การใช้น้ำมันหอมระเหยในโคเพื่อการปรับเปลี่ยนกระบวนการหมักของจุลินทรีย์ในกระเพาะหมัก

นายอธิฏฐาน นานนท์

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรดุษฎีบัณฑิต
สาขาวิชาเทคโนโลยีการผลิตสัตว์
มหาวิทยาลัยเทคโนโลยีสุรนารี
ปีการศึกษา 2557

USE OF ESSENTIAL OILS IN CATTLE FOR MANIPULATION OF RUMEN MICROBIAL FERMENTATION

Atitthan Nanon

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 2014

USE OF ESSENTIAL OILS IN CATTLE FOR MANIPULATION OF RUMEN MICROBIAL FERMENTATION

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

_____

(Dr. Wittawat Molee)

Chairperson

_____

(Assoc. Prof. Dr. Wisitiporn Suksombat)

Member (Thesis Advisor)

_____

(Asst. Prof. Dr. Pipat Lounglawan)

Member

_____

(Assoc. Prof. Dr. Pramote Paengkoum)

Member

_____

(Asst. Prof. Dr. Pakanit Kupittayanant)

Member

_____

(Assoc. Prof. Dr. Suthipong Uriyapongson)

Member

_____

(Prof. Dr. Sukit Limpijumnong)

Vice Rector for Academic Affairs and Innovation

_____

(Asst. Prof. Dr. Suwayd Ningsanond)

Dean of Institute of Agricultural Technology

อธิฏฐาน นานนท์ : การใช้น้ำมันหอมระเหยในโคเพื่อการปรับเปลี่ยนกระบวนการหมักของจุลินทรีย์ในกระเพาะหมัก (USE OF ESSENTIAL OILS IN CATTLE FOR MANIPULATION OF RUMEN MICROBIAL FFRMENTATION) อาจารย์ที่ปรึกษา : รองศาสตราจารย์ ดร.วิศิษฐิพร สุขสมบัติ, 148 หน้า.

วัตถุประสงค์ของงานวิจัยนี้คือ เพื่อศึกษาผลของการใช้น้ำมันหอมระเหยต่อการย่อยได้ของอาหารและกระบวนการหมักในห้องปฏิบัติการและตัวสัตว์

การทดลองที่ 1 การทดลองแบบ batch culture ทั้ง 2 ครั้ง วางแผนการทดลองแบบ complete randomized design โดยมี 2 ซ้ำต่อกลุ่มการทดลอง กลุ่มการทดลองใน batch culture แรก ได้แก่ กลุ่มควบคุม เสริมน้ำมันอบเชย เสริมน้ำมันกานพลู เสริมน้ำมันกระเทียม เสริมน้ำมันขิง เสริมน้ำมันตะไคร้ ระดับของการเสริมน้ำมันหอมระเหยแต่ละชนิดคือ 200 400 800 และ 1600 มิลลิกรัม/กิโลกรัม ในการทดลอง batch culture ที่สอง จะใช้กลุ่มควบคุมเหมือน batch culture แรก แต่ระดับของการเสริมน้ำมันหอมระเหยคือ 50 100 150 และ 200 มิลลิกรัม/กิโลกรัม ในการทดลอง batch culture ที่สอง อาหารที่ใช้ในการทดลองนี้เป็นอาหารในรูปแบบอาหารของโคนมซึ่งประกอบด้วย อาหารหยาบ 50% และอาหารข้น 50% การเสริมน้ำมันหอมระเหยเพิ่มการย่อยได้ของวัตถุแห้งแต่ไปลดความเข้มข้นของแอมโมเนียไนโตรเจนด้วยการเพิ่มระดับการใช้น้ำมันหอมระเหยจาก 0 200 400 800 ถึง 1600 มิลลิกรัม/กิโลกรัม ใน batch culture แรก จากผลการทดลองบ่งชี้ว่าที่ระดับการเสริม 200 มิลลิกรัม/กิโลกรัม จะเป็นระดับที่มีผลคุ้มค่าใช้ที่สุดของแต่ละชนิดของน้ำมันหอมระเหย ซึ่งผลการทดลองของการย่อยได้ของวัตถุแห้งและความเข้มข้นของแอมโมเนียไนโตรเจนใน batch culture ที่สองยังให้ผลดังเช่นเดิม

การทดลองที่ 2 มี 2 ส่วน โดยส่วนแรกคือการทดลองแบบ batch culture ส่วนที่ 2 คือการทดลองในโคเจาะกระเพาะ การทดลองแบบ batch culture วางแผนการทดลองแบบ complete randomized design ร่วมด้วยการจัดเรียงแบบ 2 × 4 factorial ของกลุ่มการทดลอง น้ำมันหอมระเหยที่ใช้คือ น้ำมันตะไคร้และน้ำมันหอมระเหยผสมระหว่างน้ำมันกระเทียมและขิงในสัดส่วน 1 ต่อ 1 ระดับการใช้ของน้ำมันหอมระเหยคือ 0 100 200 และ 300 มิลลิกรัม/กิโลกรัม อาหารประกอบด้วยข้าวสาลี DDGS เมล็ดข้าวบาร์เลย์ หญ้าแห้ง และอาหารผสม ในการทดลองในโคเจาะกระเพาะใช้โคสาวเจาะกระเพาะ 3 ตัว โดยให้อาหารผสมแบบไม่จำกัด น้ำมันหอมระเหยจะใช้เหมือนกับการทดลองแบบ batch culture แต่จะใช้เพียงระดับเดียว คือ 200 มิลลิกรัม/กิโลกรัม ระดับของน้ำมันหอมระเหยถูกเลือกจากผลการทดลอง batch culture อาหารที่ใช้ก็เหมือนกับการทดลองแบบ batch culture ผลการทดลองแสดงให้ทราบว่าการเสริมน้ำมันตะไคร้และน้ำมันหอมระเหยผสมระหว่างน้ำมันกระเทียมและขิงที่ระดับ 200 มิลลิกรัม/กิโลกรัม เพิ่มการย่อยได้ของวัตถุแห้ง

และเยื่อใยทั้งในการทดลองในห้องปฏิบัติการและโคเจาะกระเพาะซึ่งสนับสนุนโดยการเพิ่มการจับของจุลินทรีย์กับหญ้าแห้งในการทดลองโคเจาะกระเพาะ

การทดลองที่ 3 การทดลองนี้ทำเพื่อประเมินผลของน้ำมันตะไคร้ต่อการหมักและการย่อยสลายโปรตีนโดยใช้ RUSITEC วางแผนการทดลองแบบ complete randomized designโดยมี 4 ซ้ำต่อกลุ่มการทดลอง อาหารที่ใช้เป็นอาหารในรูปแบบอาหารของโคนม ประกอบด้วย อาหารหยาบ 50% และอาหารข้น 50% กลุ่มการทดลองคือ กลุ่มควบคุม เสริมน้ำมันตะไคร้ 100 มิลลิกรัม/กิโลกรัม เสริมน้ำมันตะไคร้ 200 มิลลิกรัม/กิโลกรัม และโมเนนซิน 30 มิลลิกรัม/กิโลกรัม น้ำมันตะไคร้ไม่มีผลต่อการย่อยได้ของอาหารและผลผลิตสุดท้ายจากการหมัก อย่างไรก็ตามน้ำมันตะไคร้เพิ่ม large peptide N (LPep N) และ small peptide plus amino acid N (SPep + AA N) แต่ลดความเข้มข้นของแอมโมเนียไนโตรเจน

การทดลองที่ 4 การทดลองทำเพื่อประเมินผลของสารออกฤทธิ์หลักของกระเทียม ขิง และตะไคร้ ต่อการกินได้ การย่อยได้ของอาหาร และการหมักในกระเพาะหมัก ในโคเจาะกระเพาะ กลุ่มการทดลองคือ กลุ่มควบคุม และการเสริม 2 มิลลิลิตร ของอัลลิซิน (allicin) ซินจิเบอร์รีน (zingiberene) และซิตรัล (citral) โคเจาะกระเพาะ 4 ตัวจะถูกแยกไปอยู่เดี่ยว โดยวางแผนการทดลองแบบ 4 × 4 Latin squares design อาหารข้น มีโปรตีน 21% ถูกให้วันละ 3 กิโลกรัม แบ่งเป็น 2 มื้อร่วมกับการให้ข้าวโพดหมักแบบไม่จำกัด การกินได้ไม่แตกต่างระหว่างกลุ่มการทดลอง แต่การย่อยได้ของวัตถุแห้งและเยื่อใยเพิ่มขึ้นจากการเสริมน้ำมันหอมระเหย น้ำมันหอมระเหยไม่มีผลต่อการหมักของกระเพาะหมักรวมถึงแอมโมเนียไนโตรเจนด้วยเช่นกันแต่ลดยูเรียไนโตรเจนในเลือด

สาขาวิชาเทคโนโลยีการผลิตสัตว์ ลายมือชื่อนักศึกษา _____

ปีการศึกษา 2557 ลายมือชื่ออาจารย์ที่ปรึกษา _____

ลายมือชื่ออาจารย์ที่ปรึกษาร่วม _____

ATITTHAN NANON : USE OF ESSENTIAL OILS IN CATTLE FOR MANIPULATION OF RUMEN MICROBIAL FERMENTATION. THESIS ADVISOR : ASSOC. PROF. WISITIPORN SUKSOMBAT, Ph.D., 148 PP.

ESSENTIAL OILS/FEED DIGESTION/RUMEN FERMENTATION

The objectives of the present study were to determine the effect of essential oils (EOs) on feed digestion and rumen fermentation in animals using laboratory experiments.

Experiment I, two batch cultures with a completely randomized design were used with three replicates per treatment. Treatments were control (CON), cinnamon oil (CIN), clove oil (CLO), garlic oil (GAR), ginger oil (GIN), and lemongrass oil (LEM). Four different doses were used for each EO; 200, 400, 800, and 1600 mg/kg. Treatments were the same as used in the first batch culture but the dosages were 50, 100, 150, and 200 mg/kg DM in the second batch culture. The feed was a dairy type ration consisting of 50% roughage and 50% concentrate. Supplementing EOs increased DM digestibility (DMD) but reduced ammonia N concentration with increasing EOs from 0, 200, 400, 800, to 1600 mg/kg in the first batch culture. The results suggested that the dose of 200 mg/kg was cost-effective for each EO, which is consistent with DMD and ammonia N concentration in the second batch culture.

Experiment II, there were two parts, firstly a batch culture, and secondly in situ. The batch culture was a completely randomized design with 2 × 4 factorial arrangement of treatments. The EOs were LEM and a combination of garlic oil and ginger oil at a ratio of 1 : 1 (CEO). The dosages of EO were 0, 100, 200, and 300 mg/kg. The substrates included wheat dried distillers' grain with solubles, barley grain, grass hay, and total mixed ration (TMR). For the in situ trial, three ruminally fistulated beef heifers were used and animals were fed ad libitum a TMR. The EOs

were the same as used in the batch culture, only one dosage (200 mg/kg) was tested. The feeds used were the same feedstuffs as in the batch culture. The results demonstrated that 200 mg/kg LEM or CEO were consistent with increased *in vitro* and *in situ* rumen DMD and NDF digestibility (NDFD) which were supported by increased microbial attachment of grass hay *in situ*.

In Experiment III, the trial was designed to evaluate the effect of LEM on *in vitro* fermentation characteristics and the protein degradation using RUSITEC. The experiment used a completely randomized design with four treatments and four replicates of each treatment. The substrate was a dairy ration consisting of 50% forage and 50% concentrate. Treatments were control, 100 mg/kg LEM, 200 mg/kg LEM, and 30 mg/kg MON. LEM had no effect on feed digestion and rumen end products. However, LEM increased large peptide N (LPep N) and small peptide plus amino acid N (SPep + AA N) but decreased concentration of ammonia N.

In Experiment IV, the trial evaluated the effect of the main active components of garlic, ginger, and lemongrass on feed intake, feed digestion, and rumen fermentation in fistulated cows. Treatments were control and 2 ml/d of allicin, zingiberene, and citral. Four fistulated crossbred Holstein Friesian non-lactating dairy cows housed in individual pens were assigned to each of four treatments in 4 × 4 Latin squares design. Diets consisted of 3 kg/d of concentrate containing 21 % CP, divided into 2 equal meals together with ad libitum corn silage. Feed intake was not different among treatments but DMD and NDFD were increased with EOs added. EOs had no effect on rumen fermentation including ammonia N but decreased blood urea N.

School of Animal Production Technology Student's Signature_____

Academic Year 2014 Advisor's Signature_____

Co-advisor's Signature_____

ACKNOWLEDGEMENTS

First of all, I would like to express my sincere thanks to my thesis advisor, Assoc. Prof. Dr. Wisitiporn Suksombat for his teaching, advice, invaluable help, continuous guidance and encouragement throughout the course of this research. I would also like to thank Asst. Prof. Dr. Pipat Lounglawan, my co-advisor, for helping, guidance and supports of the thesis. In addition, I would like to express my sincere thanks to Prof. Dr. Karen Beauchemin (senior scientist of Lethbridge Research Centre, Agriculture and Agri-Food Canada) for her teaching, advice, invaluable help, continuous guidance and encouragement throughout the course of this research. I would not have achieved this far and this thesis would not have been completed without all the support that I have always received from them. I would like to thank staff of dairy group, University farm and the Center of Scientific and Technological Equipment and my friends in Animal Production Technology, Suranaree University of Technology for helpful suggestion. Most importantly, I would like to give the biggest thank to my family for their love, support, suggestion and giving me the opportunity to pursue my graduate studies.

Noppharat Phakachoed

CONTENTS

Page

CONTENTS (Continued)

Page

CONTENTS (Continued)

Page

CONTENTS (Continued)

Page

CONTENTS (Continued)

Page

LIST OF TABLES

LIST OF TABLES (Continued)

LIST OF TABLES (Continued)

LIST OF TABLES (Continued)

Table **Page**

LIST OF ABBREVIATIONS

ADF	=	Acid detergent fiber
ADFD	=	Acid detergent fiber digestibility
C2	=	Acetate
C2 : C3	=	Acetate to propionate ratio
C3	=	Propionate
C4	=	Butyrate
CP	=	Crude protein
CPD	=	Crude protein digestibility
DM	=	Dry matter
DMD	=	Dry matter digestibility
Eos	=	Essential oils
GP	=	Gas production
LAB	=	Liquid-associated bacteria
NDF	=	Neutral detergent fiber
NDFD	=	Neutral detergent fiber digestibility
NH_3-N	=	Ammonia nitrogen
OM	=	Organic matter
SAB	=	Solid-associated bacteria
TMR	=	Total mixed ration
VFA	=	Volatile fatty acids

CHAPTER I

INTRODUCTION

1.1 Rationale of the study

Ruminants consume forage and concentrate feeds, as a result, rumen microorganisms degrade feed to produce volatile fatty acids (VFA) as energy source while they synthesize microbial protein as source of protein supplied for host animals. However, this process is inefficient to use nutrients due to losses of methane and ammonia N during fermentative process. Consequently, animals receive inadequate nutrients for maintenance and production. In the rumen, protein is hydrolyzed to oligo-peptides by proteolytic bacteria afterwards prevotella degrades oligo-peptides to dipeptides. Then various species of bacteria produce dipeptidases and metaloproteases for degrading dipeptides to amino acids afterwards deamination present, change amino acids to ammonia by hyper ammonia-producing bacteria (HAPB) including *Clostridium sticklandii* and *Peptostreptococcus anaerobius*. Plant essential oils (EOs) are volatile and lipophilic compound mixtures extracted from plants through distillation (Benchaar et al., 2008). Plant essential oils from variety of sources have been intensively studied during the last decades by ruminant scientists aiming to develop rumen modifiers for manipulating rumen fermentation as documented by several review papers (Calsamiglia et al., 2007; Hart et al., 2008; Benchaar and Greathead, 2011). EOs have bioactivity properties, such as antibacterial activity, antimethanogenesis, as well as enhancement of ruminal propionate proportion and by

pass protein to the intestine as reported by several previous reports (Wallace, 2004; Macheoeuf et al., 2008). Chemically, EOs are variable mixtures comprising a variety of compounds with low molecular weight, such as aliphatic hydrocarbons, acids, alcohols, aldehydes, phenols, acyclic esters or lactones, and others (Lin et al., 2013). Each type of EO has one or several main compounds that determine its key bioactivities. In vitro experiments reported that main components of EO had similar effects on rumen fermentation as their corresponding natural EO (Castillejos et al., 2006; Macheboeuf et al., 2008). The effects of EO on ruminal fermentation vary with their main active components (Busquet et al., 2006). The objective of this study was to determine the effect of EO supplementation on gas production (GP), fermentation characteristics, nutrient digestibility and microbial attachment *in vitro*, *in situ*, and *in vivo.*

1.2 Research objectives

1. To screen for the effects of various essential oils supplementation on gas production, fermentation characteristics, and nutrient disappearance using batch culture technique.

