nitrate; S × N = interaction between Sunflower oil and Nitrate; SEM = standard error of the mean; *P<0.05; **P<0.01; ***P<0.001; NS = not significantly different (P>0.05).

There was the effect on concentrate intake on increased when nitrate (P<0.01 for g/day and P<0.05 g/BW$^{0.75}$/day and % g/BW/day). However, there were

not found the effect in the interaction between sunflower oil and nitrate. There was the significant difference (P<0.001) on nutrient intake, but, there was did not affected by the interaction between sunflower oil and nitrate (P>0.05). There was a significant difference on DM and ADF intakes by nitrate could be decreased when increased levels of nitrate in the diet.

Table 4.11 Effect of sunflower oil and nitrate on nutrient intake and digestibility (Exp. 2.2).

Items	S3		S6		SEM	P-value		
	N2	N3	N2	N3		S	N	S × N
Nutrient intake, g/day								
OM	771.88	833.56	903.32	937.23	10.648	***	***	NS
CP	88.06	95.04	102.49	106.138	1.194	***	***	NS
EE	21.71	23.43	25.28	26.19	0.932	***	***	NS
NDF	523.51	565.62	615.56	639.72	7.444	***	***	NS
ADF	275.18	297.26	323.01	335.48	3.864	***	***	NS
Appearance digestibility, %								
DM	75.70	74.58	75.91	74.87	0.304	NS	**	NS
OM	78.02	77.39	76.76	77.94	0.394	NS	NS	NS
CP	66.50	66.14	66.81	65.48	0.700	NS	NS	NS
EE	76.13	75.47	66.20	64.89	0.390	NS	NS	NS
NDF	64.75	62.03	64.54	64.34	0.626	NS	NS	NS
ADF	45.55	42.58	43.86	41.76	0.529	NS	***	NS

DM = Dry matter; OM = Organic matter; CP = Crude protein; EE = Ether extract; NDF = Neutral detergent fiber; ADF = Acid detergent fiber; S = Sunflower oil; N = Nitrate; S × N = interaction between Sunflower oil and Nitrate; SEM = standard error of the mean; *P<0.05; **P<0.01; ***P<0.001; NS = not significantly different (P>0.05).

4.4.7 Rumen fermentation parameters

The effect of sunflower oil level and nitrate levels on total volatile fatty acid (TVFA), the concentration of VFA and acetate : propionate ratio have been shown in the Table 4.12. The interaction between sunflower oil and potassium nitrate did not effect on TVFA, concentration of volatile fatty acids and acetate : propionate ratio. Except for there was the significant difference (P<0.05) on butyrate at 0h and 3 h after morning feeding. However, there was the significant difference on total VFA could increase concentration in an increased level of sunflower oil at 0h and 3 h after morning feeding. The concentrate of acetate, butyrate and acetate : propionate did not affect by sunflower oil. The nitrate had affected to the concentration of acetate and acetate : propionate increased and the concentration of propionate decreased when increased level of nitrate. Ammonia nitrogen did not effect in the interaction between sunflower oil and nitrate. However, there significant different by sunflower oil and nitrate alone at 6h after morning tend to decreased when increased level of nitrate. There were a significant difference in the rumen pH by sunflower oil added in diets. The blood urea nitrogen did not affect with all of the treatment diets.

Table 4.12 Effect of sunflower oil and nitrate on total volatile fatty acid (TVFA) and acetate : propionate ratio (Exp. 2.2).

Items	S3		S6		SEM	P-value		
	N2	N3	N2	N3		S	N	S × N
Total volatile fatty acids (mM/L)								
0 h post feeding	92.90	90.23	95.75	95.41	0.89	*	NS	NS
3 h post feeding	96.19	93.75	99.01	97.91	0.85	*	NS	NS
6 h post feeding	86.56	84.33	88.57	89.00	0.87	NS	NS	NS

Table 4.12 Effect of sunflower oil and nitrate on total volatile fatty acid (TVFA) and acetate : propionate ratio (Exp. 2.2) (Continued).

Items	S3		S6		SEM	P-value		
	N2	N3	N2	N3		S	N	S × N
Volatile fatty acids proportion (mole/100 mole)								
Acetate (C2)								
0 h post feeding	60.42	63.93	61.14	63.75	0.60	NS	*	NS
3 h post feeding	61.23	64.91	62.09	65.03	0.71	NS	*	NS
6 h post feeding	68.09	72.17	69.31	71.84	0.71	NS	*	NS
Propionate (C3)								
0 h post feeding	33.04	27.52	31.71	30.13	0.66	NS	**	NS
3 h post feeding	32.80	26.79	31.40	29.92	0.73	NS	**	NS
6 h post feeding	25.77	21.87	24.69	22.56	0.71	NS	**	NS
Butyrate (C4)								
0 h post feeding	6.54	8.55	7.15	6.12	0.34	NS	NS	*
3 h post feeding	5.97	8.28	6.51	5.05	0.40	NS	NS	*
6 h post feeding	6.14	5.96	6.00	5.60	0.49	NS	NS	NS
Acetrate : Propionate ratio (C2 : C3)								
0 h post feeding	1.85	2.38	1.95	2.12	0.07	NS	**	NS
3 h post feeding	1.89	2.49	2.00	2.19	0.07	NS	**	NS
6 h post feeding	2.72	3.29	2.88	3.22	0.08	NS	**	NS

S = Sunflower oil; N = Nitrate; S × N = interaction between sunflower oil and nitrate; SEM = standard error of the mean; *P<0.05; **P<0.01; ***P<0.001; NS = not significantly different (P>0.05).

Table 4.13 Effect of sunflower oil and nitrate on methane production, ammonia nitrogen, pH and blood urea nitrogen (Exp. 2.2).

Items	S3		S6		SEM	P-value		
	N2	N3	N2	N3		S	N	S × N
CH_4 (mol/100 mol)								
0 h post feeding	22.88	25.52	24.35	26.01	0.49	NS	*	NS
3 h post feeding	21.80	24.45	23.28	24.90	0.48	NS	*	NS
6 h post feeding	24.09	26.75	25.52	27.22	0.48	NS	*	NS
Ammonia-N (NH_3-N; mg %)								
0 h post feeding	12.68	7.94	16.99	11.71	0.82	***	***	NS
3 h post feeding	16.43	17.35	18.30	17.70	0.52	NS	NS	NS
6 h post feeding	14.18	13.50	14.81	12.88	0.56	NS	*	NS
pH								
0 h post feeding	6.41	7.42	7.66	6.59	0.03	*	NS	NS
3 h post feeding	6.20	7.20	7.40	7.40	0.03	*	NS	NS
6 h post feeding	6.67	6.68	6.87	6.79	0.07	*	NS	NS
Blood urea nitrogen (BUN; mg %)								
0 h post feeding	3.30	3.35	3.29	3.32	0.06	NS	NS	NS
3 h post feeding	5.39	5.66	5.41	6.14	0.36	NS	NS	NS
6 h post feeding	5.03	5.66	5.59	5.54	0.06	NS	NS	NS

S = Sunflower oil; N = Nitrate; S × N= interaction between sunflower oil and nitrate; SEM = standard error of the mean; *P<0.05; **P<0.01; ***P<0.001; NS = not significantly different (P>0.05); CH_4 = (0.45 × acetate) − (0.275 × propionate) + (0.4 × butyrate) according to Moss et al. (2000).

Table 4.14 Effect of sunflower oil and nitrate on N- balance (Exp. 2.2).

Items	S3		S6		SEM	P-value		
	N2	N3	N2	N3		S	N	S × N
N intake (g)	15.62	16.86	18.17	18.82	0.221	***	***	NS
N excretion (g)								
Fecal	5.38	5.72	6.05	6.50	0.151	***	NS	NS
Urine	4.24	4.86	5.07	5.40	0.173	**	NS	NS
N absorption (g)	10.24	11.14	12.13	12.32	0.150	***	**	NS
N retention (g)	5.99	6.28	7.06	6.93	0.194	***	NS	NS
N absorption (%)	66.50	66.14	66.81	65.48	0.645	NS	NS	NS
N retention (%)	38.42	37.20	38.89	36.74	1.031	NS	NS	NS

S = Sunflower oil; N = Nitrate; S × N = interaction between sunflower oil and nitrate; SEM = standard error of the mean; *P<0.05; **P<0.01; ***P<0.001; NS = not significantly different (P>0.05).

4.4.8 Nitrogen balance.

Effect of sunflower oil and nitrate levels on daily nitrogen balance have significantly (P<0.01) affect on total nitrogen intake and excretion and nitrogen absorption and nitrogen retention, except urinary nitrogen excretion significant difference (P>0.05) when increased level of sunflower oil. The nitrogen balance did not affect by the interaction between sunflower oil and nitrate.

Table 4.15 Effect of sunflower oil and nitrate levels on population of microbial in rumen fluid of direct count methods (Exp. 2.2).

Items	S3		S6		SEM	P-value		
	N2	N3	N2	N3		S	N	S × N
Protozoa count (10^6 cells/ml)								
0 h post feeding	2.41	2.34	2.37	2.34	0.01	NS	NS	NS
3 h post feeding	2.59	2.29	2.57	2.28	0.06	NS	**	NS
6 h post feeding	2.42	2.34	2.41	2.35	0.04	NS	NS	NS
Mean	2.47	2.32	2.45	2.32	0.04	NS	**	NS
Bacteria count (10^{12} cells/ml)								
0 h post feeding	4.93	4.43	3.31	4.62	0.71	NS	NS	NS
3 h post feeding	6.68	4.43	2.43	4.12	1.01	**	NS	**
6 h post feeding	3.62	2.81	1.93	2.56	0.38	**	NS	**
Mean	5.08	3.88	2.55	3.78	0.67	**	NS	**

S = Sunflower oil; N = Nitrate; S × N = interaction between sunflower oil and nitrate; SEM = standard error of the mean; *P<0.05; **P<0.01; ***P<0.001; NS = not significantly different (P>0.05).