2. To determine the effect of essential oils supplementation on *in vitro* and *in situ* feed digestion in beef cattle.

3. To determine the effect of lemongrass oil for manipulation of ruminal fermentation using Rusitec technique.

4. To determine the effect of essential oils supplementation on feed intake, ruminal disappearance, and rumen fermentation profile using *in vivo* technique.

1.3 Research hypotheses

1. Supplementation of essential oils *in vitro* batch culture can reduce ammonia N concentration of total mixed ration.

2. Supplementation of essential oils can increase feed digestibility and microbial attachment of high fiber feed *in vitr*o and *in situ*.

3. Supplementation of lemongrass oil can increase nutrient digestibility but decrease ammonia N concentration of total mixed ration in Rusitec technique.

4. Supplementation of essential oils *in vivo* can increase nutrient disappearance but reduce ammonia N and BUN concentration.

1.4 Scope and limitation of the study

1. A 48 h *in vitro* batch culture method was used to examine the effects of essential oils on gas production, nutrient disappearance and rumen fermentation profile of individual feed or total mixed ration.

2. Fistulated ruminally Spayed beef heifers from Agriculture and Agri-Food Canada Research Centre, Lethbridge, Canada farm were used to examine the effects of essential oils on nutrient disappearance and microbial attachment of individual feed and total mixed ration.

3. Fistulated ruminally crossbred Holstein Friesian cows from Suranaree University's dairy farm were used to examine the effects of essential oils on feed intake, ruminal disappearance, and rumen fermentation profile of total mixed ration.

1.5 Expected results

1. Supplementing essential oils reduced ammonia N concentration using batch culture technique.

2. Supplementing essential oils increased feed digestibility and microbial attachment of high fiber feed both of *in vitro* and *in situ*.

3. Supplementation of lemongrass oil increased nutrient digestibility but decreased ammonia N concentration of total mixed ration in Rusitec technique.

4. Supplementation of essential oils *in sacco* increased nutrient disappearance but reduced ammonia N and BUN concentration.

1.6 References

Benchaar, C., Calsamiglia, S., Chaves, A. V., Fraser, G. R., Colombatto, D., McAllister, T. A., and Beauchemin, K. A. (2008). A review of plant-derived essential oils inruminant nutrition and production. **Anim. Feed Sci. Technol.** 145: 209-228.

Benchaar, C., and Greathead, H. (2011). Essential oils and opportunities to mitigate enteric methane emissions from ruminants. **Anim. Feed Sci. Technol.** 166-167: 338-355.

Busquet, M., Calsamiglia, S., Ferret, A., and Kamel, C. (2006). Plant extracts affect *in vitro* rumen microbial fermentation. **J. Dairy Sci.** 89: 761-771.

Calsamiglia, S., Busquet, M., Cardozo, P. W., Castillejos, L., and Ferret, A. (2007). Invited review : essential oils as modifiers of rumen microbial fermentation. **J. Dairy Sci.** 90: 2580-2595.

Castillejos, L., Calsamiglia, S., and Ferret, A. (2006). Effect of essential oil active compounds on rumen microbial fermentation and nutrient flow in in vitro systems. **J. Dairy Sci.** 89: 2649-2658.

Hart, K. J., Yanez-Ruiz, S. R., Duval, S. M., McEwan, N. R., and Newbold, C. J. (2008). Plant extracts to manipulate rumen fermentation. **Anim. Feed Sci. Technol.** 147: 8-35.

Lin, B., Wang, J. H., Lu, Y., Liang, Q., and Liu, J. X. (2013). *In vitro* rumen fermentation and methane production are influenced by active components of essential oils combined with fumarate. **J. Anim. Physiol. Anim. Nutr.** 97: 1-9.

Macheboeuf, D., Morgavi, D. P., Papon, Y., Mousset, J. L., and Arturo-Schaan, M. (2008). Dose-response effects of essential oils on *in vitro* fermentation activity of the rumen microbial population. **Anim. Feed Sci. Technol.** 145: 335-350.

Wallace, R. J. (2004). Antimicrobial properties of plant secondary metabolites. **Proc. Nutr. Soc.** 63: 621-629.

CHAPTER II

LITERATURE REVIEW

In the last decades, antibiotic ionophores have been using in ruminants for reducing losses of energy and protein resulted from inefficiency fermentative process as methane or ammonia N formed in the rumen. This inefficiency fermentation process not only decreases animal performance but also increases pollutant such as methane that directly associates with greenhouse effect. Using antibiotic such as ionophores in ruminants results in lower energy and protein losses in the rumen (Van Nevel and Demeyer, 1988). However, using antibiotics in animal feed is facing reduced social acceptation. During the last decade, using routine of antibiotics in livestock nutrition increases public concern because they might increase the emergence of antibiotic resistant bacteria that may increase risk of consumers' health. Using antibiotics in animal feed has been banned in European Union since January 2006 (Calsamiglia et al., 2007). Therefore, the new alternative additive should be unharmful for human and not be remained in animal products. For this reason, plant extracts such as essential oil or saponin are used to modify rumen fermentation by using their antimicrobial properties. Essential oils are classified as safe for human and animal consumption, and recognized as safe (GRAS; FDA, 2004) in the USA (Benchaar et al., 2008). Essential oils have antimicrobial properties against a wide range both of gram-positive and gram-negative microorganisms, including bacteria, protozoa, and fungi (Benchaar et al., 2008). Plant essential oils (EOs) from variety of sources have been intensively studied during the last decades by ruminant scientists

aiming to develop rumen modifiers for manipulating rumen fermentation as documented by several review papers (Calsamiglia et al., 2007; Hart et al., 2008; Benchaar and Greathead, 2011).

2.1 Essential oils

The extraction of essential oils can obtain from steam distillation from many parts of plant such as leaves, flowers, stem, seed, roots, and bark (Benchaar et al., 2008). However, essential oil composition can vary even when it is extracted from the same plant. In addition, age of plant, part of plant, environment that plant grows, or harvesting season also influences on chemical composition of essential oils (Benchaar et al., 2008). Essential oils are secondary metabolites that are divided into 2 groups of active compounds including terpenoids and phenylpropanoids. Terpenoids are 5 carbons (C5H8) basic structure but phenylpropanoids are compounds of chain 3 carbons bound to aromatic ring of 6 carbons (Calsamiglia et al., 2007). Burt et al. (2004) described mode of action of essential oil as essential oil interfere with bacterial cell membrane and mitochondria, interrupting the structure and permeable. Ions and cell contents can leak afterward. Essential also disrupting the proton motive force, electron flow, active transport and coagulation of cell contents. Bacterial cells will die after extensive loss of cell contents or the exit of critical molecules.

2.1.1 Effects of essential oils on ruminal fermentation

Since the announcement of the ban on antibiotics as feed additives in the European Union, there has been renewed interest in studying the effects and mechanisms of action of essential oils on rumen microbial fermentation (Calsamiglia et al., 2007)

Cinnamon oil (Cinnamaldehyde)

Cinnamaldehyde is a main component of cinnamon oil. Cinnamon oil or cinnamaldehyde has been intensive studies in last decade in both *in vitro* and *in vivo*. However, the results were varying depend on dosages and other factors such as substrate.

In vitro

In early, cinnamaldehyde was tested in batch culture or continuous culture for screening for optimal dose however the results were varied with the doses that used by different authors. Busquet et al. (2005c) reported that total VFA and nutrients digestibility were not affected with cinnamaldehyde supplementation but decreased acetate proportion and increased propionate proportion. Cardozo et al. (2006) reported that cinnamon leaf oil had no effect on total VFA and individual VFA proportion and large peptides (LPep) while decreased ammonia N (NH_3-N) and Holotrichs (protozoa) population but increased small peptides plus amino acids nitrogen (SPep + AA N). In contrast, total VFA, SPep + AA N and digestibility of DM, OM, CP, and starch were decreased but increased digestibility of NDF and unaffected NH_3-N as reported by Li et al. (2012). Fraser et al. (2007) also reported that total VFA concentration and ammonia N concentration reduced with 500 mg/L cinnamon leaf oil.

In vivo

Supplementation with cinnamaldehyde had no effect on any of the ruminal fermentation as well as the proportion of acetate, propionate and butyrate were not different in lamb as reported by Chaves et al. (2008a). Similarly with Chaves et al. (2008b), who observed that 200 mg/kg diet cinnamaldehyde had no effect on the molar proportion of individual VFA and ammonia N concentrations however reduced

ruminal pH which reflected the higher total VFA concentrations for lamb. Cardozo et al. (2006) demonstrated that ammonia N concentration and acetate proportion decreased while SPep + AA N increased with cinnamon oil supplementation in Holstein heifers.

Table 2.1 Effects of cinnamon oil on pH and volatile fatty acids concentration.

References	Treatments[1/]		pH	Total VFA (*mM*)	VFA (mol/100 mol)[3/]			
					C_2	C_3	C_4	$C_2 : C_3$
Busquet et al.	CON	0 mg/L		87.4^{b}	61.2^{a}	20.5^{d}	10.8^{c}	3.0^{a}
(2005c)	CDH	31.2 mg/L		85.7^{b}	55.8^{c}	24.2^{bc}	13.1^{bc}	2.3^{b}
	CDH	312 mg/L		88.0^{b}	57.0^{bc}	21.6^{cd}	14.3^{b}	2.6^{ab}
	SEM[2/]			3.28	0.94	1.24	1.47	0.14
Fraser et al.	CON	0 mg/L	6.88^{B}	43.3^{A}	52.9	26.1^{A}	14.2^{B}	2.04^{B}
(2007)	CIN	500 mg/L	6.94^{A}	25.5^{B}	53.8	13.0^{B}	25.8^{A}	4.13^{A}
	SEM[2/]		0.008	1.70	1.05	0.62	0.81	0.685
Li et al.	CON	0 mg/L	6.31^{B}	46.8^{a}	38.7^{B}	19.2^{A}	19.3	2.06^{B}
(2012)	CDH	300 mg/L	6.42^{A}	28.4^{b}	42.9^{A}	15.2^{B}	19.4	2.88^{A}
	SEM[2/]		0.021	2.89	1.09	0.62	0.78	0.094
Chaves et al.	CON	0 g/kg	6.38	81.0	53.6	29.5	11.0	1.8
(2008a)	CDH	200 g/kg	6.13	94.1	51.3	34.6	8.4	1.5
	SEM[2/]		0.132	7.64	1.4	2.4	1.4	0.17
Chaves et al.	CON	0 g/kg	5.93^{a}	97.2^{b}	49.1	39.8	6.6	1.24
(2008b)	CDH	200 g/kg	5.67^{b}	115.7^{a}	47.8	42.4	6.2	1.13
	SEM[2/]		0.101	4.85	1.24	1.75	0.73	0.094

[A–B] Means within a column with different superscripts differ ($P<0.01$).

[a–d] Means within a column with different superscripts differ ($P<0.05$).

[1/]CON = control; CDH = cinnamaldehdye; CIN = cinnamon oil.

[2/]SEM = standard error of the mean.

[3/]C_2 = acetate; C_3 = propionate; C_4 = butyrate; $C_2 : C_3$ = acetate to propionate ratio.

Table 2.2 Effects of cinnamon oil on nutrient digestibility and N fractions.

References	Treatments[1/]	Dose	Digestibility (%)				N fractions[3/]		
			DM	NDF	ADF	CP	NH_3-N	LPep	SPep + AA N
Busquet et al. (2005c)	CON	0 mg/L	62.0	38.8	36.7	49.6	21.5		
	CDH	31.2 mg/L	60.0	33.3	30.8	54.4	18.5		
	CDH	312 mg/L	61.4	33.5	29.9	65.7	20.6		
	SEM[2/]		2.37	3.49	4.01	5.33	1.67		
Fraser et al. (2007)	CON	0 mg/L	54.2	39.3^{a}	13.8^{A}		18.6		
	CIN	500 mg/L	53.2	35.3^{b}	11.2^{B}		19.9		
	SEM[2/]		0.39	0.91	0.15		0.60		
Li et al. (2012)	CON	0 mg/L	48.3^{A}	23.2^{B}		52.9^{A}	10.6	11.0	5.4^{A}
	CDH	300 mg/L	42.5^{B}	25.5^{A}		47.6^{B}	8.9	12.1	3.5^{B}
	SEM[2/]		0.94	1.10		1.07	0.80	1.46	0.67

[A–B] Means within a column with different superscripts differ ($P<0.01$).

[1/]CON = control; CDH = cinnamaldehdye; CIN = cinnamon oil.

[2/]SEM = standard error of the mean.

[3/]N fractions : NH_3-N = ammonia N; LPep = large peptides; SPep + AA N = Small peptides plus amino acids N.

Clove oil (Eugenol)

The main active component of clove oil is eugenol. Clove oil or eugenol is another one of essential oil that was interested by ruminant nutritionists for improve rumen fermentation. The last decades, clove oil or eugenol was tested both of *in vitro* and *in vivo*. However, the results still unclear like cinnamaldehyde for nutrient digestibility and rumen fermentation.

In vitro

In some of the studies reported that total VFA concentration, butyrate proportion, and ammonia N concentration were reduced by eugenol at high dosage (Cardozo et al., 2005; Busquet et al., 2006). Similarly with Catillejos et al. (2006) who observed that the concentration of ammonia N and total VFA were reduced with 5000 mg/L eugenol but increased pH without effect on nutrients digestibility and individual VFA proportion. In contrast, at the dose 800 mg/L eugenol increased pH but decreased digestibility of DM and NDF, gas production, whereas total VFA concentration, individual VFA proportion, and ammonia N were not affected (Benchaar et al., 2007). The increased pH was associated with a reduction of total VFA concentrations, reflecting a decrease in diet fermentability, which is a consistent with the antimicrobial activity of phenolic compounds (Benchaar et al., 2007).

In vivo

The results from *in vivo* experiments are the same direction. Supplementation with eugenol had on effect on rumen fermentation and nutrients digestibility (Benchaar et al., 2012; Lourenco et al., 2008). However, ruminal degradability of NDF linearly decreased and degradation of N in the rumen tended to linearly decrease with increasing EUG supplementation, whereas total VFA concentration, individual VFA proportion, ammonia N, protozoa, and blood metabolites were not affected (Yang et al., 2010).

Table 2.3 Effects of clove oil on pH and volatile fatty acids concentration.

References	Treatments[1/]	Dose	pH	Total VFA (*mM*)	VFA (mol/100 mol)[3/]			
					C_2	C_3	C_4	$C_2 : C_3$
Benchaar et al. (2007)	CON	0 mg/L	5.58	89.8	5.54	24.6^{a}	16.3^{b}	2.3^{b}
	CLO	200 mg/L	5.64	104.4	5.64	24.0^{a}	15.9^{b}	2.4^{b}
	EUG	800 mg/L	5.92	76.0	48.4	12.5^{b}	33.1^{a}	3.9^{a}
	SEM[2/]		0.029	5.31	1.43	0.68	1.26	0.28
Busquet et al. (2006)	CON	0 mg/L	5.9^{B}	187.3^{A}	57.9	27.0^{B}	9.4^{B}	
	EUG	3 mg/L	5.9^{B}	182.7^{A}	57.9	27.0^{B}	9.5^{B}	
	EUG	30 mg/L	5.9^{B}	186.0^{A}	57.8	27.2^{B}	9.4^{B}	
	EUG	300 mg/L	6.0^{B}	175.5^{A}	57.6	26.6^{B}	10.5^{A}	
	EUG	3000 mg/L	7.1^{A}	107.4^{B}	57.4	29.5^{A}	8.9^{C}	
	SEM[2/]		0.13	4.93	1.96	1.86	0.12	
Castillejos et al. (2006)	CON	0 mg/L	6.46^{b}	140.4^{a}	64.9	20.6^{a}	10.5	3.55
	EUG	5 mg/L	6.42^{b}	134.8^{a}	63.8	21.0^{a}	11.0	3.36
	EUG	50 mg/L	6.43^{b}	137.0^{a}	64.0	20.9^{a}	10.9	3.38
	EUG	500 mg/L	6.56^{b}	133.4^{a}	65.1	19.8^{b}	11.3	3.66
	EUG	5000 mg/L	7.35^{a}	66.1^{b}	64.7	21.0^{a}	10.5	3.45
	SEM[2/]		0.08	3.95	0.46	0.29	0.36	0.08

Table 2.3 Effects of clove oil on pH and volatile fatty acids concentration (Continued).

References	Treatments[1/]	Dose	pH	Total VFA (*mM*)	VFA (mol/100 mol)[3/]			
					C_2	C_3	C_4	$C_2 : C_3$
Benchaar et al. (2012)	LC	0 mg/L	6.22	130.0	63.9	19.3	13.1	3.37
	LC + EUG	50 mg/L	6.23	124.0	63.5	19.6	13.0	3.30
	HC	0 mg/L	6.05	135.0	59.8	23.1	13.5	2.68
	HC + EUG	50 mg/L	6.03	135.0	59.6	23.4	13.0	2.60
	SEM[2/]		0.053	3.9	0.78	1.05	0.36	0.138
Yang et al. (2010)	CON	0 mg/d	6.13	122.1	65.1	17.3	13.0	4.26
	EUG	400 mg/d	6.12	120.8	64.0	16.2	15.3	4.06
	EUG	800 mg/d	6.21	116.4	62.1	19.0	14.6	3.35
	EUG	1600 mg/d	6.23	114.6	62.3	20.9	12.7	3.23
	SEM[2/]		0.087	6.45	2.0	2.30	1.41	0.591

[A–C] Means within a column with different superscripts differ ($P<0.01$).

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

[1/]CON = control; CLO = clove oil; EUG = eugenol; LC = low concentrate-the forage : concentrate is 65 : 35; LC + EUG = low concentrate with 50 mg/kg eugenol; HC = high concentrate-the forage : concentrate is 35 : 65; HC + EUG = high concentrate with 50 mg/kg eugenol.

[2/]SEM = standard error of the mean.

[3/]C_2 = acetate; C_3 = propionate; C_4 = butyrate; $C_2 : C_3$ = acetate to propionate ratio.

Table 2.4 Effects of clove oil on nutrient digestibility and N fractions.

References	Treatments[1/]	Dose	Digestibility (%)				N fractions[3/]		
			DM	NDF	ADF	CP	NH_3-N	LPep	SPep+AA N
Benchaar et al. (2007)	CON	0 mg/L	31.7[a]	27.7[a]			11.7		
	CLO	200 mg/L	32.0[a]	19.8[b]			13.3		
	EUG	800 mg/L	24.5[b]	7.2[c]			8.4		
	SEM[2/]		1.07	1.35			2.61		
Busquet et al. (2006)	CON	0 mg/L					32.2[A]		
	EUG	3 mg/L					31.7[A]		
	EUG	30 mg/L					31.8[A]		
	EUG	300 mg/L					28.6[A]		
	EUG	3000 mg/L					16.9[B]		
	SEM[2/]								
Castillejos et al, (2006)	CON	0 mg/L	51.0	201.0	27.8		21.9[a]		
	EUG	5 mg/L	49.4	23.0	31.3		19.9[a]		
	EUG	50 mg/L	53.9	18.7	30.4		17.1[a]		
	EUG	500 mg/L	61.4	12.3	20.1		16.9[a]		
	EUG	5000 mg/L					10.4[b]		
	SEM[2/]		1.97	6.15	5.86		1.94		

Table 2.4 Effects of clove oil on nutrient digestibility and N fractions (Continued).

References	Treatments[1/]	Dose	Digestibility (%)				N fractions[3/]		
			DM	NDF	ADF	CP	NH_3-N	LPep	SPep+AA N
Benchaar et al. (2012)	LC	0 mg/L	69.3		56.5	69.3	5.97		
	LC + EUG	50 mg/L	69.4		58.4	68.3	5.86		
	HC	0 mg/L	67.1		55.8	65.2	5.80		
	HC + EUG	50 mg/L	66.6		54.2	66.7	6.36		
	SEM[2/]		0.53		0.99	0.64	0.260		
Yang et al. (2010)	CON	0 mg/d		47.8		66.9	3.86		
	EUG	400 mg/d		45.3		63.0	3.99		
	EIG	800 mg/d		41.0		60.6	4.30		
	EUG	1600 mg/d		38.5		59.0	4.39		
	SEM[2/]			4.69		4.18	0.770		

[A–C] Means within a column with different superscripts differ (P<0.01).

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

[1/]CON = control; CLO = clove oil; EUG = eugenol; LC = low concentrate-the forage : concentrate is 65 : 35; LC + EUG = low concentrate with 50 mg/kg eugenol; HC = high concentrate-the forage : concentrate is 35 : 65; HC + EUG = high concentrate with 50 mg/kg eugenol.

[2/]SEM = standard error of the mean.

[3/]N fractions : NH_3-N = ammonia N; LPep = large peptides; SPep + AA N = Small peptides plus amino acids N.

Garlic oil (Allicin)

Garlic oil is a complex mix of many different compounds present in the plant or derived from processing. The garlic oil and 4 purified active components (allicin, diallyl sulfide, diallyl disulfide, and allyl mercaptan) thought to play a major role in its antimicrobial activity. It has antimicrobial activity against a wide spectrum of gram-positive and gram-negative bacteria and its potential effect on modifying rumen microbial fermentation has been studied recently (Calsamiglia et al., 2007; Kongmun et al., 2010).

In vitro

At the high dose of garlic oil (3000 mg/L) seems to be a toxic for rumen microbial fermentation such as reduced total VFA concentrations as reported in previous studies (Busquet et al., 2005a; Busquet et al., 2006). Addition with 300 or 312 mg/L garlic oil had no effect on DM, NDF, and ADF digestion (Busquet et al., 2005a; Busquet et al., 2005c; respectively). Garlic oil decreased total VFA concentration and acetate proportion but increased propionate and butyrate proportion (Busquet et al., 2005a; Busquet et al., 2005c; Busquet et al., 2006; Cardozo et al., 2005). The proportion of propionate is increase that is more efficient for beef production system.

In vivo

Garlic oil had no effect on feed digestibility, rumen end products, and protozoa as reported by previous studies (Chaves et al., 2008a; Yang et al., 2007). Although, garlic oil and the organosulfur are known to exhibit a number of antimicrobial activities. However, garlic oil unaffected methane production and protozoa, although ammonia N was decreased (Klevenhusen et al., 2011).

Table 2.5 Effects of garlic oil on pH and volatile fatty acids concentration.

References	Treatments[1/]		Dose	pH	Total VFA (*mM*)	VFA (mol/100 mol)[3/]			
						C_2	C_3	C_4	$C_2 : C_3$
Busquet et al. (2005a)	**Exp. 1**	CON	0 mg/L	6.2^{b}	123.1^{a}	65.3^{a}	17.3^{b}	13.1^{d}	
		GAR	3 mg/L	6.1^{b}	127.9^{a}	64.5^{a}	17.7^{b}	13.4^{d}	
		GAR	30 mg/L	6.2^{b}	124.9^{a}	62.9^{a}	18.3^{b}	14.3^{c}	
		GAR	300 mg/L	6.4^{a}	110.1^{b}	58.5^{b}	20.1^{a}	16.9^{b}	
		GAR	3000 mg/L	6.6^{a}	92.0^{c}	59.9^{b}	16.4^{c}	19.2^{a}	
		SEM[2/]		0.14	4.95	0.27	0.20	0.33	
	Exp. 2	CON	0 mg/L		110.4	62.7^{a}	20.5^{b}	11.6^{b}	
		GAR	300 mg/L		100.9	46.1^{b}	32.0^{a}	15.6^{a}	
		SEM[2/]			3.82	1.24	1.33	0.91	
Busquet et al. (2005c)		CON	0 mg/L		87.4	61.2^{a}	20.5^{b}	10.8^{b}	3.0^{a}
		GAR	31.2 mg/L		93.8	58.5^{a}	22.6^{b}	11.3^{b}	2.6^{a}
		GAR	312 mg/L		94.3	46.8^{b}	27.4^{a}	19.4^{a}	1.7^{b}
		SEM[2/]			3.28	0.94	1.24	1.47	0.14
Chaves et al. (2008a)		CON	0 mg/kg	6.38	81.0	53.6	29.5	11.0	1.8
		GAR	200 mg/kg	6.08	100.8	52.9	30.9	9.0	1.7
		SEM[2/]		0.132	7.64	1.4	2.4	1.4	0.17

Table 2.5 Effects of garlic oil on pH and volatile fatty acids concentration (Continued).

References	Treatments[1/]	Dose	pH	Total VFA (*mM*)	VFA (mol/100 mol)[3/]			
					C_2	C_3	C_4	$C_2 : C_3$
Yang et al. (2007)	CON	0 g/d	6.12	128.7	60.7	24.7	10.2	2.72
	GAR	5 g/d	6.15	126.8	60.1	25.6	10.0	2.60
	SEM[2/]		0.13	6.30	3.30	3.70	0.60	0.12

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

[1/]CON = control; GAR = garlic oil.

[2/]SEM = standard error of the mean.

[3/]C_2 = acetate; C_3 = propionate; C_4 = butyrate; $C_2 : C_3$ = acetate to propionate ratio.

Table 2.6 Effects of garlic oil on nutrient digestibility and N fractions.

References	Treatments[1/]		Dose	Digestibility (%)				N fractions[3/]		
				DM	NDF	ADF	CP	NH_3-N	LPep	SPep+AA N
Busquet et al. (2005a)	**Exp. 1**	CON	0 mg/L					21.9[b]		
		GAR	3 mg/L					23.7[b]		
		GAR	30 mg/L					24.7[a]		
		GAR	300 mg/L					23.1[b]		
		GAR	3000 mg/L					20.9[b]		
		SEM[2/]						0.7		
	Exp. 2	CON	0 mg/L	59.8	36.7	36.0		5.7	3.6	2.9
		GAR	300 mg/L	55.3	33.5	29.6		6.8	5.1	3.4
		SEM[2/]		2.57	1.83	1.82		1.24	0.91	0.50
Busquet et al. (2005c)		CON	0 mg/L	62.0	38.8	36.7	49.6	21.5	6.8	1.9[b]
		GAR	31.2 mg/L	64.1	39.4	36.0	56.1	20.1	7.5	3.4[ab]
		GAR	312 mg/L	58.9	30.8	25.0	45.9	19.0	5.5	4.6[a]
		SEM[2/]		0.37	3.49	4.01	5.33	1.67	1.41	2.12
Chaves et al. (2008a)		CON	0 mg/kg					8.4		
		GAR	200 mg/kg					6.9		
		SEM						2.63		

Table 2.6 Effects of garlic oil on nutrient digestibility and N fractions (Continued).

References	Treatments[1/]	Dose	Digestibility (%)				N fractions[3/]		
			DM	NDF	ADF	CP	NH_3-N	LPep	SPep+AA N
Yang et al. (2007)	CON	0 g/d	49.4[B]	42.9	40.7		5.45		
	GAR	5 g/d	55.2[A]	39.9	38.0		5.51		
	SEM[2/]		2.4	2.8	3.6		0.72		

[A–B] Means within a column with different superscripts differ ($P<0.01$).

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

[1/]CON = control; GAR = garlic oil.

[2/]SEM = standard error of the mean.

[3/]N fractions : NH_3-N = ammonia N; LPep = large peptides; SPep + AA N = Small peptides plus amino acids N.

Ginger oil (Zingiberene)

The effect of ginger oil on rumen fermentation is limited. Ginger is a herb that also known for antimicrobial activities. Hammer et al. (1999) showed that ginger have effect for inhibit 10 different microorganisms. Garlic oil has many compounds such as α-pinene, camphene, β-pinene, linalool, borneol, γ-terpineol, nerol, geraniol, geranial, zingiberene, etc. At our best knowledge, there is one *in vitro* study that investigates the effect of ginger oil on rumen fermentation. Busquet et al. (2005b) reported that 180 g/kg ginger oil had unaffected rumen fermentation and N fractions.

Table 2.7 Effects of ginger oil on pH, volatile fatty acids concentration, and N fractions.

Items	Treatments[1/]		SEM[2/]
	CON	GIN	
Total VFA (*mM*)	114.1	108.0	3.48
VFA (mol/100 mol)[3/]			
C_2	61.9	60.5	1.14
C_3	23.2	24.2	1.36
C_4	9.6	10.0	0.79
C_2: C_3	2.7	2.5	0.12
N fractions[4/] (mg/100 mL)			
NH_3-N	7.5	7.1	1.57
LPep	3.8	4.8	3.19
SPep + AA N	5.2	4.0	1.19

[1/]CON = control; GIN = ginger oil.

[2/]SEM = standard error of the mean.

[3/]C_2 = acetate; C_3 = propionate; C_4 = butyrate; C_2 : C_3 = acetate to propionate ratio.

[4/]N fractions : NH_3-N = ammonia N; LPep = large peptides; SPep + AA N = Small peptides plus amino acids N.

Source : Busquet et al. (2005b).

Lemongrass oil (Citral)

Lemongrass is a herb that widely used in tropical countries food composition and antibacterial agent. Citral is an active component of lemongrass oil. Lemongrass products have properties to be antibacterial and antihyper-ammonia producing bacteria (Valero and Salmeroìn, 2003; McIntosh et al., 2003). Blood metabolites and rumen fermentation in Holstein steers also change with lemongrass products (Hosoda et al., 2006). However, the study of lemongrass was little evaluated on its effects on ruminal fermentation compared with other essential oil, such as cinnamon oil, clove oil, and garlic oil.

Wanapat et al. (2008) showed that digestibility of nutrients is dose dependent manner. At the dose 100 g/d lemongrass powder improved digestibility of DM but reduced CP without effect on NDF and ADF in beef cattle steers. At the same time, Digestibility of DM, NDF, and ADF had no effect with 200 or 300 g/d lemongrass powder. Wanapat et al. (2013) also reported 100 g/d lemongrass meal unchanged DM digestibility in beef cattle.

Table 2.8 Effects of lemongrass oil on pH and volatile fatty acids concentration.