4.4.9 Ruminal microbial population

The number of protozoa in rumen fluid of direct count methods did not affect by sunflower oil and the interaction but there was significant difference by nitrate, it tends to decrease when increased level of nitrate. However, there was on effect on a number of protozoa when added nitrate alone diets. In addition, there was affected on bacteria by sunflower oil and the interaction and rumen microbial population using real-time PCR was influenced.

Table 4.16 Effect of sunflower oil and nitrate levels on population of rumen microbial population using real-time PCR (Exp. 2.2).

Items	S3		S6		SEM	P-value		
	N2	N3	N2	N3		S	N	S × N
Quantity real-time PCR, copies/ml of rumen content								
Total Protozoa, $\times 10^6$	4.34	4.19	4.37	4.60	0.08	NS	NS	NS
Total bacteria, $\times 10^{11}$	2.15	2.04	2.18	2.35	0.05	NS	NS	NS
Total Fungi, $\times 10^8$	3.16	3.03	3.19	3.41	0.07	NS	NS	NS
F. succinogenes, $\times 10^8$	5.80	6.04	5.86	5.98	0.04	NS	NS	NS
R. flavefaciens, $\times 10^9$	3.67	2.98	3.52	3.16	0.11	NS	NS	NS
R. albus, $\times 10^8$	3.55	2.13	2.47	2.07	0.19	NS	NS	NS
Prevotella bryantii, $\times 10^4$	9.23	8.77	7.23	6.46	0.35	**	NS	NS
Prevotella ruminicola, $\times 10^7$	9.34	8.91	7.47	6.47	0.31	**	NS	NS
S. ruminantium, $\times 10$	3.33	3.77	3.45	3.63	0.12	NS	NS	NS
Archae mcrA, $\times 10^3$	1.47	1.46	0.86	1.44	0.03	NS	NS	NS

S = Sunflower oil; N = Nitrate; S × N = interaction between sunflower oil and nitrate; SEM = standard error of the mean; *P<0.05; **P<0.01; ***P<0.001; NS = not significantly different (P>0.05).

4.5 Discussion

4.5.1 Nutrient intake and nutrient digestibility

Both study there was an interaction between sunflower oil and nitrate on nutrient intake and nutrient digestibility, there was agreed with Van Zijderveld et al. (2010) who reported that fed 26 g nitrate/kg DM in sheep found no difference. Pal et

al. (2015) reported that, nitrate did not reduce feed intake, which was low for exhibiting any adverse effect on intake. Also, similar with Van Zijderveld et al. (2010) reported nitrate on effect on apparent digestibility of NDF, starch, and crude fat. But, there was effect on DMI and nutrient digestibility in sunflower oil alone there were low quality roughage (Exp. 2.1) tendency to less than high quality roughage (Exp. 2.2) because low quality slowly degradable. Mewara et al. (2008) reported that digestibility of DM, CP and fiber components were unaffected and that of EE was increased by addition of sunflower oil to a concentrate mixture.

4.5.2 Rumen fermentation

The results showed that sunflower oil had effect on total VFA, pH and DMI. According to Jenkins and McGuire (2006), the main effects of the addition of lipids on intake reduction are related to modifications in rumen fermentation. Which, in this experiment rumen VFA concentration was significant difference on acetic acid when increased sunflower oil can increased concentration. However, there found on effect on ammonia nitrogen, pH, N- balance and total VFA concentration did not effect and the tendency decreased propionic. Similar results Farra and Satter (1971) who found decreased concentration of total VFA fed a nitrate in diet to dairy cow. Contrast with Nolan et al. (2010) who reported that a greater total VFA concentration in animal compared an isonitrogenous amount of urea. The proportion of butyrate decreased, possibly due to the electron utilized when nitrate was reduced, that upon supplying nitrate (Alaboudi and Jones, 1985; Sar et al., 2005). This contributory acetyl- CoA to shift from butyrate synthesis to acetate formation (Alaboudi and Jones, 1985). In this experiment showed that the interaction between sunflower oil and nitrate to reduce a number of bacteria. Marais et al. (1988) observed a reduction

in total bacteria count with nitrate, the toxic effect of the intermediate nitrate may have affected ruminal fermentation. Overall, current data suggest that inclusion of sunflower oil alone in the diet has no negative effect on acetate, propionate, methane and blood urea nitrogen when compare with nitrate alone (Kucuk et al., 2004; Shingfield et al., 2010). Czerkawski et al. (1975) Oil supplements tend to replace acetate:propionate ratios and methane production in the rumen.

4.5.3 Methane and rumen microbial

In this experiment, the interaction did not effect on methane production and protozoal population. The population of bacteria decreased related with to endo-symbiosis living between bacteria. It has been shown that sunflower added in the feed has an inhibitory effect on methane production in the rumen (Beauchemin et al., 2009; Macmuller et al., 2000). Dohme et al. (2001) showed a methane reduction *in vitro* upon incubation of C18 : 2 with rumen fluid of some 25%, which is consistent with a reduction in methane production of 27% *in vitro* in lambs given sunflower bean (Beauchemin et al., 2009).

4.6 Conclusion

The present experiment was aimed to study the effects of sunflower oil and nitrate levels on rumen fermentation, nitrogen utilization, rumen microorganisms and nutrient digestibility in meat goats of fed on rice straw and panggola grass hay did not effect on feed intake, nutrient digestibility, N-balance, number of protozoa and pupation of ruminal microbial. There was significant on methane production by the interaction between sunflower oil and nitrate decreased when received rice straw, but did not significant by pangola grass hay. The sunflower oil and the interaction

between sunflower oil could be decreased number of bacteria when received both of roughage source.

4.7 References

Alaboudi A., and Jones, G. (1985). Effect of acclimation to high nitrate intakes on some rumen fermentation parameters in sheep. **Canadian Journal of Animal Science**. 65: 841-849.

Anderson, R.C., and Rasmussen, M.A. (1998). Use of a novel nitrotoxinmetabolizing bacterium to reduce ruminal methane production. **Bioresource Technology**. 64: 89-95.

AOAC. (1990). **Official Methods of Analysis.15th edn. Association of Official Analytical Chemists**. Washington, DC. USA.

Beauchemin, K.A., McGinn, S.M., Benchaar, C., and Holtshausen, L. (2009). Crushed sunflower, flax, or canola seeds in lactating dairy cow diets: effects on methane production, rumen fermentation, and milk production. **Journal of Dairy Science**. 92(5): 2118-2127.

Bromner, J.M., and Keeney, D.R. (1965) Steam distillation methods of determination of ammonium, nitrate and nitrite. **Analytica Chimica Acta**. 32: 485.

Czerkawski J.W., Christie W.W., Breckenridge G., and Hunter L. (1975): Changes in the rumen metabolism of sheep given increasing amounts of linseed oil in their diet. **British Journal of Nutrition**. 34: 25-44.

Crocker, C.L. (1967). Rapid determination of urea nitrogen in serum or plasma without deprotenization. **American Journal of Medical Technology**. 33: 361.

Czerkawski, J.W., Christie, W.W., Breckenridge, G., and Hunter, L. (1975). Changes in the rumen metabolism of sheep given increasing amounts of linseed oil in their diet. **British Journal of Nutrition**. 34: 25-44.

Denman, S. E., Tomkins, N., and McSweeney, C. S. (2006). Monitoring the effect of bromochloromethane on methanogen populations within the rumen using qPCR. **The 2nd International Conference on Greenhouse Gases and Animal Agriculture GGAA2005 Working papers**. pp. 112-114.

Domh, F., Machmuller, A., Wasserfallen, A., and Kreuzer, M. (2001). Ruminal *methanogenasis* as influence by individual fatty acids supplemented to complete ruminant diet. **Letters in Applied Microbiology**. 32: 47-51.

Farra, P.A., and Satter, L.D. (1971). Manipulation of the ruminal fermentation. III. Effect of nitrate on ruminal volatile fatty acid production and milk composition. **Journal of Dairy Science**. 54: 1018-1024.

Galbraith, H., and Miller, T.B. (1973). Physicochemical effects of long chain fatty acids on bacterial cells and their protoplasts. **Journal of applied bacteriology**. 36: 647.

Grant, R., and Kubik, D. (1990). **Supplemental fat for high producing dairy cows. Institute of Agriculture and Natural Resources**, University of Nebraska-Lincoln. USA.

Hillman, K., Lloyd, D., Williams, A.G. (1988) Interactions between the methanogens *Methanosarcina barkeri*and the rumen holotrich ciliate protozoa. **Letters in Applied Microbiology**. 7: 49-53.

Hulshof, R.B.A., Berndt, A., Gerrits, W.J.J., Dijkstra, J., Van Zijderveld, S.M., Newbold, J.R., and Perdok, H.B. (2012). Dietary nitrate supplementation

reduces methane emission in beef cattle fed sugarcane based diets. **Journal of Animal Science**. 90: 2317-2323.

Hungate, R.E. (1966). **The rumen and its microbes**. 272-273. Academic Press Inc., New York.

Johnson, K.A., and Johnson, D. E. (1995). Methane emissions from cattle. **Journal of Animal Science**. 73: 2483-2492.

Kobayashi, Y., M. Yamada and Yamamoto, M. (2001). Survival of a recombinant rumen bacterium in the rumen of sheep. **Journal of Animal Science**. 72: 344-346.

Jenkins, T.C., and McGuire M.A. (2006). Major advances in nutrition: impact on milk composition. **Journal of Dairy Science**. 89: 1302-1310.

Kucuk, O., Hess, B.K., and Rule, D.C. (2004). Soybean oil supplementation of high concentrate diet dose not affect site and extent of organic matter, starch, neutral detergentergent fiber or nitrogen digestion but influences both ruminal metabolism and intestinal flow of fatty acids in limit-fed lambs. **Journal of Animal Science.** 82: 2985-29.

Marais, J. P., Therion, J. J., Mackie R. I., Kistner, A., and Dennison, C. (1988). Effect of nitrate and its reduction products on the growth and activity of the rumen microbial population. **British Journal of Nutrition**. 59: 301-313.

Mewar koushal, K., Anil kumar., Raman Rao, P. V., and Tiwari, D. P. (2008). Effect of soybean oil supplementation on nutrient utilization, milk yield and its quality in lactating crossbred cows. **Indian journal of animal sciences**. 78: 751-757.

Machmu¨ller, A., Soliva, C.R., Razminowicz, R., Duleba, S.J., and Kreuzer, M. (2002). Evaluating the dietary pre-conditions for a significant methane-suppressing effect of myristic acid in ruminants. **In Greenhouse Gases and Animal Agriculture.** pp. 155-159

Nolan, J.V., Hegarty, J.,Godwin, I.R., and Woodgate, R. (2010). Effects of dietary nitrate on fermentation, methane production and digesta kinetics in sheep. **Livestock production in a changing environment**. 50: 5-6.

Pal, K., Patra, A. K., Sahoo, A., and Soren, N. M. (2015). Effects of nitrate and fumarate in tree leaves-based diets on nutrient utilization, rumen fermentation, microbial protein supply and blood profiles in sheep. **Livestock Science**. 172: 5-15.

SAS, (1996). **User's Guide: Statistic**, Version 5. Edition. SAS. Inst, Cary, NC., U.S.A.

Sar, C., Santoso, B., Mwenya, B., Gamo, Y., Kobayashi, T., Morikawa, R., Kimura, K., Mizukoshi, H., and Takahashi, J. (2004). Manipulation of rumen *methanogenesis* by the combination of nitrate with β 1-4 galacto-oligosaccharides or nisin in sheep. **Animal Feed Science and Technology**. 115: 129-142.

Sar, C., Mwenya, B., Santoso, B., Takaura, K., Morikawa, R., Isogai, N., Asakura, Y., Toride, Y., and Takahashi, J. (2005). Effect of Escherichia coli wild type or its derivative with high nitrite reductase activity on *in vitro* ruminal *methanogenesis* and nitrate/nitrite reduction. **Journal of Animal Science**. 83: 644-652.

Steel, R.G.D., and Torrie, J.H. (1980). **Principles and Procedures of Statistics: A**

Biometrical Approach, 2nd Ed. McGraw-Hill Inc, New York.

Soliva, M., and Felipó, M.T. (2003). Organic wastes as a resource for Mediterranean soils. **In: Langenkamp, H., Marmo, M. (eds). Biological Treatment of Biodegradable Wastes. Technical Aspects. Workshop in Brussels (B)**. 249-272.

Shingfield, K.J., Lee, M.R.F., Humphries, D.J., Scollan, N.D., Toivonen, V., Beever, D. E., and Reynolds, C.K. (2011). Effect of linseed oil and fish oil alone or as an equal mixture on ruminal fatty acid metabolism in growing steers fed maize silage-based diets**. Journal of Animal Science.** 89: 3728-374.

Takahashi, J., and Young, B.A. (1991). Prophylactic effect of L-cysteine on nitrate-induced alteration in respiratory exchange and metabolic rate in sheep. **Animal Feed Science and Technology.** 35: 105-113.

Tajima, K., Aminov, R. I., Nagamine, T., Matsui, H., Nakamura, M. and Benno, Y. (2001). Diet-dependent shifts in the bacterial population of the rumen revealed with real-time PCR. Applied and Environmental Microbiology. 67:2766-2774.

Ushida, K. and J. P. Jouany. (1996). Methane production associated with rumen-ciliated protozoa and its effect on protozoan activity. **Letters in Applied Microbiology**. 23:129-132.

Ungerfeld, E.M., Rust, S.R., Burnett, R.J., Yokoyama, M.T., and Wang, J.K. (2005). Effects of two lipids on *in vitro* ruminal methane production. **Animal Feed Science and Technology**. 119: 179-185.

Van Zijderveld, S.M., Gerrits, W.J.J., Apajalahti, J.A., Newbold, J.R., Dijkstra, J., Leng, R.A., and Perdok, H.B. (2010). Nitrate and sulfate: Effective alternative

hydrogen sinks for mitigation of ruminal methane production in sheep. **Journal of Dairy Science**. 93: 5856-5866.

Van Nevel, C.J., and Demeyer, D.I. (1996). Influence of pH on lipolysis and biohydrogenation of soybean oil by rumen contents *in vitro*. **Reproduction Nutrition Development, EDP Sciences**. 36(1): 53-63.

Yu, Z., and Morrison, M. (2004). Improved extraction of PCR-quality community DNA from digesta and fecal samples. **Bio Techniques.** 36(5): 808-812.

CHAPTER V

EXPERIMENT III

EFFECT OF SUNFLOWER OIL AND NITRATE LEVELS ON FEED INTAKE, RUMEN FERMENTATION, CARCASS TRAITS, MEAT QUALITY AND MUSCLE FATTY ACID PROFILES IN MEAT GOAT

5.1 Abstract

The objective of this study was to investigate the effect of nitrate supplementation and roughage quality on feed intake, nutrient digestibility, rumen fermentation, carcass traits and muscle fatty acid profile in meat goats. Thirty-two crossbred (Thai native × Anglo-Nubian) growing goats with weighed 17.10 ± 1.03 kg (mean ± SD), were used in 2 × 2 factorial arrangements in Randomized complete block design (RCBD). Goat was fed individually and slaughtered at the end of the fattening period (120 days). Results showed that most of the dissected both of level nitrate compare with roughage sources had no effect on total feed intake (g/day) and nutrient digestibility. Muscle pH and the temperature value of the loin area measured at 24 mines and 24 hours postmortem were not affected by the different level of nitrate and roughage sources. Cooking loss, % and muscle color components (CIE; L*, a*, and b* value) were not affected by roughage sources and levels of nitrate. However, drip loss of goats fed with pangola grass hay were significantly higher

(P<0.001) than rice straw. The slaughter performance there was significant on heart and kidney on low-quality roughage (rice straw) when increase nitrate in diets. The treatments did not influence on the proportion of conjugated linoleic acid (CLA) and ratio of ω6 : ω3 in the *longissimus dorsi* muscle when to compare the level of nitrate but had affected to C18 : 1 *trans* 9, C18 : 2n6 and C20 : 2n6 by the different quality of roughage.

Keyword : nitrate, quality roughage, nutrient digestibility, muscle fatty acid profile, meat goats

5.2 Introduction

Livestock production systems contribute 12-18% of the global greenhouse gas (GHG) emissions when expressed as CO_2 equivalents (Steinfeld et al., 2006; Westhoek et al., 2011). Methane emission is the largest source of GHG from agriculture rector. Kurihara et al. (1999) also reported that methane emission rates ranging from 6.7% to 11.4% measured in ruminant fed tropical forage-based diets. Pangola grass hay as a tropical forage species especially in Thailand. Pangola grass (*Digitaria eriantha Steud*., *synonym D. decumbens*) has been shown to be a highest quale grass. However, during the long dry periods of the year, ruminants are mainly dependent on crop residues such as rice straw. Rice straw is a low protein content and high indigestible parts or slowly fermentable which result in low voluntary intake and digestibility. Use of NPN in a low-CP crop residues is a primarily strategy to supplely nitrogen for rumen microbial to convert into microbial protein (Leng, 1990). Nitrate

is a nitrogen source which derived from NPN source and in addition can be used as methane inhibitor (Zhou et al., 2011; Holshof et al., 2012).

Nitrate acting as an electron sink and competing with CO_2 for electrons, and nitrite, the first intermediate of nitrate reduction, exerting toxicity to methanogens (Bozic et al., 2009; Zhou et al., 2011). Nitrate suppresses methane production by acting as a hydrogen sink as well as directly inhibiting the methanogens (Patra and Yu, 2014). Increased ammonia concentration in the nitrate supplemented group may be represented to the conversion of nitrate to ammonia ruminants requires an adequate supply of N for ruminal microorganisms for the synthesis of microbial protein. Oils and fats have often been used in ruminant rations to increase their metabolizable energy (ME) value (Clapperton and Steele, 1983). While, in recent years, dietary fat supplementation has become a common practice, fat supplemented diets have had variable effects on animal performance and carcass characteristics. McGinn et al. (2004), reported who studied sunflower oil, reduced methane emissions by 17.1%. The loss of energy from methane in ruminant livestock is also a critical priority regarding feed energy utilization and animal performance (Blaxter, 1989; Johnson and Johnson, 1995; Kurihara et al., 1999) and to increase their metabolizable energy (ME) value (Clapperton and Steele, 1983), have variable effects on animal performance and carcass characteristics.

5.3 Objective

The objective of this study on the effect of nitrate supplementation and quality of roughage on feed intake, nutrient digestibility, carcass traits, and muscle fatty acid profile in meat goats.

5.4 Materials and methods

5.4.1 Animals and feeding management

Thirty-two meat goats crossbred (Thai native × Anglo-Nubian) growing goats with weighed 17.10 ± 1.03 kg (mean ± SD), were used in 2 × 2 factorial arrangements in RCBD. Dietary treatments were two sources of roughage and two levels of nitrate (2% and 3 % level of potassium nitrate) for each quality of roughage, were purchased from the local market at Nakhon Ratchasima province. The starting the experiment, the animals were injected with Ivomic (Merial Ltd., Iselin, NJ) for anti-internal parasite, and housed in individual pens (0.9 × 1.4 m^2) where the animals could have an easy access to rice straw and clean water *ad libitum*. The pens were cleaned and disinfected before animals were housed. Animals were fed *ad libitum* of rice straw and were fed concentrate twice daily at 08.00 am and 16.00 pm. Experiment period lasted for 120 days. The first 21 days was used for animal adaptation and following by 90 days for parameters measurement and in the last 7 days for total collection. Feed refusal was weighed daily prior to the morning feeding to determine daily dry matter intake (DMI). Body weight (BW) of each animal was measured weekly immediately before the morning feeding. The goats were randomly allocated to 8 experimental groups of 4 each. Group 1. Eight goats received the rice straw with nitrate compound at 2% in concentrate, Group 2 Eight goats received the rice straw with nitrate compound at 3% in concentrate, Group 3. Eight goats received the pangolar hay with nitrate compound at 2% in concentrate and Group 4. Eight goats received the pangolar hay with nitrate compound at 3% in concentrate respectively.