References	Treatments[1/]	Dose	pH	Total VFA (*mM*)	VFA (mol/100 mol)[3/]			
					C_2	C_3	C_4	$C_2 : C_3$
Wanapat et al. (2008)	CON	0 g/d	6.5	105.6	69.3	22.0	8.6	3.2
	LP	100 g/d	6.5	114.1	67.7	24.1	8.3	2.8
	LP	200 g/d	6.4	112.8	70.7	21.0	8.3	3.4
	LP	300 g/d	6.4	106.5	71.0	20.4	8.6	3.6
	SEM[2/]		0.04	4.30	1.42	1.18	0.49	0.29
Wanapat et al. (2013)	CON	0 g/d	6.54	109.1	73.2^{a}	2.7^{b}	13.2	3.2^{A}
	LM	100 g/d	6.73	105.0	70.0^{b}	22.6^{b}	12.4	3.1^{A}
	LM + LP	100 + 10 g/d	6.81	102.0	66.7^{c}	22.8^{b}	12.4	2.9^{B}
	LM + LP + GP	100 + 10 + 40 g/d	6.90	101.9	66.9^{c}	23.2^{a}	11.7	2.9^{B}
	SEM[2/]		0.08	2.47	0.50	0.47	0.28	0.07

[A–B] Means within a column with different superscripts differ ($P<0.01$).

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

[1/]CON = control; LP = lemongrass powder; LM = lemongrass meal; GP = garlic powder.

[2/]SEM = standard error of the mean.

[3/]C_2 = acetate; C_3 = propionate; C_4 = butyrate; $C_2 : C_3$ = acetate to propionate ratio.

Table 2.9 Effects of clove oil on nutrient digestibility, ammonia N concentration, and blood urea N.

References	Treatments[a]	Dose	Digestibility (%)				NH_3-N[3/]	BUN[3/]
			DM	NDF	ADF	CP		
Wanapat et al. (2008)	CON	0 g/d	64.7^{b}	71.0^{ab}	61.2	77.9^{a}	19.1^{A}	13.5^{a}
	LP	100 g/d	74.8^{a}	72.5^{a}	68.2	74.0^{b}	17.5^{B}	11.1^{b}
	LP	200 g/d	66.8^{b}	71.9^{ab}	61.2	70.8^{bc}	16.7^{BC}	10.8^{b}
	LP	300 g/d	62.0^{b}	66.6^{b}	60.3	69.7^{c}	15.7^{C}	12.3^{ab}
	SEM[2/]		2.14	2.03	3.62	0.95	0.43	0.55
Wanapat et al. (2013)	CON	0 g/d	61.0	65.0	54.0	60.0^{ab}	22.8^{a}	13.4^{a}
	LM	100 g/d	67.0	69.0	57.0	65.0^{a}	21.2^{a}	11.2^{b}
	LM + LP	100+10 g/d	60.0	59.0	54.0	57.0^{ab}	18.7^{b}	9.8^{c}
	LM + LP + GP	100+10+40 g/d	61.0	56.0	53.0	53.0^{b}	18.3^{b}	9.5^{c}
	SEM[2/]		2.7	1.3	0.2	6.4	0.53	0.73

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

[1/]CON = control; LP = lemongrass powder; LM = lemongrass meal; GP = garlic powder.

[2/]SEM = standard error of the mean.

[3/]NH_3-N = ammonia N; BUN = blood urea N.

2.2 References

Benchaar, C., Calsamiglia, S., Chaves, A. V., Fraser, G. R., D. Colombatto, McAllister, T. A., and Beachemin, K. A. (2008). A review of plant-derived essential oils in ruminant nutrition and production. **Anim. Feed Sci. Technol.** 145: 209-228.

Benchaar, C., Chaves, A. V., Fraser, G. R., Wang, Y., Beauchemin, K. A., and McAllister, T. A. (2007). Effects of essential oils and their components on *in vitro* rumen microbial fermentation. **Can. J. Anim. Sci.** 87: 413-419.

Burt, S. (2004). Essential oils : their antibacterial properties and potential applications in food-a review. **Int. J. Food Microbiol.** 94: 223-253.

Busquet, M., Calsamiglia, S., Ferret, A., Carro, D., and Kamel, C. (2005a). Effect of garlic oil and four of its compounds on rumen microbial fermentation. **J. Dairy Sci.** 88: 4393-4404.

Busquet, M., Calsamiglia, S., Ferret, A., Cardozo, P. W., and Kamel, C. (2005c). Effects of cinnamaldehyde and garlic oil on rumen microbial fermentation in a dual flow continuous culture. **J. Dairy Sci.** 88: 2508-2516.

Busquet, M., Calsamiglia, S., Ferret A., and Kamel, C. (2005b). Screening for effects of plants on dairy cattle rumen microbial fermentation in a continuous culture system. **Anim. Feed Sci. Technol.** 123: 597-613.

Busquet, M., Calsamiglia, S., Ferret, A., and Kamel, C. (2006). Plant extracts affect *in vitro* rumen microbial fermentation. **J. Dairy Sci.** 89: 761-771.

Calsamiglia, S., Busquet, M., Cardozo, P. W., Castillejos, L., and Ferret, A. (2007). Invited review : Essential oils as modifiers of rumen microbial fermentation. **J. Dairy Sci.** 90: 2580-2595.

Cardozo, P. W., Calsamiglia, S., Ferret, A., and Kamel, C. (2005). Screening for the effects of natural plant extracts at different pH on *in vitro* rumenmicrobial fermentation of a high-concentrate diet for beef cattle. **J. Anim. Sci.** 83: 2572-2579.

Cardozo, P. W., Calsamiglia, S., Ferret, A., and Kamel, C. (2006). Effects of alfalfa extract, anise, capsicum, and a mixture of cinnamaldehyde and eugenol on ruminal fermentation and protein degradation in beef cattle heifers fed a high-concentrate diet. **J. Anim. Sci.** 84: 2801-2808.

Castillejos, L., Calsamiglia, S., and Ferret, A. (2006). Effect of essential oil active compounds on rumen microbial fermentation and nutrient flow in *in vitro* systems. **J. Dairy Sci.** 89: 2649-2658.

Chaves, A. V., Stanford, K., Dugan, M. E. R., Gibson, L. L., McAllister, T. A., Van Herk, A., and Benchaar, A. (2008a). Effects of cinnamaldehyde, garlic and juniper berry essential oils on rumen fermentation, blood metabolites, growth performance, and carcass characteristics of growing lambs. **Livest. Sci.** 117: 215-224.

Chaves, A. V., Stanford, K., Gibson, L. L., McAllister, T. A., and Benchaar, A. (2008b). Effects of carvacrol and cinnamaldehyde on intake, rumen fermentation, growth performance, and carcass characteristics of growing lambs. **Anim. Feed Sci. Technol.** 145: 396-408.

Fraser, G.R., Chaves, A.V., Wang, Y., McAllister, T.A., Beauchemin, K.A., and Benchaar, C. (2007). Assessment of the effects of cinnamon leaf oil on rumen microbial fermentation using two continuous culture systems. **J. Dairy Sci.** 90: 2315-2328.

Hammer, K. A., Carson, C. F., and Riley, T. V. (1999). Antimicrobial activity of essential oils and other plant extracts. **J. Appl. Microbiol.** 86: 985-990.

Hosoda, K., Kuramoto, K., Eruden, B., Nishida, T., and Shioya, S. (2006). The effects of three herbs as feed supplements on blood metabolites, hormones, antioxidant activity, IgG concentration, and ruminal fermentation in Holstein steers. **Asian-Aust. J. Anim. Sci.** 19: 35-41.

Klevenhusen, K., Zeitz, J. O., Duval, S., Kreuzer, M., and Soliva, C. R. (2011). Garlic oil and principal component diallyl disulfide fail to mitigate methane, but improve digestibility in sheep. **Anim. Feed Sci. Technol.** 166: 356-363.

Kongmun, P., Wanapat, M., Pakdee, P., and Navanukraw, C. (2010). Effect of coconut oil and garlic powder on *in vitro* fermentation using gas production technique. **Livest. Sci.** 127: 38-44.

Li, Y. L., He, M. L., Li, C., Forster, R., Beauchemin, K. A., and Yang, W. Z. (2012). Effects of wheat dried distillers' grains with solubles and cinnamaldehyde on *in vitro* fermentation and protein degradation using the Rusitec technique. **Arch. Anim. Nutr.** 66: 131-148.

Lourenço, M., Cardozo, P. W., Calsamiglia, S., and Fievez, V. (2008). Effects of saponins, quercetin, eugenol, and cinnamaldehyde on fatty acid biohydrogenation of forage polyunsaturated fatty acids in dual-flow continuous culture fermenters. **J. Anim. Sci.** 86: 3045-3053.

McIntosh, F. M., Williams, P., Losa, R., Wallace, R. J., Beever, D. A., and Newbold, C. J. (2003). Effect of essential oil on ruminal metabolism and their protein metabolism. **Appl. Environ. Microbiol.** 69: 5011-5014.

Valero, M., and Salmeroìn, M. C. (2003). Antibacterial activity of 11 essential oils against Bacillus cereus in tyndallized carrot broth. **Int. J. Food Microbiol.** 85: 73-81.

Wanapat, M., Cherdthong, A., Pakdee, P., and Wanapat, S. (2008). Manipulation of rumen ecology by dietary lemongrass (Cymbopogon citratus Stapf.) powder supplementation. **J. Anim. Sci.** 86: 3497-3503.

Wanapat, W., Kang, S., Khejornsart, P., and Wanapat, S. (2013). Effects of plant herb combination supplementation on rumen fermentation and nutrient digestibility in beef cattle. **Asian-Aust. J. Anim. Sci.** 26: 1127-1136.

Yang, W. Z, Benchaar C, Ametaj, B. N., and Beauchemin K. A. (2010). Dose response to eugenol supplementation in growing beef cattle : Ruminal fermentation and intestinal digestion. **Anim. Feed Sci. Technol.** 158: 57-64.

Yang, W. Z., Benchaar, C., Ametaj, B. N., Chaves, A. V., He, M. L., and McAlister, T. A. (2007). Effects of garlic and juniper berry essential oils on ruminal fermentation and on the site and extent of digestion in lactating cows. **J. Dairy Sci.** 90: 5671-5681.

CHAPTER III

USE OF ESSENTIAL OILS FOR MANIPULATION OF RUMEN MICROBIAL FERMENTATION USING BATCH CULTURE

3.1 Abstract

The objective of this study was to evaluate the effects of essential oils on feed digestibility, gas production and rumen fermentation. Two batch cultures were designed for screening various doses of each essential oil (EO). Treatments were control (CON), cinnamon oil (CIN), clove oil (CLO), garlic oil (GAR), ginger oil (GIN), and lemongrass oil (LEM). The dosages were 200, 400, 800, and 1600 mg/kg DM in Experiment 3.1 (1st batch) and 50, 100, 150, and 200 mg/kg DM in Experiment 3.2 (2nd batch). Digestibility of DM (DMD), neutral detergent fiber (NDFD) and acid detergent fiber (ADFD) were measured at 24 h and 48 h post incubation, while gas production (GP) was read at 3, 6, 12, 24, 36, and 48 h post incubation. The feed was a dairy type ration consisting of 50% roughage (35% grass hay and 15% alfalfa hay) and 50% concentrate (20% barley grain, 10% corn DDGS, 10% wheat DDGS, 5% canola meal, and 5% vitamin and mineral supplements). All essential oils could improve DM disappearance with consistency result both of the Experiment 3.1 and Experiment 3.2. Meanwhile, essential oils had no effect on NDF and ADF digestibility. Total VFA concentration and individual VFA proportion in the

Experiment 3.1 were unaffected by essential oils but 200 mg/kg DM of each EO increased total VFA concentration without effect on individual VFA proportion in the Experiment 3.2. Ammonia N concentration was reduced by essential oils both in the Experiment 3.1 and 3.2 which confirmed the effect of essential oils on deamination. However, the effect of EO on methane production was apparently negligible. These results suggested that EO used in the present study could be potentially developed as rumen modifier to improve feed digestibility in the rumen.

3.2 Introduction

Plant essential oil (EO) from variety of sources have been intensively studied during the last decades by ruminant scientists aiming to develop rumen modifiers for manipulating rumen fermentation as documented by several review papers (Calsamiglia et al., 2007; Hart et al., 2008; Benchaar and Greathead, 2011). Many studies focused on cinnamon oil (cinnamaldehyde) or clove oil (eugenol) to evaluate the effects on rumen fermentation characteristics in particular (Busquet et al., 2006; Cardozo et al., 2005; Cardozo et al., 2006; Fraser et al., 2007). Garlic, ginger, and lemongrass are plant extract and herb of interest. These herbs are widely used in tropical countries as for one of human food composition. Garlic oils and garlic oil compounds have been explored as an alternative to antibiotics to manipulate rumen fermentation due to their well-known antimicrobial effects (Ramos-Morales et al., 2013). Garlic oil and garlic derived compounds have been demonstrated to have antimethanogenic property with mixed effects on rumen fermentation (Busquet et al., 2005a; Chaves et al., 2008c). In addition, ginger oil can decrease ammonia N without affecting on VFAs (Busquet et al., 2006). Ginger oil has also been detected to have

inhibitory effects for 10 different micro-organisms (Hammer et al., 1999) but limited studies showed no effect on rumen VFA concentration in a continuous culture (Busquet et al., 2005b). Lemongrass has been shown antibacterial (Valero and Salmeroìn, 2003), antioxidant (Cheel et al., 2005), and antihyper-NH3-producing ruminal bacterial (McIntosh et al., 2003) activities as well as the effects on changes of blood metabolites and rumen fermentation in Holstein steers (Hosoda et al., 2006). However, lemongrass EO was little evaluated on its effects on ruminal fermentation. Lemongrass powder can decrease ammonia N without affecting VFAs. Moreover, lemongrass powder can decrease protozoal population (Wanapat et al., 2008).

3.3 Objectives

The objective of this study was to determine the effect of EOs supplementation on gas production (GP), fermentation characteristics and nutrient digestibility using batch culture.

3.4 Materials and methods

Experiment 3.1 (Exp. 3.1) was a complete randomized design with three replicates per treatment. Treatments were control (CON), cinnamon oil (CIN), clove oil (CLO), garlic oil (GAR), ginger oil (GIN), and lemongrass oil (LEM). The EOs were purchased commercially (purity >99%; Phodé S.A., Albi, France). Four different doses were used for each EO; 200, 400, 800, and 1600 mg/kg substrate DM. Experiment 3.2 (Exp. 3.2) was a complete randomized design with three replicates per treatment. Treatments were the same as used in the Exp. 3.1; the highest dose (200 mg/kg DM) for each EO was selected based on the results from the batch culture

(Exp. I; *i.e.* digestibility of DM and ammonia N). The dosages of EO were 50, 100, 200, and 200 mg/kg substrate DM in Exp. 3.2. Microbial fermentation, and DMD were evaluated in batch culture. The feed was a dairy type ration consisting of 50% roughage (35% grass hay and 15% alfalfa hay) and 50% concentrate (20% barley grain, 10% corn DDGS, 10% wheat DDGS, 5% canola meal, and 5% vitamin and mineral supplement) (Table 3.1).

Source of rumen fluid for *in vitro* incubations

Inoculum for the batch culture was obtained from three ruminally fistulated beef heifers (Spayed beef heifer) fed a diet consisting of 64% barley silage, 6% grass hay, 27% dry-rolled barley grain, and 3% vitamin and mineral supplement. Rumen fluid was collected from different sites within the rumen approximately 2 h after the morning feeding, pooled, and squeezed through PeCAP® polyester screen (pore size 355 µm; B & S Thompson, Ville Mont-Royal, QC, Canada) into an insulated thermos, and transported immediately to the laboratory. All animal procedures were in accordance with the guidelines of the Canadian Council on Animal Care (CCAC 1993).