5.4.2 Feeding trial

Feed offered and left after eating were weighed on two consecutive days of each period. Feed samples were then taken for proximate analysis (AOAC, 1990), detergent analysis (Georing and Van Soest, 1970). One metabolism trial of 7 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 2 days, prior to actual collection of 5 days to acclimatize the animals to the new surroundings. The appropriate aliquots of feed offered, the residue left, feces were preserved animal wise for the day for chemical analysis. Body weight of the animals was recorded before and after the metabolism trials. Measurement data of feed offer and residue 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°C until the end of the experiment. Feces from the 7 days were thoroughly mixed and then samples were taken and dried at 60°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°C for 24 h, ash content was assayed by incinerating samples at 550°C, and organic matter (OM) could, therefore, be obtained. Nitrogen (N) was determined by the Macro Kjeldahl technique and crude protein calculated as N*6.25. Neutral detergent fiber (NDF) and acid detergent fiber (ADF) were analyzed followed the procedure described by Goering and Van Soest (1970).

5.4.3 Rumen fermentation and blood urea nitrogen in plasma

After 120 days of the experiment, the rumen contents was collected before morning feeding (0, 3, and 6 hours after feeding using a stomach tube attached to a suction pump, pH measured immediately using a glass electrode pH meter. After

recording pH, an aliquot of the samples was strained through 4 layers of cheese cloth. The rumen fluid was then acidified with H_2SO_4 (10%, v/v) and stored at -20°C for subsequently quantifying NH_3-N and volatile fatty acids (VFAs) concentration. Volatile fatty acids (VFA) by using gas chromatography (GC) analysis (Erwin et al., 1961). With that, all samples were kept at -20°C until further analysis. The supernatant fluid was analyzed for VFAs (acetate, propionate, and butyrate) concentrations by gas chromatography (Hewlett Packard GC system HP6890 A; Hewlett Packard, Avondale, PA) equipped with a 30 m × 0.25 mm × 0.25 μm film (DB-FFAP). Blood samples were taken from the jugular vein at 0 (prior to feeding), 3 and 6 hours post feeding. Then, the blood samples were prior to plasma separation by centrifugation (3,000 × g for 15 min) and plasma samples were then stored at -20°C for determining blood urea nitrogen (BUN) concentration.

5.4.4 Preparation of samples for gas chromatography (GC) analysis

The ruminal fluid samples that used to determine total VFA and molar proportion of main VFA mix (acetate, propionate, and butyrate) were centrifuged at 3500 x rpm for 10 min at 4°C to get rid of food particles and ruminal microbe, with that measured 1 ml supernatant into a 2 ml vial for gas chromatography (GC) analysis.

5.4.5 Feeding trial

The metabolism trial of 7 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 2 days, prior to actual collection of 5 days to acclimatize the animals to the new surroundings. The appropriate aliquots of feed offered, the residue left, feces were

preserved animal wise for the day for chemical analysis. Body weight of the animals was recorded before and after the metabolism trials. Measurement data of feed offer and residue 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°C until the end of the experiment. Feces from the 7 days were thoroughly mixed and then samples were taken and dried at 60°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°C for 24 h, ash content was assayed by incinerating samples at 550°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*6.25. Neutral detergent fiber (NDF) and acid detergent fiber (ADF) were analyzed followed the procedure described by Goering and Van Soest (1970).

5.4.6 Analysis of fatty acids by Gas chromatography (GC)

Total VFA and molar proportion of acetic, propionic, and butyric acids in ruminal fluid and fatty acid profile of plasma samples were determined by HP6890 gas chromatography (GC) (made in the USA) that fitted with a Flame Ionization Detector (FID). In addition, a J and W 122~3232 column was applied for the determination of VFA, whereas a 100 m × 0.25 mm fused silica capillary column (SP2560, Supelco Inc, ellefonte, PA, USA) for determination the plasma fatty acid profiles. The column temperature was fixed at 70°C for 4 min, then it increased at 13°C /min to 175°C which lasted for 27 min. Continually it increased at 4°C /min to 215 °C and kept for 31 min. Nitrogen was adopted as a carrier gas with a 60 ml/min flow rate and the oven temperature was 250°C. FID and injection temperature were fixed at 280°C, and a 1µL injection was done with a 10-µL injector.

5.4.7 Slaughter procedure and carcass characteristics

At the end of 120 days fattening period, 4 goats have randomly chosen from each group and were stunned with a captive-bolt pistol at the experimental slaughter unit at Suranaree University of Technology (SUT). After slaughtering, non-carcass components were removed from the carcass and weighed, and then the rest of component was hot carcass weighed. Hot carcass included kidneys and perinephric-pelvic fat as described by Colomer-Rocher et al. (1987). Cold carcass weight was obtained after chilling the hot carcass at 4°C for 24 hours. Dressing percentage was calculated as hot carcass weight divided by slaughter BW. Carcass length was measured from the top point of shoulder (anterior part of scapula) to the tuber ischium (pelvic bone) and loin eye area were measured from both sides of each carcass, at the 12^{th} and 13^{th} rib using a one-centimeter grid (each dot on the grid represents 0.1 square inches of measurement). Lean weight was estimated from formula: Lean weight (kg) = -1.09 + (0.8 × Cold carcass weight (kg)); R^2 = 0.98 (Hopkins-Shoemaker, 2006). The lean percentage was calculated as lean weight (kg) ×100 / hot carcass weight (kg).

5.4.8 Meat quality analysis

Instrumental meat quality characteristics investigated in the current study were carcass pH, drip loss (%), water holding capacity (%), shear force (kg) and meat color (L*, a*, b*). Carcass pH was measured at 45 min after slaughter (pH_{45min}) and at 24 hour post-slaughter (pH_{24h}) using a digital pH meter. The pH measurement was performed directly on *longissimus thoracic* muscle between 12^{th} and 13^{th} thoracic vertebrae. The *longissimus dorsi* muscle was removed from the right side of the carcass at 24 h post-mortem in order to assess instrumental meat quality

characteristics. *Longissimus thoracic* muscle between 6th and 13th ribs was used for shear force determination, while samples from the *longissimus lumborum* muscle used for meat color and drip loss measurements. Meat color was measured after 1 h storage (first measurement) and finally after 24 h storage (second measurement) on the cut surface of 2.5 cm thick samples from the fat-free area. During the storage period, samples were kept at 5°C in a polystyrene tray and over wrapped with oxygen permeable PVC film to allow blooming. The color was evaluated using the CIELAB color space. L* (lightness), a* (redness) and b* (yellowness) using a Minolta CM-2006 d spectrophotometer (Konica Minolta Holdings, Inc, Osaka, Japan). Drip loss was determined using the method described by Honikel (1998). Briefly, meat samples were weighed and then suspended in an inflated polyethylene bag without any contact with the bag. After a 24 hours storage period at 5°C, the samples were gently dried with paper towels and reweighed. Drip loss (%) was estimated by the ratio of weight loss to initial sample weight. The cooking loss has estimated the meat in a plastic cook bag in an 80°C water bath to an internal temperature of 75°C. After cooking, the chops were placed in ice water and cooled to room temperature in order to prevent additional cooking once the desired temperature of 75°C was achieved. Chops were weighed before and after cooking in order to calculate cooking loss.

5.4.9 Fatty acid methyl ester of oil samples

The samples of *longissimus dorsi* muscle and peritoneal fat of the meat goats were collected and were immediately frozen at -20°C until analysis. All samples were prepared for FA analysis by gas chromatography (GC) of fatty acid methyl ester (FAME). The lipids were extracted from the forages using the chloroform/methanol (2/1) method procedure of Folch et al. (1957). For

quantification of CLA isomers, lipids extracted from samples were methylated (sodium methoxide) following the method of Li and Watkins (1998). Methylation of samples by the procedure described by Metcalfe (1966) was used. Fatty acid composition was measured after methylation of samples. Fatty acid methyl esters were analyzed on a Perkin Elmer Auto system gas chromatograph equipped with a flame-ionization detector (FID) using a capillary column (SPTM - 2560, 100 m × 0.25 mm ID, 0.20 μm film). This analyzed adopted a split injection (split ratio 100 : 1). The GC analysis was temperature programmed, at 140 ˚C held at 5 min, and raised from 140°C to 240°C at a rate of 4°C/min and then held 240°C for 40 min. The injection port and detector temperatures were set at 260°C. Helium was used as the carrier gasses at a rate of 20 cm/sec. Identification of the FA was based upon retention times using standards of methyl esters. A mixture of the standards of the individual FAME was used to determine response factors. The areas of the peaks in the chromatogram were calculated and normalized using response factors. Proportions of individual FA were calculated.

5.4.10 Statistical analysis

Data were statistically analyzed according to 2 × 2 factorial in RCBD using the PROC GLM procedure (SAS, 1990). Significant differences ($P<0.05$) among treatments were determined using Duncan's News Multiple Range test according to Steel and Torrie (1980).

5.5 Experimental location

The experiment was conducted at Suranaree University of Technology's dairy farm, The Center for Scientific and Technological Equipment's Building 1 and 3, and 10 Suranaree University of Technology.

5.6 Duration

The duration of the present experiment was from September, 2013 to May, 2014.

5.7 Results

5.7.1 Chemical composition of concentrate and roughages

The chemical of dietary concentrate and roughages has been shown in Table 5.1. The diet was adequate to meet the requirements of crude protein, growth net energy, and dry matter intakes of the goats under the condition of maintenance. As to the concentrate, it contained DM 93.91%, CP 14.63%, NDF 42.59%, and ADF 45.65 whereas the rice straw contained DM 91.82%, CP 3.35%, NDF 76.31 %, and ADF 52.34% (DM basis) the fatty acids mass were showed in Table 5.2.

5.7.2 Feed intake

The concentrate, roughage, and total intake of the goat were shown that Table 5.3. There were no significant difference by interaction between the quality roughage and level of nitrate. But, there were significantly difference ($P<0.001$) in quality roughage and levels of nitrate ($P<0.01$). In our study, appearance digestibility did not effect by levels of nitrate and interaction, but, there were significant on CP, and EE tendency increased with high quality roughage more than fed with low quality

upon levels of nitrate could be increased when increased concentration nitrate in diets.

Table 5.1 The ingredient and chemical composition of concentrate and roughage (rice straw and pangola grass hay) used in the experiment.

Item	Concentrate	Rice straw	Panggola grass hay
Ingredient, % dry matter			
Cassava distillers dried meal	32.0	-	-
Soybean meal	20.0	-	-
Corn distillers dried grains	17.5	-	-
Rice bran	10.0	-	-
Wheat bran	10.0	-	-
Molasses	8.0	-	-
Mineral and vitamin mixture[1]	2.5	-	-
Chemical composition			
Dry matter, %	92.2	91.8	87.5
% of dry matter			
Ash	7.0	12.3	8.4
Crude protein	14.6	3.3	7.3
Ether extract	4.0	1.0	1.9
Crude fiber	17.1	39.7	32.0
Neutral detergent fiber	42.5	76.3	73.4
Acid detergent fiber	26.3	52.3	35.9
Acid detergent lignin	10.9	6.3	4.0
TDN (%)[2/]	60.2	46.1	50.9
NFC[3/]	31.7	6.9	8.8

[1/]Mineral and vitamin mix : provided per kg of concentrate including vitamin A, 5,000 IU; vitamin D3, 2,200 IU; vitamin E, 15 IU; Ca, 8.5 g; P, 6 g; K, 9.5 g; Mg, 2.4

g; Na, 2.1 g; Cl, 3.4 g; S, 3.2 g; Co, 0.16 mg; Cu, 100 mg; I, 1.3 mg; Mn, 64 mg; Zn, 64 mg; Fe, 64 mg; Se, 0.45 mg.

[2/]Total digestible nutrients, TDN = tdNFC + tdCP + (tdFA x 2.25) + tdNDF – 7 (NRC, 2001).

[3/]Non Fiber Carbohydrate, NFC = 100 – (CP – NDF – EE – ash.

Table 5.2 Fatty acid profiles of sunflower oil, concentrate, rice straw and pangola grass hay (% DM basis).

Item	Sunflower oil	Concentrate	Rice straw	Pangola hay
C12 : 0	-	15.06	-	-
C14 : 0	0.48	5.92	1.28	1.41
C16 : 0	6.07	6.28	47.49	31.3
C17 : 0	1.36	19.79	-	-
C18 : 0	7.71	2.31	8.57	26.5
C18 : 1n9c	38.33	14.47	16.76	24.5
C18 : 2n6c	31.43	30.37	19.87	2.57
C18 : 3n3	2.34	1.82	6.03	8.98
C20 : 0	4.12	-	-	1.05
C22 : 0	3.85	-	-	-
Others	4.31	3.98	-	3.69

Table 5.3 The effect of nitrate and quality of roughage on feed intake.

Items	L		H		SEM	P-value		
	N2	N3	N2	N3		RQ	N	RQ × N
Roughage DMI								
g/day	335.17	335.04	408.70	412.80	3.83	***		NS
%BW	2.08	2.09	2.20	2.22	0.001	***	**	NS
g/kg $BW^{0.75}$	41.66	42.40	45.74	46.07	0.15	***	**	NS
Concentrate DMI								
g/day	205.88	217.88	233.00	235.25	7.20	***	**	NS
%BW	1.28	1.28	1.27	1.27	0.61	***	**	NS
g/kg $BW^{0.75}$	25.64	26.10	26.13	26.31	0.09	***	**	NS
Total DMI								
g/day	541.46	573.56	642.13	648.59	6.11	***	**	NS
%BW	3.36	3.38	3.47	3.48	0.001	***	**	NS
g/kg $BW^{0.75}$	67.30	68.50	71.87	72.39	0.23	***	**	NS

L = Low quality roughage (Rice straw); H = High quality roughage (pangola grass hay); S = Sunflower oil; N = Nitrate; RQ = quality of roughage, RQ × N = interaction quality of roughage and between nitrate, BW = Body weight, SEM = standard error of the mean; *P<0.05; **P<0.01; ***P<0.001; NS = not significantly different (P>0.05).

Table 5.4 The effect of nitrate and quality of roughage on growth performance.

Items	L		H		SEM	P-value		
	N2	N3	N2	N3		RQ	N	RQ x N
Initial BW, kg	17.38	17.30	17.41	17.58	0.17	NS	NS	NS
Final BW, kg	25.08	24.95	26.73	27.48	0.17	***	***	NS
BWC, kg	7.69	7.65	9.31	9.88	0.08	***	**	NS
FCR	8.44	9.00	8.27	7.86	0.66	***	**	NS
ADG, g/day	64.06	63.75	77.60	82.40	0.70	***	**	**

BWC = Body weight change; FCR = feed conversion ratio; ADG = Average Daily Gain; L = Low quality roughage (Rice straw); H = High quality roughage (Pangola grass hay); [1/]Effect of low quality roughage (Rice straw); [2/]High quality roughage

(Pangola grass hay); RQ = quality of roughage, RQ × N = interaction between quality of roughage and nitrate; SEM = standard error of the mean; *P<0.05; **P<0.01; ***P<0.001; NS = not significantly different (P>0.05).

5.7.3 Animal performance

For animal performance in this experiment under the effect of quality of roughage and nitrate supplementation on growth performance of finishing goats are present in (Table 5.4). There were no significant difference on the final body weight, body weight change significantly affected, feed conversion ratio (FCR) by interaction between quality roughage and levels of nitrate, but there affected on average dairy gain (ADG). The quality roughage had effect on final body weight, body weight change significantly affected, feed conversion ratio (FCR) could be increased when received high quality with 3% of nitrate the best in experiment.

Table 5.5 The effect of nitrate and quality of roughage on total volatile fatty acid (TVFA), proportion of volatile fatty acids, and acetate:propionate ratio.

Items	L		H		SE	P-value		
	N2	N3	N2	N3	M	RQ	N	RQ × N
Total volatile fatty acids (mM/L)								
0 h post feeding	78.85	79.25	76.12	79.26	1.28	*	NS	NS
3 h post feeding	89.56	83.85	87.16	88.48	1.33	NS	NS	NS
6 h post feeding	83.74	82.62	80.86	88.33	1.50	NS	NS	NS
Volatile fatty acids proportion (mol/100 mol)								
Acetate (C2)								
0 h post feeding	71.07	69.70	70.47	70.15	0.36	NS	NS	NS
3 h post feeding	69.78	71.08	71.99	70.94	0.58	NS	NS	NS
6 h post feeding	70.74	69.79	71.50	70.27	0.42	NS	NS	NS

Table 5.5 The effect of nitrate and quality of roughage on total volatile fatty acid (TVFA), proportion of volatile fatty acids, and acetate : propionate ratio (Continued).

Items	L		H		SE	P-value		
	N2	N3	N2	N3	M	RQ	N	RQ × N
Total volatile fatty acids (mM/L)								
0 h post feeding	78.85	79.25	76.12	79.26	1.28	*	NS	NS
3 h post feeding	89.56	83.85	87.16	88.48	1.33	NS	NS	NS
6 h post feeding	83.74	82.62	80.86	88.33	1.50	NS	NS	NS
Volatile fatty acids proportion (mol/100 mol)								
Acetate (C2)								
0 h post feeding	71.07	69.70	70.47	70.15	0.36	NS	NS	NS
3 h post feeding	69.78	71.08	71.99	70.94	0.58	NS	NS	NS
6 h post feeding	70.74	69.79	71.50	70.27	0.42	NS	NS	NS
Propionate (C3)								
0 h post feeding	23.32	23.65	21.81	23.00	0.40	NS	NS	NS
3 h post feeding	23.91	23.11	20.38	20.98	0.62	*	NS	NS
6 h post feeding	23.25	24.12	21.93	22.61	0.52	NS	NS	NS
Butyrate (C4)								
0 h post feeding	5.60	6.64	7.71	6.84	0.40	NS	NS	NS
3 h post feeding	6.30	5.79	7.62	8.09	0.43	*	NS	NS
6 h post feeding	5.99	6.08	6.56	7.11	0.42	NS	NS	NS
Acetate : Propionate (C2 : C3)								
0 h post feeding	3.06	2.69	3.25	3.11	0.06	NS	NS	NS
3 h post feeding	2.94	3.13	3.67	3.84	0.12	*	NS	NS
6 h post feeding	3.08	2.95	3.33	3.15	0.09	NS	NS	NS

L = Low quality roughage (Rice straw); H = High quality roughage (Pangola grass hay); RQ = quality of roughage, RQ × N = interaction between quality of roughage and nitrate; Sunflower oil; N = Nitrate; SEM = standard error of the mean; *P<0.05; **P<0.01; ***P<0.001; NS = not significantly different (P>0.05).

Table 5.6 The effect of nitrate and quality of roughage on methane production, ammonia nitrogen, pH and blood urea nitrogen.