Table 3.1 Ingredient and chemical composition of the diet.

Ingredient composition (%)	
Grass hay	35.0
Alfalfa hay	15.0
Barley grain	20.0
Corn DDGS	10.0
Wheat DDGS	10.0
Canola meal	5.0
Vitamin and mineral supplement[1/]	5.0

Table 3.1 Ingredient and chemical composition of the diet (Continued).

Chemical composition (%)	
Dry matter	93.2
Neutral detergent fiber	41.8
Acid detergent fiber	20.5
Crude protein	16.1

[1/]Supplied per kilogram of dietary DM : 15 mg of Cu, 65 mg of Zn, 28 mg of Mn, 0.7 mg of I, 0.2 mg of Co, 0.3 mg of Se, 6000 IU of vitamin A, 600 IU of vitamin D, and 47 IU of vitamin E.

Rumen fluid was added to medium at a ratio of 1 : 3 (rumen fluid : medium). Anaerobic buffer medium (45 mL; Goering and Van Soest, 1970) contained tryptone, buffer, macro and micro mineral solution, resazurin and water. Forty-five milliliters of prewarmed media and 15 mL of inoculum were added anaerobically to the 100 mL bottles by flushing with oxygen free CO_2. Bottles were sealed immediately with a 14 mm butyl rubber stopper plus aluminum crimp cap and incubated at 39 ^{o}C for 24 or 48 h. The incubation was repeated with two runs. Needle was inserted through rubber stopper of each vial for about 5 seconds to release small amount of gas that might have built up and created starting point for incubation. All vials were returned to the incubator. Rotary shaker was turned turn on (~120 rpm). Negative control (rumen fluid plus anaerobic buffer medium) and blanks (filter bags plus anaerobic buffer medium and rumen fluid) were also incubated using 4 replications for correction of gas production and disappearance, respectively.

Sample collection and processing

At pre-determined time points, headspace gas production (GP) was measured at 3, 6, 12, 24, 36, and 48 h post incubation by inserting a 23 gauge (0.6 mm) needle attached to a pressure transducer (model PX4200-015GI, Omega Engineering, Inc., Laval, QC., Canada), connected to a visual display (Data Track, Christchurch, UK). A volume of 15 mL gas was sampled using a syringe and transferred into 6.8 mL Exetainer vials (Labco Ltd., Wycombe, Bucks, UK) for immediate measurement of CH_4. Methane concentration was determined using a gas chromatography (Varian 4900 GC; Agilent Technologies Canada Inc., Mississauga, ON, Canada). Pressure values, corrected by the amount of substrate OM incubated and the gas released from negative controls, were used to generate volume using the equation of Mauricio et al. (1999) as :

$$\text{Gas volume} = 0.18 + (3.697 \times \text{gas pressure}) + (0.0824 \times \text{gas pressure}^2)$$

The kinetic parameters of GP were calculated using the equation of France et al. (2000) as :

$$A = b \times (1 - e^{-c(t-L)})$$

Where A is the volume of GP at time *t*; b is the asymptotic GP (mL/g DM); *c* is the rate of GP (/h), and *L* (h) is the discrete lag time prior to gas produced.

After 24 h and 48 h of incubation, the bags were removed from the vials and washed under stream of cold water until the water runs clear. The bags were dried in an oven at 55 °C for 48 h to determine DM digestibility. The NDF concentration in the residue was determined as described by Van Soest et al. (1991) using heat-stable α-amylase (Termamyl 120 L, Novo Nordisk Biochem, Franklinton, NC, USA) and

sodium sulfite. Procedures to analyze NDF and ADF were adapted for use in an ANKOM200 fiber analyzer (Ankom Technology Corp., Macedon, NY).

Chemical analyses were performed on each sample in duplicate, and analysis was repeated when the CV was >0.05. Analytical DM was analyzed by drying samples at 135 °C for 2 h, followed by hot weighing (AOAC, 1995; method 930.15). The NDF analysis was conducted using an ANKOM^{200} Fiber analyzer unit based on the procedure described by Van Soest et al. (1991) using heat-stable amylase and sodium sulfite. The NDF values are expressed inclusive of residual ash. Concentration of CH_4 was determined using a Varian 4900 gas chromatograph equipped with 10 m PPU column and thermal conductivity detector (Varian Inc., Middelburg, the Netherlands).

At the end of incubation, vials were removed from incubator. Gas pressure and gas samples were then taken into vials and placed on ice to stop fermentation. Vials should be opened as soon as possible for measuring of end fermentation pH and taking of supernatant aliquots for VFA and NH_3-N analyses.

The volatile fatty acid (VFA) and ammonia N (NH_3- N) concentration were measured for the 24 and 48 h incubation after measuring gas and pH. Two 5 mL samples were taken from the bottle directly at the end of time point, and placed in screw-capped vials preserved with 1 mL of 25% (wt/wt) metaphosphoric solution, or with 1 mL of 1% H_2SO_4, and immediately frozen at -20 °C for VFA and NH_3-N analysis, respectively. Concentration of VFA was quantified using gas chromatograph (model 5890, Hewkett-Packard Lab, Palo Alto, CA) with a capillary column (30 m × 0.32 mmi.d., 1 µm phase thickness, Zeborn ZB-FAAP, Phenomenex, Torrance, CA), and flame ionization detection, and crotonic acid (trans-2-butenoic acid) was used as the internal standard. The NH_3-N was determined as described by Rhine et al. (1998).

Five mL of samples from vial was added to 1 mL of 1.07 N sulfuric acid and centrifuged at 14000 g for 15 minutes.

3.5 Statistical analysis

Data were analyzed using the mixed model procedure of SAS (SAS Inst. Inc., Cary, NC) to account for the fixed effect of EO source, EO dosage, interaction between EO and dosage, and run was random effect (experimental unit). The effect of increasing levels of EO from 0, 200, 400, 800, to 1600 mg/kg DM or 0, 50, 100, 150 to 200 mg/kg DM in the substrate was examined through linear and quadratic orthogonal contrasts using the CONTRAST statement of SAS. Differences were declared significant at $P \leq 0.05$. Trends were discussed at $0.05 < P \leq 0.10$ unless otherwise stated.

3.6 Experimental site

The experiment was conducted at Research Centre, Agriculture and Agri-Food Canada, Lethbridge, Alberta, Canada.

3.7 Duration

The duration of this trial was from March to May 2012.

3.8 Results

Feed digestibility

In Experiment 3.1, DM digestibility (DMD) increased in all treatments both at 24 and 48 h when compared with the control (Table 3.2). Addition of 200 and 400

mg/kg DM CIN linearly increased but 1600 mg/kg DM decreased DMD ($P<0.01$) at 24 h incubation. Only 800 mg/kg DM CIN quadratically increased DMD at 48 h incubation ($P<0.01$). All CLO doses linearly increased DMD at either 24 or 48 h post incubation ($P<0.01$). The dose of 200 and 400 mg/kg DM GAR linearly increased but 1600 mg/kg DM linearly decreased both at 24 and 48 h ($P<0.01$). The dose of 200 to 800 mg/kg DM GIN and LEM quadratically improved DMD either at 24 or 48 h ($P<0.05$) but at the dose of 1600 mg/kg DM did not affect DMD. NDF and ADF digestibility were unaffected by all EO treatments (Table 3.2).

In Experiment 3.2, DM digestibility also increased in all treatments at both 24 and 48 h when compared with the control (Table 3.6). In addition, the highest dose of 200 mg/kg DM EOs linearly improved DMD in all treatments at either 24 or 48 h incubation ($P<0.05$) when compared with the control. The dose of EOs below 200 mg/kg DM also linearly improved DMD, including 150 mg/kg DM CIN at 24 h ($P<0.01$), 150 mg/kg DM CLO at 48 h ($P<0.01$), and 150 mg/kg DM GAR at 24 h ($P<0.05$). However, disappearances of NDF and ADF were unaffected by the treatments (Table 3.6).

Rumen fermentation

In Experiment 3.1, cumulative gas production was not different in most treatments but quadratically increased at 200, 400, and 800 mg/kg DM CIN, GAR and GIN at 24 h (Table 3.3). Total VFA, individual VFA, and methane production were similar in all treatments (Table 3.4 and Table 3.5). The ammonia N concentration linearly reduced in all treatments ($P<0.05$) (Table 3.5). Methane production was quadratically reduced at 200 ($P<0.05$), 400 ($P<0.05$), and 800 ($P<0.05$) mg/kg DM CIN and GAR at 24 h whereas 200 ($P<0.05$) or 800 ($P<0.05$) mg/kg DM GIN increased methane production. In contrast, CLO and LEM did not affect methane

production. At 48 h of incubation, VFA and methane production were not significantly different among the treatments (Table 3.4 and Table 3.5, respectively). In Experiment 3.2, at the dose of 200 mg/kg DM, all EOs linearly increased cumulative gas production at 24 h and 48 h (Table 3.7). However, 50 mg/kg DM of all EOs had no effect but 100 or 150 mg/kg DM CLO, GAR, and LEM linearly increased cumulative GP ($P<0.01$). Table 3.8 showed that 200 mg/kg DM of all EOs linearly increased total VFA at 48 h of incubation, however, only 200 mg/kg DM GAR and LEM improved total VFA at 24 h ($P<0.05$ and $P<0.05$, respectively). All of the treatments did not affect individual VFA. The ammonia N concentration was linearly decreased at 200 mg/kg DM of all EOs (Table 3.9). The doses of 200 mg/kg DM CLO, GIN, and LEM linearly increased (Table 3.9) methane production ($P<0.01$) at 24 h while at 200 mg/kg DM CIN and LEM increased methane production at 48 h ($P<0.01$ and $P<0.05$, respectively).

Table 3.2 Effect of essential oils on digestibility of DM, NDF, and ADF in batch culture (Experiment 3.1).

	EO[1/]	Dose (mg/kg DM)					SEM[2/]	P-value	
		0	200	400	800	1600		Linear	Quadratic
		DM digestibility (%)							
24 h	CIN	54.9^{b}	56.9^{ab}	57.1^{a}	54.4^{b}	48.3^{c}	0.75	0.01	0.01
	CLO	54.9^{b}	58.0^{a}	59.4^{a}	59.0^{a}	54.8^{b}	0.53	0.01	0.01
	GAR	54.9^{b}	58.5^{a}	55.9^{b}	52.1^{c}	49.8^{d}	0.33	0.01	0.14
	GIN	54.9^{c}	60.4^{a}	59.7^{ab}	58.1^{b}	54.2^{c}	0.69	0.76	0.01
	LEM	54.9^{c}	56.2^{b}	56.7^{b}	58.8^{a}	54.3^{c}	0.47	0.59	0.01
48 h	CIN	62.9^{b}	62.6^{b}	63.2^{b}	66.3^{a}	62.6^{b}	0.98	0.68	0.01
	CLO	62.9^{c}	64.7^{b}	67.2^{a}	67.0^{a}	62.2^{c}	0.55	0.01	0.01
	GAR	62.9^{bc}	65.5^{a}	63.8^{b}	61.9^{c}	59.8^{d}	0.44	0.01	0.06
	GIN	62.9^{c}	65.2^{b}	67.4^{a}	66.5^{ab}	62.3^{c}	0.71	0.01	0.05
	LEM	62.9^{c}	65.6^{b}	65.7^{b}	67.6^{a}	64.1^{c}	0.65	0.41	0.01
		NDF digestibility (%)							
24 h	CIN	34.4	36.3	37.8	34.2	34.0	3.00	0.77	0.77
	CLO	34.4	35.5	36.2	40.9	33.5	2.03	0.23	0.22
	GAR	34.4	36.3	34.6	31.3	32.6	3.32	0.34	0.60
	GIN	34.4	32.3	36.9	39.4	31.3	3.57	0.61	0.33
	LEM	34.4	34.2	30.3	36.5	31.7	3.70	0.70	0.23

Table 3.2 Effect of essential oils on digestibility of DM, NDF, and ADF in batch culture (Experiment 3.1) (Continued).

	EO[1/]	Dose (mg/kg DM)					SEM[2/]	P-value	
		0	200	400	800	1600		Linear	Quadratic
					NDF digestibility (%)				
48 h	CIN	44.1	42.9	45.3	47.5	45.8	3.39	0.45	0.52
	CLO	44.1	46.9	47.9	48.8	43.5	2.65	0.22	0.65
	GAR	44.1	49.2	46.3	45.8	45.8	2.50	0.86	0.24
	GIN	44.1	43.4	49.5	50.8	43.6	1.83	0.28	0.42
	LEM	44.1	50.0	43.9	51.7	40.7	2.60	0.92	0.36
					ADF digestibility (%)				
24 h	CIN	27.4	29.3	30.1	27.5	25.4	4.66	0.48	0.64
	CLO	27.4	27.4	28.1	34.3	27.1	2.89	0.32	0.12
	GAR	27.4	30.7	27.4	23.7	24.6	3.11	0.16	0.56
	GIN	27.4	25.5	28.5	32.9	25.1	4.98	0.89	0.23
	LEM	27.4	27.5	23.9	30.1	25.2	5.18	0.84	0.71
48 h	CIN	37.0	35.1	38.4	40.3	36.8	4.40	0.50	0.56
	CLO	37.0	41.0	41.5	41.1	36.2	3.09	0.27	0.42
	GAR	37.0	42.9	41.1	38.7	36.5	2.93	0.63	0.46

Table 3.2 Effect of essential oils on digestibility of DM, NDF, and ADF in batch culture (Experiment 3.1) (Continued).

	EO[1/]	Dose (mg/kg DM)					SEM[2/]	P-value	
		0	200	400	800	1600		Linear	Quadratic
					ADF digestibility (%)				
48 h	GIN	37.0	36.2	43.3	44.2	36.4	2.71	0.29	0.37
	LEM	37.0	44.2	41.5	46.8	36.9	3.65	0.93	0.29

[a-d]Within a row means without a common superscript letter differ.

[1/]EO : CIN = cinnamon oil; CLO = clove oil; GAR = garlic oil; GIN = ginger oil; LEM = lemongrass oil.

[2/]SEM = standard error of the mean.

Table 3.3 Effects of essential oils on gas kinetics and cumulative gas production in batch culture (Experiment 3.1).

EO[2/]	Dose	Gas production parameters[1/]			*In vitro* gas production (mL/g DM)					
		b	c	L	GP_3	GP_6	GP_{12}	GP_{24}	GP_{36}	GP_{48}
CIN	0	177	0.043	0.127	20.9^{b}	34.8^{c}	61.1^{b}	94.6^{b}	117.2	133.7
	200	156	0.059	0.117	29.4^{a}	46.7^{a}	75.8^{a}	112.2^{a}	131.3	147.4
	400	152	0.060	0.129	28.5^{ab}	46.1^{ab}	76.4^{a}	113.6^{a}	129.5	145.8
	800	149	0.052	0.051	27.4^{ab}	44.9^{ab}	73.8^{a}	112.3^{a}	125.8	140.5
	1600	147	0.042	0.180	22.9^{ab}	37.5^{bc}	65.5^{ab}	100.9^{ab}	118.1	133.6
	SEM[3/]	14.4	0.005	0.090	2.91	3.15	4.21	5.33	8.78	9.08
	Linear	0.20	0.574	0.680	0.55	0.47	0.62	0.85	0.52	0.46
	Quadratic	0.32	0.007	0.389	0.05	0.02	0.02	0.02	0.23	0.28
CLO	0	177	0.043	0.127	20.9^{c}	34.8^{c}	61.1	94.6	117.2	133.7
	200	167	0.050	0.081	25.9^{ab}	43.8^{ab}	73.9	111.6	135.7	152.5
	400	160	0.054	0.028	28.5^{a}	46.0^{a}	74.5	111.0	134.3	150.1
	800	142	0.060	0.004	27.1^{a}	42.6^{ab}	69.3	105.0	118.3	133.8
	1600	150	0.056	0.334	21.8^{bc}	38.0^{bc}	67.8	108.0	120.8	135.6
	SEM[3/]	14.4	0.005	0.090	2.19	2.41	4.29	5.59	7.16	7.90
	Linear	0.14	0.029	0.030	0.40	0.64	0.89	0.34	0.34	0.28
	Quadratic	0.24	0.033	0.014	0.01	0.01	0.09	0.19	0.44	0.50

Table 3.3 Effects of essential oils on gas kinetics and cumulative gas production in batch culture (Experiment 3.1) (Continued).