Items	L		H		SEM	P-value		
	N2	N3	N2	N3		RQ	N	RQ × N
Methane ,CH_4 (mole/100 mole)								
0 h post feeding	25.09	24.58	22.44	23.46	0.39	*	NS	NS
3 h post feeding	26.18	25.15	27.26	27.22	0.58	NS	NS	NS
6 h post feeding	25.01	24.09	24.78	26.34	0.53	NS	NS	NS
Ammonia-N (NH_3-N%mg)								
0 h post feeding	10.92	9.53	10.36	9.65	0.92	NS	NS	NS
3 h post feeding	17.25	17.88	18.23	18.36	0.33	NS	NS	NS
6 h post feeding	13.89	15.54	15.15	14.27	0.42	NS	NS	*
pH								
0 h post feeding	6.97	7.12	7.11	7.12	0.02	NS	NS	NS
3 h post feeding	6.57	6.76	6.56	6.23	0.04	**	NS	**
6 h post feeding	6.43	6.67	6.61	6.30	0.04	NS	NS	**
Blood urea nitrogen (BUN, mg %)								
0 h post feeding	5.65	5.77	6.14	6.24	0.01	***	***	NS
3 h post feeding	12.80	12.75	14.96	15.72	0.11	***	**	*
6 h post feeding	7.96	8.16	8.46	8.44	0.04	***	*	NS

L = Low quality roughage (Rice straw); H = High quality roughage (Pangola grass hay); Q = quality of roughage, Q × N = interaction between quality of roughage and nitrate; N = Nitrate; SEM = standard error of the mean; *P<0.05; **P<0.01; ***P<0.001; NS = not significantly different (P>0.05).; CH_4 = (0.45 × acetate) − (0.275 × propionate) + (0.4 × butyrate) according to Moss et al. (2000).

5.7.4 Rumen fermentation and blood urea nitrogen in plasma

Results in this study on effects of nitrate supplementation and quality of roughage on total volatile fatty acid (TVFA) and concentration of volatile fatty acids have been shown in Table 5.6. There was no effect on total volatile fatty acids

(P>0.05) by level of nitrate and the interaction between nitrate and quality of roughage, except for the quality of roughage had affected on total volatile fatty acid (0h P<0.05 for at 0 hour), propionate, butyrate and acetate : propionate (P<0.05 for at 3h post- after feeding the experimental diets). There was the effect on methane production (P<0.05 at 0 hour) and pH (P<0.01 at 3 hours) by quality roughage. However, there were have an effect on ammonia nitrogen and blood urea nitrogen (P<0.05 at 3 hours post-feeding) and pH (P<0.01 at 3 and 6 hours post-feeding) in the interaction between nitrate and quality of roughage.

Table 5.7 The effect of nitrate supplementation and quality of roughage on nitrogen balance.

Items	L		H		SEM	P-value		
	N2	N3	N2	N3		RQ	N	RQ × N
N intake (g)	6.61	7.01	10.26	10.36	0.086	***	*	NS
N excretion (g)								
Fecal	2.47	2.75	2.59	2.86	0.114	NS	NS	NS
Urine	1.90	1.16	2.26	1.90	0.136	***	***	NS
N absorption (g)	4.14	4.25	7.66	7.50	0.149	***	NS	NS
N balance (g)	2.25	3.10	5.41	5.61	0.169	***	**	NS
N absorption (%)	62.63	60.71	74.60	72.37	1.331	***	NS	NS
N retention (%)	33.99	44.16	52.75	53.98	1.959	***	*	NS

L = Low quality roughage (Rice straw); H = High quality roughage (Pangola grass hay); RQ = quality of roughage, RQ × N = interaction quality of roughage and between potassium Nitrate; Sunflower oil; N = Potassium Nitrogen; SEM = standard error of the mean; *P<0.05; **P<0.01; ***P<0.001; NS = not significantly different (P>0.05).

5.7.5 Nitrogen balance

The effect of dietary treatment on daily nitrogen balance have been shown in Table 5.8. The total nitrogen intake and faecal nitrogen excretion and nitrogen absorption were not significant by interaction nitrate and quality of roughage. There were significant difference on nitrogen balance by quality of roughage and levels of nitrate tendency increased when increased levels of nitrate.

5.7.6 Ruminal microbe population

The effect of nitrate supplementation and quality of roughage on population of microbial in rumen fluid of direct count methods have been shown Table 5.9. The quality of roughage and the interaction between nitrate and quality of roughage did not effect on population of microbes, However, there were significant on protozoa ($P<0.01$ at 0h and $P<0.05$ at 3h after morning feeding) and there were had effect on bacteria ($P<0.05$ at 6 h after morning feeding).

5.7.7 Slaughter performance and meat quality characteristics and carcass composition for *longissimus dorsi* muscle

The interaction between quality roughage and levels of nitrate did not effect on living weight, but there were significant difference when received different quality roughage tend to decrease in low quality and increase in high quality roughage when increased levels of nitrate. Carcass weight (hot carcass, dressing carcass and cold carcass) were significant difference ($P<0.01$) by the interaction. However, there were no significant in body composition. The interaction between quality roughage and levels of nitrate did not effect in meat quality characteristics (cooking loss and drip loss) and on significant changes in physicochemical characteristics (pH values, colour, moisture, fat, ash), except for protein content.

Table 5.8 The effect of nitrate supplementation and quality of roughage on population of microbial in rumen fluid of direct count methods.

Items	L		H		SEM	P-value		
	N2	N3	N2	N3		RQ	N	RQ × N
Protozoa count (10^6 cells/ml)								
0 h post feeding	2.25	2.10	2.21	2.10	0.02	NS	**	NS
3 h post feeding	2.34	2.19	2.34	2.19	0.09	NS	*	NS
6 h post feeding	2.26	2.20	2.20	2.11	0.06	NS	NS	NS
Bacteria count (10^{12} cells/ml)								
0 h post feeding	3.56	3.06	3.31	3.87	0.86	NS	NS	NS
3 h post feeding	4.25	3.56	4.68	3.50	0.55	NS	NS	NS
6 h post feeding	2.43	1.75	2.56	1.87	0.20	NS	*	NS

L = Low quality roughage (Rice straw); H = High quality roughage (Pangola grass hay); RQ = quality of roughage, RQ × N = interaction quality of roughage and between potassium Nitrate; N = Nitrate; SEM = standard error of the mean; *P<0.05; **P<0.01; ***P<0.001; NS = not significantly different (P>0.05).

Table 5.9 The effect of nitrate and quality of roughage on slaughter performances.

Items	L		H		SEM	P-value		
	N2	N3	N2	N3		RQ	N	RQ x N
Living weigh (kg)	25.08	24.95	26.73	27.48	0.41	**	*	NS
Carcass characteristics								
Hot carcass (kg)	12.87	13.25	12.96	13.57	0.13	**	*	*
Dressing carcass, %	45.30	45.45	48.64	51.34	1.15	**	*	*
Cold carcass (kg)	12.11	12.49	12.20	12.81	0.15	**	*	*
Length Carcass (cm)	62.00	61.25	61.00	62.12	0.59	NS	NS	NS

Table 5.9 The effect of nitrate and quality of roughage on slaughter performances.

Items	L		H		SEM	P-value		
	N2	N3	N2	N3		RQ	N	RQ x N
Body component, % of slaughter weight								
Head	12.27	11.22	11.10	11.71	0.12	NS	NS	NS
Skin	12.25	13.00	12.49	12.93	0.16	NS	NS	NS
Blood	7.64	4.05	5.62	5.31	0.29	NS	NS	NS
Heart	0.47	0.47	0.58	0.55	0.01	*	NS	NS
Spleen	2.17	1.92	1.56	1.64	.008	NS	NS	NS
Liver	2.47	1.99	1.90	2.10	0.03	NS	NS	NS
Lungs	1.70	1.72	1.32	1.74	0.04	NS	NS	NS
Kidney	0.41	0.35	0.32	0.34	.005	*	NS	NS
GI tract	53.79	57.56	57.78	56.35	0.98	NS	NS	NS

L = Low quality roughage (Rice straw); H = High quality roughage (Pangola grass hay); Q=quality of roughage, Q × N = interaction between quality of roughage and nitrate; N = Nitrate; SEM = standard error of the mean; *P<0.05; **P<0.01; ***P<0.001; ns = not significantly different (P>0.05).

Table 5.10 The effect of nitrate and quality of roughage on meat quality.

Items	L		H		SEM	P-value		
	N2	N3	N2	N3		Q	N	Q × N
Meat quality characteristics, % of slaughter weight								
Cooking loss	26.13	25.35	24.64	24.75	1.54	*	*	*
Drip loss	1.57	1.58	1.56	1.57	0.07	NS	NS	NS
pH_{45min}	6.29	6.28	6.27	6.30	0.18	NS	NS	NS
pH_{24h}	5.79	5.78	5.77	5.80	0.18	NS	NS	NS
Color *Longissimus dorsal* muscle								
L*	48.15	46.93	46.32	44.11	0.50	*	NS	NS
a*	5.38	5.77	5.71	5.75	0.25	NS	NS	NS
b*	8.74	8.28	8.26	7.22	0.25	NS	NS	NS

Table 5.10 The effect of nitrate and quality of roughage on meat quality (Continued).

Items	L		H		SEM	P-value		
	N2	N3	N2	N3		Q	N	Q × N
Carcass composition,% of slaughter weight								
Moisture	75.18	74.82	75.46	74.67	0.37	NS	NS	NS
ASH	1.76	1.84	1.81	1.85	0.03	NS	NS	NS
CP	22.29	22.20	24.04	23.47	0.12	**	*	**
EE	3.14	3.08	2.77	2.86	0.45	*	NS	NS

L = Low quality roughage (Rice straw); H = High quality roughage (Pangola grass hay); RQ = quality of roughage, RQ × N = interaction between quality of roughage and Nitrate; N = Nitrate; SEM=standard error of the mean; *P<0.05; **P<0.01; ***P<0.001; NS = not significantly different (P>0.05).

5.7.8 Fatty acids composition of *logissimus dorsi muscle* and fatty acid profile.

Fatty acid (FA) profiles in the *longissimus* meat samples were collected from meat goats at the end of the experiment (120 days) showed in Table 5.12. The proportion of C14 : 0, C17 : 1, C18 : 1*trans*9, C18 : 2n6, C22:0, MUFA/SFA and n-6 was significant higher (P<0.05) in an interaction between quality roughage and levels of potassium nitrate. Quality roughage received in die had effect on the proportion of total.

Table 5.11 The effect of nitrate supplementation and quality of roughage on fatty acid profiles (g/100 g total fat) in meat goats.

Items	L		H		SEM	P-value		
	N2	N3	N2	N3		Q	N	Q x N
C12 : 0	0.16	0.14	0.19	0.15	0.004	**	NS	NS
C14 : 0	2.70	4.21	2.51	4.47	0.168	**	**	**
C15 : 0	0.28	0.34	0.27	0.27	0.017	NS	NS	NS
C16 : 0	24.39	24.09	24.20	23.29	0.157	**	**	NS
C16 : 1	1.38	1.40	1.24	1.23	0.023	NS	NS	NS
C17 : 0	0.70	0.74	0.64	0.65	0.028	NS	NS	NS
C17 : 1	0.55	0.80	0.81	0.77	0.031	**	**	**
C18 : 0	14.40	15.22	15.07	14.75	0.212	**	**	NS
C18 : 1	41.46	39.03	39.31	37.04	0.090	**	NS	NS
C18 : 1*trans*9	0.40	0.38	0.39	0.34	0.003	NS	NS	**
C18 : 1 *trans* 11	1.71	1.58	1.84	1.92	0.044	**	NS	NS
C18 : 2n6	5.43	5.46	5.35	6.63	0.164	**	**	**
C18 : 2*cis*9*trans*11	0.33	0.40	0.28	0.79	0.040	NS	NS	NS
C18 : 2*cis*12*trans*10	0.13	0.32	0.39	0.46	0.042	**	NS	NS
C18 : 3n3	1.73	1.80	2.80	2.83	0.110	**	*	NS
C18 : 3n6	0.27	0.20	0.22	0.25	0.016	NS	NS	NS
C20 : 0	0.14	0.14	0.14	0.13	0.002	NS	NS	NS
C20 : 2n6	0.12	0.20	0.15	0.08	0.010	*	NS	NS
C20 : 3n6	0.16	0.26	0.47	0.55	0.034	**	**	NS
C20 : 4n6	2.53	2.37	2.53	2.49	0.029	**	NS	NS
C22 : 0	0.22	0.21	0.38	0.15	0.019	**	**	**
C22 : 6n3	0.40	0.18	0.19	0.16	0.045	NS	NS	NS
C24 : 0	0.42	0.52	0.63	0.57	0.027	**	NS	NS
SFA	42.55	42.77	42.04	41.56	0.434	**	NS	NS
MUFA	43.68	44.03	42.90	42.64	0.420	**	NS	NS
PUFA	13.76	13.19	15.06	15.80	0.462	**	NS	NS
CLA	0.46	0.72	0.67	1.25	0.082	**	NS	NS

L = Low quality roughage (Rice straw); H= High quality roughage (Pangola grass hay); RQ=quality of roughage, RQ × N = interaction between quality of roughage and nitrate; N=Nitrate; SEM=standard error of the mean; *P<0.05; **P<0.01; ***P<0.001; NS= not significantly different (P>0.05); SFA: saturated fatty acid = C12 : 0 + C14 : 0 + C15 : 0 + C16 : 0 + C17 : 0 + C18 : 0 + C20 : 0 + C22 : 0 + C24 : 0, MUFA: monounsaturated fatty acid = C16 : 1 + C17 : 1 + C18 : 1n9 and PUFA : polyunsaturated fatty acid = C18 : 2n6 + CLA + C18 : 3n3 + C20 : 3n6 + C20 : 4n6 + C22 : 2 + C22 : 6n3, CLA = Conjugated linoleic acid = *cis-9, trans-11*CLA + *trans-10, cis-12*CLA.

5.8 Discussion

5.8.1 Feed intake and animal performance

Dry matter intake (DMI) from low quality roughage with high nitrate supplementation may result in an increased rate and extent of fiber digestion in the rumen. In the present study, supplementation with different levels of nitrate and different quality roughage sources had no effect on feed intake. This, in turn, did not alter the growth performance of the goats ($P<0.05$). These results are in accordance with Weston et al. (1989) and Vega et al. (2000) and are most likely related to the difference in particle size of the forage. It is well-established that a reduction in forage particle size increases voluntary intake because of a reduction in rumination time and in the retention time of food in the rumen caused by the small particles.

5.8.2 Rumen fermentation and blood urea nitrogen

In the present study, there were no significant effects due to treatments on acetate, propionate, butyrate and acetate : propionate ratio but there was a tendency to decrease the concentration of acetate, propionate, and butyrate when the level of nitrate was increased. A possible mechanism to explain this is that nitrate reduction rapidly consumes electrons leading to decreased concentrations of NADH and increased concentrations of NAD^+ cations which, in turn, favours production of acetate over production of more reduced VFAs such as butyrate (Nolan et al., 2010). However, Zhou et al. (2012) report that an increased percentage of acetate with a decreased percentage of butyrate triggered by an increase in nitrate is well-documented. Patra and Yu (2014) reported that a nitrate supplement alone resulted in a significantly higher ammonia concentration than with other treatments.

5.8.3 Slaughter performance, meat quality characteristics and carcass composition

In the present study, enriching goat meat with nitrate and low quality roughage had no effect on the drip loss and cooking loss over the designated aging time. The influence of fatty acids on meat colour comes mainly from the ability of UFA to oxidize rapidly, which results in changing the red oxymyoglobin to the brown metmyoglobin. This reaction commonly occurs in parallel with that of rancidity (Wood et al., 2004, 2008). There were no significant in lightness (L*), redness (a*) and yellowness (b*). This finding is in agreement with previous studies on lamb by Moloney et al. (2012) and on beef by Corazzin et al. (2012). They found that the inclusion of linseed in the diets did not influence the meat colour.

5.8.4 Fatty acid composition of the *longissimus dorsi* muscle and fatty acid profile.

Sunflower oil supplementation as a source of PUFA in the diets of goats did not alter total SFA in muscle and adipose tissues. In lambs, it has been reported that total SFA concentrations in muscle and adipose tissues did not change with PUFA-rich oil supplementation (Bessa et al., 2008; Boles et al., 2005; Kott et al., 2003; Radunz et al., 2009). Bolte et al. (2002) demonstrated that total SFA contents were similar in muscle. In the present experiment, PUFA and CLA concentrations in muscle were increased by high quality roughage (Pangola grass hay). The CLA cis-9, trans-11 enhancement can be partially attributed to the increased concentrations of VA (C18 : 1, *trans*-11) arising from partial biohydrogenation of oleic, linoleic and linolenic acids by ruminal microorganisms.

5.9 Conclusion

This study examined the effect of nitrate supplementation and quality of roughage on feed intake, nutrient digestibility, carcass traits, and muscle fatty acid profiles in meat goats. High quality roughage supplemented with 3% nitrate had an effect on nitrogen balance and % BW and Final BW, but had no effect on appearance/nutrient digestibility. Low quality roughage with nitrogen supplementation can improve nitrogen balance and total VFAs. The meat quality characteristics and carcass composition for the *longissimus dorsi* muscle were influenced by high quality roughage when the level of KNO_3 in the goats' diets was increased. Improvements were noted in drip loss and CP in carcass composition, but there was no effect on cooking loss, colour and pH for the *longissimus dorsi* muscle.

5.10 References

AOAC. (1990). **Official Methods of Analysis.15th edn. Association of Official Analytical Chemists**, Washington DC, USA.

Bessa, R. J. B., Lourenco, M., Portugal, P.V., and Santos-Silva, J. (2008). Effects of previous diet and duration of soybean oil supplementation on light lambs carcass composition, meat quality, and fatty acid composition. **Meat Science**. 80: 1100-1105.

Blaxter, K.L., and Czerkawski, J.W. (1966). Modification of methane production of sheep by supplementation of its diet. **Journal of the Science of Food and Agriculture**.17: 417- 420.

Boles, J.A., Kott, R.W., Hatfield, P.G., Bergman, J.W., and Flynn, C.R. (2005). Supplemental safflower oil affects the fatty acid profile, including conjugated linoleic acid, of lamb. **Journal of Animal Science**. 83: 2175-2181.

Bolte, M.R., Hess, B.W., Means, W.J., Moss, G.E., and Rule, D.C., (2002). Feeding lambs high-oleate or highlinoleate safflower seeds differentially influences carcass fatty acid composition. **Journal of Animal Science**. 80: 609-616.

Bozic, A.K., Anderson, R.C., Carstens, G.E., Ricke, S.C., Callaway, T.R., and Yokoyama M. T. (2009). Effects of the methane-inhibitors nitrate, nitroethane, lauric acid, Lauricidin and the Hawaiian marine algae *Chaetoceros* on ruminal fermentation *in vitro*. **Bioresource Technology**. 100: 4017-4025.

Bromner, J.M., and Keeney, D.R. (1965) Steam distillation methods of determination of ammonium, nitrate and nitrite. **Analytica Chimica Acta.** 32: 485.

Clapperton, J.L., and Steele, W. (1983). Fat supplementation in animal production-ruminants. **Proceedings of the Nutrition Society**. 42: 343-350.

Crocker, C.L. (1967). Rapid determination of urea nitrogen in serum or plasma without deprotenization. **American Journal Medical Technology.** 33: 361.

Colomer-Rocher, F., Morand-Fehr, P., and Kirton, A.H. (1987). Standard methods and procedures for goat carcass evaluation, jointing and tissue separation. **Livestock Production Science.** 17: 149-159.

Corazzin, M., Bovolenta, S., Sepulcri, A., and Piasentier, E. (2012). Effect of whole linseed addition on meat production and quality of Italian Simmental and Holstein young bulls. **Meat Science**. 90: 99-105.

Erwin, E.S., Marco, G.J., and Emery, E.M. (1961). Volatile fatty acid analyses of

blood and rumen fluid by gas chromatography. **Journal of Dairy Science.** 44: 1768-1771.

Folch, J., Lees, M., and Stanley, G.H.S. (1957). A simple method for the isolation and purification of total lipides from animal tissues. **The Journal of Biological Chemistry**. 226: 497-509.

Goering, H.K., and Van Soest. P.J. (1970). Forage Fiber Analysis (apparatus, reagent, procedures and some application). **Agriculture Handbook No. 379, ARS, USDA,** Washington, DC.