EO[2/]	Dose	Gas production parameters[1/]			*In vitro* gas production (mL/g DM)					
		b	c	L	GP_3	GP_6	GP_{12}	GP_{24}	GP_{36}	GP_{48}
GAR	0	177	0.042	0.127	20.9^{c}	34.8^{c}	61.1^{b}	94.6^{c}	117.2	133.7
	200	163	0.055	0.144	28.0^{a}	46.1^{a}	75.4^{a}	112.7^{a}	134.6	151.1
	400	141	0.063	0.300	24.9^{ab}	43.2^{ab}	72.2^{a}	109.3^{ab}	120.4	136.3
	800	152	0.056	0.028	27.4^{ab}	44.6^{a}	71.5^{a}	107.5^{ab}	126.9	143.0
	1600	148	0.052	0.042	23.7^{bc}	39.5^{b}	64.6^{b}	101.4^{bc}	118.5	134.8
	SEM[3/]	14.4	0.005	0.090	2.79	1.47	1.58	3.44	7.06	8.32
	Linear	0.25	0.648	0.116	0.73	0.73	0.17	0.80	0.53	0.58
	Quadratic	0.26	0.007	0.901	0.01	0.01	0.01	0.02	0.28	0.38
GIN	0	177	0.043	0.127	20.9^{b}	34.8^{c}	61.1^{c}	94.6^{c}	117.2	133.7
	200	177	0.046	0.059	25.4^{a}	43.3^{a}	72.4^{a}	112.2^{a}	138.8	156.9
	400	151	0.055	0.078	24.5^{a}	40.9^{ab}	66.7^{abc}	102.8^{b}	120.4	136.7
	800	144	0.061	0.069	26.7^{a}	42.7^{a}	69.6^{ab}	107.9^{ab}	119.4	135.1
	1600	153	0.049	0.014	23.7^{ab}	38.7^{bc}	64.0^{b}	101.4^{bc}	121.5	136.5
	SEM[3/]	14.4	0.005	0.090	2.59	1.18	2.18	2.92	5.19	5.89
	Linear	0.21	0.310	0.147	0.31	0.45	0.65	0.76	0.38	0.26
	Quadratic	0.22	0.007	0.996	0.02	0.01	0.02	0.02	0.99	0.99

Table 3.3 Effects of essential oils on gas kinetics and cumulative gas production in batch culture (Experiment 3.1) (Continued).

EO[2/]	Dose	Gas production parameters[1/]			*In vitro* gas production (mL/g DM)					
		b	c	L	GP_3	GP_6	GP_{12}	GP_{24}	GP_{36}	GP_{48}
LEM	0	177	0.043	0.127	20.9	34.8	61.1	94.6	117.2	133.7
	200	148	0.060	0.290	24.9	42.3	70.4	107.0	121.4	138.2
	400	135	0.062	0.269	22.7	38.1	65.6	101.8	109.4	125.5
	800	137	0.059	0.254	22.5	37.3	65.1	103.1	111.5	127.0
	1600	137	0.059	0.237	23.5	38.9	65.8	104.4	113.5	128.9
	SEM[3/]	14.4	0.005	0.090	2.87	3.72	5.41	6.59	12.59	13.27
	Linear	0.12	0.181	0.752	0.73	0.79	0.87	0.43	0.69	0.63
	Quadratic	0.11	0.068	0.451	0.85	0.77	0.64	0.49	0.61	0.60

[a-c]Within a column means without a common superscript letter differ.

[1/]Parameters : b is the theoretical maximum GP (mL/g DM); c is the rate constant of GP (/h); Lag is the initial delay before GP begins (h).

[2/]EO : CIN = cinnamon oil; CLO = clove oil; GAR = garlic oil; GIN = ginger oil; LEM = lemongrass oil.

[3/]SEM = standard error of the mean.

Table 3.4 Effects of essential oils on total VFA concentration and individual VFA proportion in batch culture (Experiment 3.1).

	EO[1/]	Dose (mg/kg DM)					SEM[2/]	P-value	
		0	200	400	800	1600		Linear	Quadratic
		Total VFA (m*M*)							
24 h	CIN	110.2	120.8	109.2	112.5	104.9	4.06	0.07	0.36
	CLO	110.2	115.0	111.3	110.3	109.1	5.43	0.55	0.87
	GAR	110.2	114.9	115.7	106.4	102.7	6.04	0.11	0.63
	GIN	110.2	114.6	107.6	112.6	105.4	4.26	0.22	0.45
	LEM	110.2	116.3	117.3	102.2	107.8	6.06	0.22	0.66
48 h	CIN	118.8	120.0	117.6	126.6	112.6	6.85	0.47	0.22
	CLO	118.8	124.6	127.9	132.9	117.8	5.64	0.66	0.06
	GAR	118.8	123.3	133.3	128.5	113.2	8.65	0.34	0.10
	GIN	118.8	118.4	119.6	119.4	118.2	5.27	0.93	0.82
	LEM	118.8	118.6	133.3	118.1	116.2	6.50	0.39	0.30
		Acetate (mol/100 mol)							
24 h	CIN	57.2	57.3	57.5	56.9	56.2	0.81	0.17	0.59
	CLO	57.2	57.1	57.5	56.1	57.0	1.08	0.65	0.54
	GAR	57.2	58.2	57.8	56.0	56.3	1.29	0.23	0.78
	GIN	57.2	59.3	57.2	56.2	56.8	1.20	0.27	0.57
	LEM	57.2	56.8	57.3	56.5	56.7	0.50	0.31	0.54

Table 3.4 Effects of essential oils on total VFA concentration and individual VFA proportion in batch culture (Experiment 3.1) (Continued).

	EO[1/]	Dose (mg/kg DM)					SEM[2/]	P-value	
		0	200	400	800	1600		Linear	Quadratic
					Acetate (mol/100 mol)[4/]				
48 h	CIN	55.9	56.2	54.7	54.6	54.0	1.19	0.14	0.61
	CLO	55.9	55.6	55.1	53.9	53.9	1.14	0.10	0.40
	GAR	55.9	55.0	54.2	53.7	54.1	2.19	0.47	0.46
	GIN	55.9	54.9	54.9	54.1	54.1	1.11	0.19	0.40
	LEM	55.9	54.3	54.9	54.3	54.2	1.99	0.53	0.68
					Propionate (mol/100 mol)[4/]				
24 h	CIN	19.7	20.0	19.9	20.0	20.3	0.70	0.52	0.99
	CLO	19.7	19.9	19.5	20.4	20.2	0.58	0.34	0.73
	GAR	19.7	19.4	19.7	20.5	20.2	0.73	0.31	0.57
	GIN	19.7	19.4	19.7	20.4	20.2	0.71	0.32	0.62
	LEM	19.7	20.1	20.0	20.2	20.1	0.49	0.53	0.55
48 h	CIN	20.5	20.3	20.6	20.7	20.7	0.17	0.13	0.48
	CLO	20.5	20.5	20.7	20.8	20.8	0.25	0.21	0.52
	GAR	20.5	20.6	20.6	20.8	20.6	0.34	0.79	0.43
	GIN	20.5	20.6	20.6	20.5	20.8	0.23	0.25	0.74
	LEM	20.5	20.8	20.4	20.4	20.7	0.48	0.86	0.70

Table 3.4 Effects of essential oils on total VFA concentration and individual VFA proportion in batch culture (Experiment 3.1) (Continued).

	EO[1/]	Dose (mg/kg DM)					SEM[2/]	P-value	
		0	200	400	800	1600		Linear	Quadratic
		A + B/P[3/]							
24 h	CIN	3.6	3.6	3.6	3.5	3.5	0.16	0.41	0.99
	CLO	3.6	3.6	3.7	3.4	3.5	0.15	0.34	0.72
	GAR	3.6	3.7	3.6	3.4	3.5	0.19	0.28	0.65
	GIN	3.6	3.7	3.6	3.4	3.5	0.18	0.31	0.61
	LEM	3.6	3.5	3.5	3.5	3.5	0.11	0.42	0.56
48 h	CIN	3.4	3.4	3.4	3.3	3.3	0.06	0.09	0.40
	CLO	3.4	3.4	3.3	3.3	3.3	0.06	0.07	0.27
	GAR	3.4	3.4	3.3	3.3	3.3	0.12	0.61	0.41
	GIN	3.4	3.3	3.3	3.3	3.3	0.07	0.21	0.77
	LEM	3.4	3.3	3.4	3.4	3.3	0.12	0.62	0.93

[1/]EO : CIN = cinnamon oil; CLO = clove oil; GAR = garlic oil; GIN = ginger oil; LEM = lemongrass oil.

[2/]SEM = standard error of the mean.

[3/]A + B/P = acetate + butyrate acid/propionate.

[4/]The proportion of individual volatile fatty acids did not include isobutyrate, isovalerate, valerate, and caproate.

Table 3.5 Effects of essential oils on CH_4 production and NH_3-N concentration in batch culture (Experiment 3.1).

	EO[1/]	Dose (mg/kg of DM)					SEM[2/]	P-value	
		0	200	400	800	1600		Linear	Quadratic
					CH_4 (mL/g DM)				
24 h	CIN	15.8^{c}	18.8^{a}	18.7^{a}	18.7^{ab}	16.0^{bc}	1.02	0.33	0.02
	CLO	15.8	18.4	18.1	17.0	17.8	1.18	0.55	0.47
	GAR	15.8^{c}	18.8^{a}	18.1^{ab}	17.5^{abc}	16.4^{bc}	0.73	0.32	0.04
	GIN	15.8^{c}	18.6^{a}	16.7^{bc}	17.9^{ab}	16.5^{bc}	0.57	0.77	0.03
	LEM	15.8	17.7	16.7	16.8	17.3	1.36	0.57	0.78
48 h	CIN	20.8	23.6	23.1	22.4	21.0	2.26	0.62	0.36
	CLO	20.8	24.6	23.9	21.6	21.3	1.84	0.41	0.40
	GAR	20.8	24.1	22.0	23.2	21.3	1.87	0.74	0.28
	GIN	20.8	24.6	22.0	21.6	21.7	1.69	0.68	0.74
	LEM	20.8	21.9	19.4	20.0	19.9	2.42	0.59	0.73
					Ammonia N (mg/100 mL)				
24 h	CIN	42.3^{a}	31.8^{b}	31.5^{b}	32.2^{b}	30.3^{b}	2.87	0.04	0.07
	CLO	42.3^{a}	31.9^{b}	32.4^{b}	32.6^{b}	31.2^{b}	2.55	0.04	0.06
	GAR	42.3^{a}	32.5^{b}	31.1^{b}	31.4^{b}	30.2^{b}	3.03	0.04	0.06
	GIN	42.3^{a}	31.9^{b}	34.3^{b}	33.0^{b}	25.2^{b}	3.28	0.01	0.57
	LEM	42.3^{a}	31.8^{b}	34.0^{b}	31.9^{b}	29.9^{b}	2.53	0.02	0.08

Table 3.5 Effects of essential oils on CH_4 production and NH_3-N concentration in batch culture (Experiment 3.1) (Continued).

	EO[1/]	Dose (mg/kg of DM)					SEM[2/]	P-value	
		0	200	400	800	1600		Linear	Quadratic
		Ammonia N (mg/100 mL)							
48 h	CIN	52.8[a]	46.3[b]	45.2[b]	45.1[b]	41.6[b]	2.01	0.01	0.11
	CLO	52.8[a]	45.9[b]	44.1[b]	44.8[b]	44.4[b]	1.92	0.03	0.03
	GAR	52.8[a]	45.6[b]	44.9[b]	46.6[b]	43.9[b]	1.34	0.01	0.04
	GIN	52.8[a]	44.2[b]	45.0[b]	45.4[b]	43.8[b]	2.00	0.04	0.07
	LEM	52.8[a]	42.6[b]	44.6[b]	43.9[b]	43.5[b]	2.08	0.04	0.04

[a-c]Within a row means without a common superscript letter differ.

[1/]EO : CIN = cinnamon oil; CLO = clove oil; GAR = garlic oil; GIN = ginger oil; LEM = lemongrass oil.

[2/]SEM = standard error of the mean.

Table 3.6 Effect of essential oils on digestibility of DM, NDF and ADF in batch culture (Experiment 3.2).

EO[1/]		Dose (mg/kg DM)					SEM[2/]	P-value	
		0	50	100	150	200		Linear	Quadratic
DM digestibility (%)									
24 h	CIN	49.9^{c}	51.2^{bc}	51.7^{bc}	53.2^{ab}	54.0^{a}	0.72	0.01	0.67
	CLO	51.1^{b}	51.8^{b}	52.3^{ab}	52.7^{ab}	53.6^{a}	0.55	0.01	0.74
	GAR	50.4^{b}	51.5^{ab}	51.6^{ab}	53.9^{a}	53.5^{a}	1.16	0.03	0.82
	GIN	50.0^{b}	50.9^{b}	52.8^{ab}	53.0^{ab}	54.5^{a}	0.86	0.01	0.55
	LEM	50.4^{b}	51.5^{ab}	53.2^{ab}	52.6^{ab}	53.7^{a}	1.10	0.03	0.52
48 h	CIN	65.1^{c}	66.9^{bc}	66.7^{bc}	65.7^{bc}	68.8^{a}	0.58	0.01	0.32
	CLO	64.7^{b}	65.5^{b}	66.7^{ab}	68.1^{a}	67.9^{a}	0.86	0.01	0.49
	GAR	64.6^{b}	65.3^{ab}	64.4^{b}	66.0^{ab}	67.6^{a}	1.14	0.05	0.23
	GIN	62.8^{b}	64.7^{b}	64.8^{ab}	64.2^{b}	66.9^{a}	0.78	0.01	0.74
	LEM	64.3^{b}	66.1^{ab}	66.0^{ab}	66.0^{ab}	67.3^{a}	1.00	0.05	0.75
NDF digestibility (%)									
24 h	CIN	27.5	26.7	28.7	29.7	29.6	1.98	0.18	0.93
	CLO	27.3	28.7	27.3	27.9	29.3	3.15	0.20	0.67
	GAR	26.3	26.6	28.7	28.9	29.2	1.59	0.99	0.69
	GIN	26.6	26.8	29.2	27.2	29.1	1.67	0.53	0.80
	LEM	26.9	27.7	28.0	27.8	28.0	2.16	0.71	0.53
48 h	CIN	43.8	44.7	44.4	44.1	44.5	3.24	0.98	0.82

Table 3.6 Effect of essential oils on digestibility of DM, NDF and ADF in batch culture (Experiment 3.2) (Continued).