Hopkins-Shoemaker, C. E. (2006). **Evaluation of sustainable forage system for meat goat production in the southern US**. Ph. D thesis. Auburn University.

Honikel, K.O. (1998). Reference methods for the assessment of physical characteristics of meat. **Meat Science**. 49: 447-457

Hulshof, R., Berndt, A., Gerrits, W., Dijkstra, J., van Zijderveld, S.M., Newbold, J.R., and Perdok, H.B. (2012). Dietary nitrate supplementation reduces methane emission in beef cattle fed sugarcane-based diets. **Journal of Animal Science**. 90: 2317-2323.

Kott, R.W., Hatfield, P.G., Bergman, J.W., Flynn, C.R., Van Wagoner, H., and Boles, J.A. (2003). Feedlot performance, carcass composition, and muscle and fat CLA concentrations of lambs fed diets supplemented with safflower seeds. **Small Ruminant Research.** 49: 11-17.

Kurihara, M., Magner, T., Hunter, R.A., and McCrabb G.J. (1999). Methane production and energy partition of cattle in the tropics. **British Journal of Nutrition.** 81: 227-234.

Leng, R.A. (2008). **The potential of feeding nitrate to reduce enteric methane production in ruminants. A report to the department of climate change**. Commonwealth Government of Australia, Canberra, Australia.

McGinn, S.M., Beauchemin, K.A., Coates, T., and Colombatto, D. (2004). Methane emissions from beef cattle: effects of monensin, sunflower oil, enzymes, yeast, and fumaric acid. **Journal of Animal Sciences**. 82: 3346-3356.

Metcalfe, L. D., Schmitz, A. A., and Pelka, J. R. (1966). **Rapid preparation of fatty acid esters from lipids for gas chromatographic analysis**. Analytical Chemistry, Washington, v. 38, n. 3, p. 514-515.

Mosley, E.E., Powell, G.L., Riley, M.B., and Jenkins, T.C. (2002). Microbial biohydrogenation of oleic acid to *trans* isomers *in vitro*. **Journal of Lipid Research**. 43: 290-296.

Nolan, J.V., Hegarty, R.S., Hegarty, J., Godwin, I.R., and Woodgate, R. (2010). Effects of dietary nitrate on fermentation, methane production and digesta kinetics in sheep. **Animal Production Science**. 50: 801-806.

Patra A. K., and Yu Z. T. (2014). Combinations of nitrate, saponin, and sulfate additively reduce methane production by rumen cultures *in vitro* while not adversely affecting feed digestion, fermentation or microbial communities. **Bioresource Technology**. 155: 129-135.

Radunz, A.E., Wickersham, L.A., Loerch, S.C., Fluharty, F.L., Reynolds, C.K., and Zerby, H.N. (2009). Effects of dietary polyunsaturated fatty acid supplementation on fatty acid composition in muscle and subcutaneous adipose tissue of lambs. **Journal of Animal Science**. 87: 4082-4091.

SAS, (1996). **User's Guide: Statistic**, Version 5. Edition. SAS. Inst, Cary, NC.,

U.S.A.

Steinfeld, H., Gerber, P., Wassenaar, T., Castel, V., Rosales, M. and de Haan, C. (2006). **Livestock's long shadow: Environmental issues and options**. Food and Agriculture Organization of the United Nations (FAO), Rome, Italy.

Steel, R.G.D., and Torrie, J.H. (1980). **Principles and Procedures of Statistics: A Biometrical Approach**, 2nd Ed. McGraw-Hill Inc, New York.

Vega, M., Urrutia, L., Iniguez, G., Gabler, F., Devoto, L., and Johnson, M.C. (2000) Nitric oxide induces apoptosis in the human corpus luteum *in vitro*. **Molecular Human Reproduction**. 6: 681-687.

Weston, R. H., Lindsay, J. R., Peter, D. W., and Buscall, D. J. (1989). Factors limiting the intake of feed by sheep. XIV Comparisons of voluntary and various transactions in the alimentary tract between lams and sheep fed roughages diets. **Australian Journal of Agricultural Research**. 40: 625-642.

Wood, J. D., Enser, M., Fisher, A.V., Nute, G.R., Sheard, P.R., Richardson, R.I., Hughes, S.I., and Whittington, F.M. (2008). Fat deposition, fatty acid composition and meat quality: A review. **Meat Science**.78: 343-358.

Zhou, Z., Meng, Q., and Yu, Z. (2011). Effects of methanogenic inhibitors on methane production and abundances of methanogens and cellulolytic bacteria in *in vitro* ruminal cultures. **Applied and Environmental Microbiology**. 77: 2634-2639.

CHAPTER VI

GENERAL CONCLUSION AND IMPLICATION

6.1 Conclusion

The aims of this thesis were to investigate the supplementation of sunflower oil and nitrate on methane production, nutrients digestibility, and growth performance in meat of goats fed with different roughage quality. For these purpose, the study was carried out consist of 3 experiments. The first experiment was subdivided into 2 past. The experiment was carried out to study the effects of sunflower oil and nitrate on methane production, *in vitro* organic matter digestibility by using gas production technique. The second experiment was conducted to investigate the effects of roughage quality on nutrient digestibility, rumen fermentation, utilization nitrogen and a number of rumen microbes. The last experiment was to study level of nitrate supplementation combine the quality of roughage on efficiency of growth performance and essential fatty acid accumulation in goat meat. The summary results from this study are presented as below:

First experiment, In conclusion, the rumen fermentation characteristic had affected at 6% of sunflower oil increases efficiency digestibility, organic matter digestibility, and metabolizable energy. The protozoa number did not affect by the interaction between sunflower oil and nitrate. However, the level of nitrate decreased total bacteria and increased concentration of ammonia nitrogen. Moreover, there was at 3% of sunflower oil increase concentration volatile fatty acids but at 6% However,

the ratio of acetate: propionate did not effect by dietary treat. Methane production had effect at 6% of sunflower oil and 3% of potassium nitrate can be decreased by 51% in low-quality roughage and 38 % in high-quality roughage.

Seconded experiment was aimed to study the effects of sunflower oil and nitrate supplementation on rumen fermentation, nitrogen utilization, rumen microorganisms and nutrient digestibility in meat goats fed with rice straw and pangola grass hay did not effect on feed intake, nutrient digestibility, N-balance, number of protozoa and pupation of ruminal microbial. There was significant on methane production by the interaction between sunflower oil and potassium nitrate decreased when received rice straw, but did not significant by pangola grass hay. The sunflower oil and the interaction between sunflower oil could be decreased number of bacteria when received both of roughage source.

The third experiment was focus on the effect of nitrate supplementation and quality of roughage on feed intake, nutrient digestibility, carcass traits, and muscle fatty acid profiles in meat goats. High quality roughage supplemented with 3% nitrate had an effect on nitrogen balance and % BW and Final BW, but had no effect on appearance/nutrient digestibility. Low quality roughage with nitrogen supplementation can improve nitrogen balance and total VFAs. The meat quality characteristics and carcass composition for the *longissimus dorsi* muscle were influenced by high quality roughage when the level of nitate in the goats' diets was increased. Improvements were noted in drip loss and CP in carcass composition, but there was no effect on cooking loss, colour and pH for the *longissimus dorsi* muscle.

Based on experimental data, 6% of sunflower oil is recommended and 3% of potassium nitrate with high quality roughage are suggested in term of reduce methane, improve ADG, % Dressing carcass and CLA.

6.2 Implications

Overall, based on experimental data, 6% of sunflower oil is recommended and 3% of nitrate with high quality roughage are suggested in term of reduce methane, improve ADG, % Dressing carcass and CLA.

The studies in this thesis have been performed to get more information and implication should be using a different kind of nitrate and lipid for meat goats, protection of oils from rumen biohydrogenation be further perspectives and encapsulation or protection of nitrate reducing the risk of nitrite toxicity for enhancing the sustainability of ruminant production should be advisable.

Future research should conduct by

- Dairy goat, supplement with 6% of sunflower oil is recommended and 3% of nitrate with high-quality roughage and should focus on milk production and milk composition.

- Meat goat, supplement with 6% of sunflower oil is recommended and 3% of nitrate with high-quality roughage and should focus on microbial community profile in the rumen, meat sensory evaluation, and n6 : n3 ratio.

Study on the methane production by respiration chambers

BIOGRAPHY

Ms. Jiravan Khotsakdee was born on June 29th, 1982 in Mahasarakham Province, Thailand. She was accepted to study M.Sc. Degree in Animal Science (Ruminant Nutrition). In 2011, she was continuing to study for her Doctor of Philosophy in Animal Production Technology, She enrolled at Suranaree University of Technology study Ruminant Nutrition in the School of Animal Production Technology, Institute of Agricultural Technology, Suranaree University of Technology, Nakhon Ratchasima, Thailand. In December, 2011, she was awarded a scholarship from The Thailand Research (TRF) though The Royal Golden Jubilee Ph.D. Program (RGJ. Ph.D. Program) under the supervision of Associate Professor Dr. Pramote Peangkoum. During her Ph.D. Degree study, Her advisor has also give very invaluable opportunity to her to attend and present research work in international Conferences. In 2015, Associate Professor Dr. Pramote Peangkoum, has supported and recommended her to undertake research at the Commonwealth Scientific and Industrial Research Organisation (CSIRO Australia) The Queensland Bioscience Precinct (QBP) in the University of Queensland (UQ) and Australian Tropical Sciences and Innovation Precinct (ATSIP) in the James Cook University, Queensland, Australia, for 6 month for training in microbial molecular techniques with Dr. Chris McSweeney under the finalcial support of the Rolyal Goldel Jubilee Ph.D. Program RGJ-Ph.D.). During her study at Commonwealth Scientific and Industrial Research Organisation (CSIRO Australia), she has gained a lot of research experiences and obtained new knowledge to enrich her Thesis work and work her vision and widening perspectives. Her Ph.D. research work focused on the Effect of sunflower oil and nitrate levels on production performance, nutrient digestibility meat and growth performant in meat goats fed with roughage quality.