	EO[1/]	Dose (mg/kg DM)					SEM[2/]	P-value	
		0	50	100	150	200		Linear	Quadratic
					NDF digestibility (%)				
48 h	CLO	41.8	42.5	43.5	43.9	43.5	2.26	0.38	0.66
	GAR	45.6	46.8	46.1	46.5	47.1	2.17	0.72	0.88
	GIN	41.3	44.3	43.7	44.1	45.6	2.97	0.27	0.81
	LEM	43.7	46.3	46.8	46.9	47.0	2.69	0.45	0.13
					ADF digestibility (%)				
24 h	CIN	16.0	16.4	17.7	18.7	19.7	1.76	0.06	0.71
	CLO	16.3	16.4	17.0	18.7	18.3	2.76	0.39	0.32
	GAR	17.3	16.9	19.3	19.6	18.9	1.65	0.89	0.86
	GIN	17.9	17.9	17.9	18.2	18.1	2.10	0.51	0.77
	LEM	16.7	18.9	18.9	19.6	19.0	1.80	0.35	0.24
48 h	CIN	33.4	33.9	33.3	33.0	35.2	3.72	0.89	0.71
	CLO	36.1	33.3	34.8	35.2	33.1	3.05	0.57	0.99
	GAR	36.5	38.3	37.2	36.5	38.4	2.32	0.73	0.93
	GIN	31.3	35.4	34.5	34.9	36.5	3.70	0.30	0.73
	LEM	34.2	38.0	37.5	38.5	37.4	3.35	0.41	0.11

[a-c]Within a row means without a common superscript letter differ. [1/]EO : CIN = cinnamon oil; CLO = clove oil; GAR = garlic oil; GIN = ginger oil; LEM = lemongrass oil. [2/]SEM = standard error of the mean.

Table 3.7 Effects of essential oils on gas kinetics and cumulative gas production in batch culture (Experiment 3.2).

EO[2/]	Dose	Gas production parameters[1/]			*In vitro* gas production (mL/g DM)					
		b	c	L	GP_3	GP_6	GP_{12}	GP_{24}	GP_{36}	GP_{48}
CIN	0	152	0.044	0.050	17.9	36.0^{b}	61.8^{b}	94.0^{b}	118.2^{b}	131.8^{c}
	50	153	0.043	0.075	18.0	36.1^{b}	62.3^{b}	94.8^{b}	119.5^{b}	133.1^{bc}
	100	153	0.043	0.056	18.2	36.2^{b}	62.4^{b}	95.1^{b}	119.7^{b}	133.7^{bc}
	150	156	0.043	0.060	18.6	37.0^{b}	63.3^{b}	96.0^{b}	121.3^{b}	135.6^{b}
	200	162	0.044	0.017	20.7	39.5^{a}	66.5^{a}	100.8^{a}	126.7^{a}	142.7^{a}
	SEM[3/]	3.85	0.002	0.047	2.29	0.76	0.80	1.32	1.16	1.19
	Linear	0.02	0.973	0.368	0.28	0.01	0.01	0.01	0.01	0.01
	Quadratic	0.26	0.897	0.283	0.52	0.05	0.03	0.07	0.03	0.01
CLO	0	157	0.042	0.040	17.9	35.9^{b}	62.1^{b}	94.2^{c}	120.1^{b}	133.7^{c}
	50	155	0.042	0.056	17.7	35.8^{b}	62.1^{b}	94.6^{bc}	120.3^{ab}	133.8^{bc}
	100	155	0.043	0.034	18.4	36.7^{ab}	63.2^{ab}	95.8^{b}	120.9^{ab}	134.6^{bc}
	150	158	0.042	0.039	18.4	36.8^{ab}	63.2^{ab}	95.8^{b}	122.3^{ab}	136.0^{ab}
	200	154	0.046	0.208	20.1	37.6^{a}	64.7^{a}	98.5^{a}	122.7^{a}	136.9^{a}
	SEM[3/]	3.85	0.002	0.047	3.13	0.67	0.75	0.56	1.08	0.85
	Linear	0.79	0.621	0.276	0.50	0.04	0.02	0.01	0.04	0.01
	Quadratic	0.94	0.725	0.336	0.73	0.64	0.40	0.08	0.70	0.39

Table 3.7 Effects of essential oils on gas kinetics and cumulative gas production in batch culture (Experiment 3.2) (Continued).

EO[2/]	Dose	Gas production parameters[1/]			*In vitro* gas production (mL/g DM)					
		b	c	L	GP_3	GP_6	GP_{12}	GP_{24}	GP_{36}	GP_{48}
GAR	0	155	0.043	0.059	18.1	36.2[b]	62.4[b]	94.8[b]	120.1[b]	133.9[b]
	50	158	0.042	0.044	18.1	36.3[b]	62.6[b]	95.0[b]	121.1[b]	135.4[b]
	100	161	0.042	0.046	18.2	36.6[b]	63.1[b]	95.6[b]	122.3[b]	136.4[b]
	150	159	0.042	0.037	18.4	37.0[b]	63.7[b]	96.7[b]	122.7[b]	137.2[b]
	200	161	0.045	0.025	19.6	39.4[a]	66.9[a]	100.6[a]	126.0[a]	141.1[a]
	SEM[3/]	3.85	0.002	0.047	3.15	0.65	0.80	0.84	1.14	1.32
	Linear	0.20	0.508	0.271	0.65	0.01	0.01	0.01	0.01	0.01
	Quadratic	0.62	0.330	0.960	0.79	0.05	0.04	0.03	0.30	0.26
GIN	0	155	0.043	0.051	18.2	36.3[c]	62.3[d]	94.7[c]	120.0[b]	133.8[b]
	50	156	0.043	0.037	18.4	36.7[c]	63.0[cd]	95.4[bc]	120.9[b]	134.7[b]
	100	157	0.043	0.039	18.2	36.7[c]	63.1[bc]	95.6[bc]	121.2[b]	135.4[b]
	150	157	0.043	0.014	18.8	37.4[b]	63.7[b]	96.4[b]	121.6[b]	136.1[b]
	200	164	0.044	0.008	20.1	39.4[a]	66.7[a]	100.8[a]	126.9[a]	141.9[a]
	SEM[3/]	3.85	0.002	0.047	3.45	0.25	0.26	0.49	1.27	1.22
	Linear	0.12	0.683	0.054	0.62	0.01	0.01	0.01	0.01	0.01
	Quadratic	0.40	0.764	0.860	0.76	0.01	0.01	0.01	0.06	0.04

Table 3.7 Effects of essential oils on gas kinetics and cumulative gas production in batch culture (Experiment 3.2) (Continued).

EO[2/]	Dose	Gas production parameters[1/]			*In vitro* gas production (mL/g DM)					
		b	c	L	GP_3	GP_6	GP_{12}	GP_{24}	GP_{36}	GP_{48}
LEM	0	155	0.042	0.034	18.1	35.9^{b}	61.8^{b}	94.0^{c}	119.1^{b}	132.9^{c}
	50	153	0.043	0.037	18.4	36.2^{b}	62.4^{b}	94.9^{bc}	119.0^{b}	133.0^{c}
	100	155	0.043	0.038	18.3	36.3^{b}	62.7^{b}	95.0^{bc}	120.2^{b}	134.3^{bc}
	150	157	0.043	0.034	18.6	36.6^{b}	63.1^{b}	96.4^{b}	121.9^{b}	135.9^{b}
	200	160	0.045	0.034	20.2	38.9^{a}	66.1^{a}	100.4^{a}	125.1^{a}	139.7^{a}
	SEM[3/]	3.85	0.002	0.047	3.46	0.46	0.58	0.67	1.04	0.90
	Linear	0.21	0.522	0.228	0.60	0.01	0.01	0.01	0.01	0.01
	Quadratic	0.49	0.663	0.271	0.75	0.03	0.03	0.01	0.07	0.03

[a-c]Within a column means without a common superscript letter differ.

[1/]Parameters : b is the theoretical maximum GP (mL/g DM); c is the rate constant of GP (/h); Lag is the initial delay before GP begins (h).

[2/]EO : CIN = cinnamon oil; CLO = clove oil; GAR = garlic oil; GIN = ginger oil; LEM = lemongrass oil.

[3/]SEM = standard error of the mean.

Table 3.8 Effects of essential oils on total VFA concentration and individual VFA proportion in batch culture (Experiment 3.2).

	EO[1/]	Dose (mg/Kg DM)					SEM[2/]	P-value	
		0	50	100	150	200		Linear	Quadratic
		Total VFA (m*M*)							
24 h	CIN	81.8	81.3	84.9	87.9	87.3	3.89	0.10	0.94
	CLO	83.8	81.1	85.8	86.0	86.8	2.98	0.16	0.77
	GAR	81.3^{b}	81.9^{b}	82.6^{ab}	82.5^{ab}	85.0^{a}	1.36	0.05	0.45
	GIN	83.1	81.1	83.0	84.5	85.2	1.98	0.16	0.38
	LEM	79.9^{b}	80.6^{ab}	81.3^{ab}	82.8^{ab}	83.5^{a}	1.13	0.02	0.77
48 h	CIN	90.2^{b}	90.1^{b}	91.6^{ab}	91.3^{ab}	92.9^{a}	0.89	0.02	0.55
	CLO	93.4^{b}	93.1^{b}	93.3^{b}	96.2^{a}	97.4^{a}	0.86	0.01	0.07
	GAR	92.5^{b}	94.6^{ab}	95.3^{ab}	95.1^{ab}	97.2^{a}	1.12	0.02	0.80
	GIN	93.2^{b}	94.1^{b}	95.8^{ab}	96.6^{a}	97.3^{a}	1.01	0.01	0.70
	LEM	95.3^{b}	96.3^{b}	97.0^{b}	96.0^{b}	99.3^{a}	0.69	0.01	0.21
		Acetate (mol/100mol)[4/]							
24 h	CIN	55.1	55.2	55.9	55.0	54.7	0.52	0.46	0.36
	CLO	55.3	53.9	55.4	54.0	55.5	0.71	0.72	0.19
	GAR	51.0	54.9	54.5	54.8	54.9	0.84	0.43	0.69
	GIN	55.2	55.1	55.2	55.3	55.2	0.71	0.91	0.99
	LEM	54.9	54.6	54.3	54.8	55.1	0.88	0.74	0.47

Table 3.8 Effects of essential oils on total VFA concentration and individual VFA proportion in batch culture (Experiment 3.2) (Continued).

	EO[1/]	Dose (mg/Kg DM)					SEM[2/]	P-value	
		0	50	100	150	200		Linear	Quadratic
		Acetate (mol/100mol)[4/]							
48 h	CIN	54.0	53.9	53.7	53.3	53.6	1.02	0.52	0.78
	CLO	54.1	54.4	54.4	54.3	54.9	0.31	0.08	0.72
	GAR	53.4	54.3	52.9	52.4	52.6	0.77	0.06	0.76
	GIN	52.8	52.5	51.4	52.3	51.8	1.20	0.43	0.65
	LEM	52.3	51.3	54.7	55.0	53.5	1.94	0.23	0.46
		Propionate (mol/100mol)[4/]							
24 h	CIN	21.0	21.0	21.3	21.2	21.1	0.22	0.46	0.35
	CLO	21.2	21.1	21.1	21.2	21.1	0.38	0.99	0.98
	GAR	21.1	21.3	20.9	21.4	21.1	0.44	0.98	0.83
	GIN	21.2	21.4	21.3	21.3	21.1	0.11	0.33	0.12
	LEM	21.3	20.9	21.2	21.1	20.4	0.43	0.18	0.43
48 h	CIN	21.6	21.7	21.4	20.9	21.2	0.39	0.27	0.75
	CLO	21.5	21.1	21.2	21.5	20.9	0.29	0.21	0.89
	GAR	21.6	21.5	21.7	21.5	21.5	0.12	0.69	0.61
	GIN	21.1	21.5	21.4	21.5	21.6	0.33	0.23	0.70
	LEM	21.3	21.3	21.7	21.8	21.6	0.33	0.25	0.55

Table 3.8 Effects of essential oils on total VFA concentration and individual VFA proportion in batch culture (Experiment 3.2) (Continued).

	EO[1/]	Dose (mg/Kg DM)					SEM[2/]	P-value	
		0	50	100	150	200		Linear	Quadratic
		A + B/P[3/]							
24 h	CIN	3.3	3.3	3.2	3.2	3.2	0.05	0.45	0.34
	CLO	3.2	3.2	3.3	3.2	3.3	0.06	0.82	0.62
	GAR	3.2	3.2	3.3	3.2	3.3	0.08	0.75	0.85
	GIN	3.2	3.2	3.2	3.2	3.2	0.02	0.28	0.23
	LEM	3.2	3.3	3.2	3.2	3.4	0.11	0.24	0.40
48 h	CIN	3.1	3.1	3.1	3.2	3.2	0.03	0.06	0.93
	CLO	3.1	3.2	3.2	3.1	3.3	0.06	0.21	0.82
	GAR	3.1	3.1	3.1	3.1	3.1	0.02	0.10	0.26
	GIN	3.2	3.1	3.1	3.1	3.0	0.06	0.17	0.54
	LEM	3.1	3.1	3.1	3.1	3.1	0.03	0.59	0.84

[a-b]Within a row means without a common superscript letter differ.

[1/]EO : CIN = cinnamon oil; CLO = clove oil; GAR = garlic oil; GIN = ginger oil; LEM = lemongrass oil.

[2/]SEM = standard error of the mean. [3/]A + B/P = acetic acid + butyric acid/propionic acid.

[4/]The proportion of individual volatile fatty acids did not include isobutyrate, isovalerate, valerate, and caproate.

Table 3.9 Effects of essential oils on CH_4 production and NH_3-N concentration in batch culture (Experiment 3.2).

	EO[1/]	Dose (mg/kg DM)					SEM[2/]	P-value	
		0	50	100	150	200		Linear	Quadratic
					CH_4 (mL/g DM)				
24 h	CIN	25.2	25.1	24.8	25.0	25.9	0.57	0.32	0.09
	CLO	24.7^{b}	23.9^{b}	24.5^{b}	25.0^{b}	26.4^{a}	0.55	0.01	0.01
	GAR	24.8	24.1	25.1	24.4	26.2	1.03	0.19	0.24
	GIN	24.0^{b}	24.5^{b}	24.3^{b}	25.0^{ab}	26.1^{a}	0.65	0.01	0.23
	LEM	24.4^{b}	25.6^{b}	25.2^{b}	25.1^{b}	27.0^{a}	0.37	0.01	0.08
48 h	CIN	32.9^{b}	33.4^{b}	32.3^{b}	33.2^{b}	34.9^{a}	0.58	0.01	0.01
	CLO	33.5	33.5	33.6	33.6	34.8	1.16	0.23	0.23
	GAR	31.5	32.5	30.2	34.3	36.1	2.47	0.06	0.23
	GIN	33.6	31.4	32.8	35.2	32.8	2.28	0.13	0.10
	LEM	27.1^{b}	28.9^{b}	30.8^{ab}	30.5^{ab}	35.2^{a}	3.27	0.02	0.18
					Ammonia N (mg/100mL)				
24 h	CIN	38.7^{a}	38.8^{a}	37.6^{ab}	37.7^{ab}	36.1^{b}	1.01	0.05	0.48
	CLO	39.0^{a}	37.3^{b}	37.4^{b}	38.0^{ab}	36.8^{b}	0.52	0.03	0.31
	GAR	37.1^{a}	36.8^{a}	36.8^{a}	36.2^{a}	34.6^{b}	0.42	0.01	0.04
	GIN	38.4^{a}	38.3^{a}	37.7^{a}	37.8^{a}	36.1^{b}	0.30	0.01	0.03
	LEM	38.0^{a}	37.3^{ab}	37.9^{a}	37.2^{ab}	35.9^{b}	0.59	0.03	0.20

Table 3.9 Effects of essential oils on CH_4 production and NH_3-N concentration in batch culture (Experiment 3.2) (Continued).

EO[1/]		Dose (mg/kg DM)					SEM[2/]	P-value	
		0	50	100	150	200		Linear	Quadratic
Ammonia N (mg/100mL)									
48 h	CIN	49.7^{a}	48.3^{ab}	46.8^{b}	47.4^{b}	47.0^{b}	0.58	0.01	0.06
	CLO	49.4^{a}	48.7^{a}	48.6^{a}	48.1^{a}	45.2^{b}	1.00	0.02	0.14
	GAR	48.1^{a}	47.2^{ab}	47.3^{ab}	47.7^{a}	45.6^{b}	0.63	0.03	0.29
	GIN	47.5^{a}	46.7^{ab}	47.1^{ab}	46.4^{bc}	45.7^{c}	0.37	0.01	0.48
	LEM	48.3^{a}	47.6^{ab}	47.5^{b}	47.2^{b}	45.6^{c}	0.26	0.01	0.04

$^{a-c}$Within a row means without a common superscript letter differ.

$^{1/}$EO : CIN = cinnamon oil; CLO = clove oil; GAR = garlic oil; GIN = ginger oil; LEM = lemongrass oil.

$^{2/}$SEM = standard error of the mean.

3.9 Discussion

CIN

The decreases in disappearance of DM, NDF, CP, and starch with 300 mg/L cinnamaldehyde were observed by Li et al. (2012) in continuous culture using a high-grain diet (90%). In contrast to the above finding, the present incubation study, supplementing 400 and 800 mg/kg DM CIN improved DMD at 24 h and 48 h incubation. However, 1600 mg/kg DM CIN decreased DMD at 24 h incubation suggesting that at this dose CIN might cause a negative effect on feed digestion. In addition, NDF and ADF digestibility were unaffected by CIN in Exp. 3.1. The result of DMD was confirmed with 200 mg/kg DM CIN at either 24 or 48 h in Exp. 3.2. However, DM and NDF digestibility were not affected by cinnamaldehyde at the doses of 31.2 or 312 mg/L as reported by Busquet et al. (2005c). Supplementing CIN had no effect on GP kinetics however there was great consistency between cumulative GP and DMD. The cumulative GP at 200 to 800 mg/kg DM CIN was higher than control at early hour, 3 to 24 h incubation (Exp. 3.1) and 3 to 48 h with 200 mg/kg DM in Exp. 3.2. The similar effect of CIN on DMD between experiment measurements suggested that CIN effectively improved feed digestion.

Although CIN improved DMD but there was no different between treatments on total VFA and individual VFA. Similarly, Fraser et al. (2007) reported that cinnamon leaf oil had no effect on total VFA in continuous culture. The concentration of VFA and individual VFA were unaffected at 200 mg/kg cinnamaldehyde in lamb (Chaves et al., 2008a). Methane production increased with 200 to 800 mg/kg DM of CIN in Exp. 3.1 only at 24 h, this is consistent with DMD and cumulative GP. Fraser et al. (2007) observed that CIN had no effect on methane production. In contrast,

cinnamaldehyde decreased methane production using continuous culture (Li et al., 2012). Cinnamaldehyde did not change total protozoa number in lactating dairy cows (Benchaar et al., 2008). The effects of EO on rumen methane production are actually not consistent (Benchaar and Greathead, 2011), depending on the number of factors such as EO source, dose, substrates used, etc. In the present study, ammonia N concentration was consistently reduced with CIN added either at 24 or 48 h post incubation both in the Experiment 3.1 and 3.2, suggesting that these additives reduced deamination of AA, and could be used as an alternative additive for reducing ammonia N loss in the rumen. This result was in agreement with previous reports that cinnamaldehyde or cinnamon oil reduced ammonia N concentration (Busquet et al., 2005c; Cardozo et al., 2005). However, several reports suggested that cinnamaldehyde or cinnamon oil had no effect on ammonia N concentration in animals (Chaves et al., 2008a; Chaves et al., 2008b).

CLO

Ruminal degradability of NDF was linearly decreased and degradation of N in the rumen tended to linearly be decreased with increasing eugenol supplementation while OM and starch degradability did not differ (Yang et al., 2010). In contrast, the present study suggested that supplementing CLO increased DMD without affecting NDF or ADF degradability. The kinetics GP was not different between treatments but cumulative GP was consistently increased, together with increasing DMD with CLO supplementation. Total VFA concentration and individual VFA were unaffected by treatments although DMD was improved. Benchaar et al. (2012) reported that adding eugenol had no effect on total VFA or individual VFA in dairy cow either with low concentrate or high concentrate ratio of dairy ration. Methane production in this present study was inconsistency, although methane production increased with 200

mg/kg DM CLO at 24 h in Exp. 3.2 but could not maintain until 48 h post incubation. This result might be relative to cumulative GP. In contrast, Yang et al. (2010) reported that molar proportion of propionate tended to linearly increase thus the ratio of acetate to propionate tended to linearly decrease with increasing dose of eugenol. In fact, the reduced methane production would result in an increase of propionate as the H^+ must have a recipient. Supplementing CLO reduced ammonia N concentration in Exp. 3.1 and Exp. 3.2 at 24 and 48 h incubation. Busquet et al. (2006) demonstrated that 3000 mg/l eugenol inhibited NH_3-N concentration using 50 : 50 ratio of forage : concentrate. Meanwhile, NH_3-N concentration was reduced in high concentrate with 300 mg/l as reported by Cardozo et al. (2005). However, the inconsistency between the reduction in ruminal degradability of CP and the lack of effect on ruminal NH_3-N, ruminal branched-chain VFA concentration, and blood urea N concentration suggests that deamination and/or proteolytic activity in the rumen might not have been inhibited by eugenol supplementation (Yang et al., 2010).

GAR

Garlic oil is a complex mix of many different compounds presented in the plant or derived from processing. It has antimicrobial activity against a wide spectrum of gram-positive and gram-negative bacteria and its potential effect on modifying rumen microbial fermentation has been studied recently (Calsamiglia et al., 2007; Chaves et al., 2008c; Kongmun et al., 2010). The garlic oil and 4 purified active components (allicin, diallyl sulfide, diallyl disulfide, and allyl mercaptan) thought to play a major role in its antimicrobial activity, were tested *in vitro* to determine their effect on rumen microbial fermentation (Busquet et al., 2005a). In the present study, supplementing garlic oil consistently improved DMD and cumulative GP but had no effect on digestibility of NDF and ADF, kinetics parameters. The result is similar to

Yang et al. (2007), who observed that supplementing 5 g/d of garlic oil increased truly digestibility of DM without effect on NDF, ADF and starch digestibility. Klevenhusen et al. (2011) reported that although garlic oil supplementation had no effect on feed digestion, its principal organosulfur compound improved feed digestion in sheep. Regarding DMD result, cumulative GP and total VFA concentration were increased with GAR added (*i.e.*, 200 mg/kg DM) in the current study. The effect of GAR on methane production was inconsistency, 200 or 400 mg/kg DM GAR increased methane production at 24 h but could not maintain until 48 h in Exp. 3.1. Kongmun et al. (2010) demonstrated that supplementing coconut oil and garlic powder affected total VFAs and individual VFAs production. Supplementation of coconut oil : garlic powder; 8 : 4, 4 : 8 and 0 : 16, reduced total VFA and methane production while adding 0 : 16 reduced NH_3-N and acetate proportion but increased propionate proportion. However, garlic oil had no effect on VFA concentration, NH_3-N concentration and protozoa in lactating dairy cows as reported by Yang et al. (2007).

GIN

Lacking with the effect of GIN on rumen fermentation, there was only one *in vitro* study reported that ginger oil had no effect on total VFA concentration, individual VFA proportion, large peptide, small peptide plus amino acid, and ammonia concentration using continuous culture (Busquet et al., 2006). In contrast, the digestibility of DM and cumulative GP were improved with GIN supplementation, resulting in increased methane production at 24 h. Total VFA concentration also increased with 150 or 200 mg/kg DM GIN at 48 h. In addition, NH_3-N was consistently reduced when 200 mg/kg DM GIN was supplemented at either 24 or 48 h incubation. The results suggested that GIN had potential to improved DMD while reduced NH_3-N by inhibiting deamination.

LEM

There are few studies that reported the effects of lemongrass supplementation on rumen fermentation and feed digestion. Wanapat et al. (2008) reported that supplementing 100 g/d lemongrass powder increased DM digestibility, quadratic changed DM and NDF digestibility without affecting digestibility of CP and ADF in steer fed high forage (73% diet DM) diets. The present study demonstrated that adding LEM at 200 mg/kg DM improved DMD, resulting in higher cumulative GP, methane production and total VFA concentration at 24 and 48 h, whereas, ammonia N concentration was lowest at 200 mg/kg DM. Similarly, Wanapat et al. (2008) suggested that ammonia N concentration was lower at 100 or 200 g/d of lemongrass powder compared with control resulting in lower plasma urea N. Urea is synthesized in the liver from ammonia absorbed from the rumen or gut, and so a urea N concentration in blood is positively correlated with the ruminal concentration of ammonia (Hosada et al., 2006). In contrast, supplementing 50 g/kg of lemongrass had no effect on rumen VFA concentration and individual VFA proportion but increased rumen ammonia concentration (Hosada et al., 2006). Furthermore, supplementing mixtures of thyme, oregano, cinnamon and lemon that varied in ratios inhibited rumen fermentation and reduced population of rumen microbes (Lin et al., 2012). Methane production was induced with 200 mg/kg DM at either 24 or 48 h followed with DMD and cumulative GP results. However, protozoa population was decreased with increasing levels of lemongrass powder from 0 to 300 g/d (Wanapat et al., 2008).

The present study demonstrated that EO consistently improved DMD at either 24 or 48 h incubation resulting in higher cumulative GP. The digestibility of DM may be relative to microbial attachment (unpublished result). The results confirmed that GP is a reliable indicator of feed fermentation in the batch culture. Ammonia N

concentration was consistently reduced in all treatments both at 24 and 48 h of incubation both of the Experiment 3.1 and 3.2, suggesting that these EOs reduced deamination of amino acids, and could be alternative for reducing ammonia N loss in the rumen.

3.10 Conclusions

Supplementing EO increased DMD but reduced NH_3-N concentration with increasing EO from 0, 200, 400, 800, to 1600 mg/kg feed DM in Exp. 3.1, indicating that the EO used in the present study affected feed digestion in a dose-dependent manner. The results suggested that the dose of 200 mg/kg DM was cost-effective for each EO, which is consistent to DMD and NH_3-N concentration in Exp. 3.2. However, the effect of EO on methane production was apparently negligible. These results suggested that the EO used in the present study could be potentially developed as rumen modifier to improve feed digestion, especially high fiber feeds in ruminant animals.

3.11 References

Association of Official Analytical Chemists (AOAC). (1995). **Official Methods of Analysis** (2 ed.). USA.

Benchaar, C., Lettat, A., Hassanat, F., Yang W. Z., Forster R. J., Petit H. V., and Chouinard, P. Y. (2012). Eugenol for dairy cows fed low or high concentrate diets : Effects on digestion, ruminal fermentation characteristics, rumen microbial populations and milk fatty acid profile. **Anim. Feed Sci. Technol.** 178: 139-150.

Benchaar, C., and Greathead, H. (2011). Essential oils and opportunities to mitigate enteric methane emissions from ruminants. **Anim. Feed Sci. Technol.** 166: 338-355.

Benchaar, C., McAllister, T. A., and Chouinard, P. Y. (2008). Digestion, ruminal fermentation, ciliate protozoal populations, and milk production from dairy cows fed cinnamaldehyde, quebracho condensed tannin, or *yucca schidigera* saponin extracts. **J. Dairy Sci.** 91: 4765-4777.

Busquet, M., Calsamiglia, S., Ferret, A., and Kamel, C. (2005b). Screening for effects of plants on dairy cattle rumen microbial fermentation in a continuous culture system. **Anim. Feed Sci. Technol.** 123: 597-613.

Busquet, M., Calsamiglia, S., Ferret, A., and Kamel, C. (2006). Plant extracts affect *in vitro* rumen microbial fermentation. **J. Dairy Sci.** 89: 761-771.

Busquet, M., Calsamiglia, S., Ferret, A., Cardozo, P. W., and Kamel, C. (2005c). Effects of cinnamaldehyde and garlic oil on rumen microbial fermentation in a dual flow continuous culture. **J. Dairy Sci.** 88: 2508-2516.

Busquet, M., Calsamiglia, S., Ferret, A., Carro, M. D., and Kamel, C. (2005a). Effect of garlic oil and four of its compounds on rumen microbial fermentation. **J. Dairy Sci.** 88: 4393-4404.

Calsamiglia, S., Busquet, M., Cardozo, P. W., Castillejos, L., and Ferret, A. (2007). Invited review : Essential oils as modifiers of rumen microbial fermentation. **J. Dairy Sci.** 90: 2580-2595.

Cardozo, P. W., Calsamiglia, S., Ferret, A., and Kamel, C. (2006). Effects of alfalfa extract, anise, capsicum, and a mixture of cinnamaldehyde and eugenol on ruminal fermentation and protein degradation in beef cattle heifers fed a high-concentrate diet. **J. Anim. Sci.** 84: 2801-2808.

Cardozo, P. W., Calsamiglia, S., Ferret, A., and Kamel. C. (2005). Screening for the effects of natural plant extracts at different pH on *in vitro* rumenmicrobial fermentation of a high-concentrate diet for beef cattle. **J. Anim. Sci.** 83: 2572-2579.

Chaves, A. V., Stanford, K., Dugan, M. E. R., Gibson, L. L., McAllister, T. A., Van Herk, A., and Benchaar, A. (2008a). Effects of cinnamaldehyde, garlic and juniper berry essential oils on rumen fermentation, blood metabolites, growth performance, and carcass characteristics of growing lambs. **Livest. Sci.** 117: 215-224.

Chaves, A. V., Stanford, K., Gibson, L. L., McAlister, T. A., and Benchaar, C. (2008b). Effects of carvacrol and cinnamaldehyde on intake, rumen fermentation, growth performance, and carcass characteristics of growing lambs. **Anim. Feed Sci. Technol.** 145: 396-408.

Chaves, A.V., He, M. L., Yang, W. Z., Hristov, A. N., McAllister, T. A., and Benchaar, C. (2008c). Effects of essential oils on proteolytic, deaminative and methanogenic activities of mixed ruminal bacteria. **Can. J. Anim. Sci.** 88: 117-122.

Cheel, J., Theoduloz, C., Rodriìguez, J., and Schmeda-Hirschmann, G. (2005). Free radical scavengers and antioxidants from lemongrass (*Cymbopogon citratus* (DC.) Stapf.). **J. Agric. Food Chem.** 53: 2511-2517.

France, J., Dijkstra, J., Dhanoa, M. S., Lopez, S., and Bannink, A. (2000). Estimating the extent of degradation of ruminant feeds from a description of their gas production profiles observed *in vitro* : Derivation of models and other mathematical considerations. **Br. J. Nutr.** 83: 143-150.

Fraser, G.R., Chaves, A. V., Wang, Y., McAllister, T. A., Beauchemin, K. A., and Benchaar, C. (2007). Assessment of the effects of cinnamon leaf oil on rumen microbial fermentation using two continuous culture systems. **J. Dairy Sci.** 90: 2315-2328.

Goering, H. H., and Van Soest, P. J. (1970). **Forage fiber analysis (apparatus, reagents, procedures and some applications)**. USA: U.S. Agricultural Research Service.

Hall, M. B., Pell, A. N., and Chase, L. E. (1998). Characteristics of neutral detergent-soluble fiber fermentation by mixed ruminal microbes. **Anim. Feed Sci. Technol.** 70: 23-39.

Hammer, K. A., Carson, C. F., and Riley, T. V. (1999). Antimicrobial activity of essential oils and other plant extracts. **J. Appl. Microbiol.** 86: 985-990.

Hart, K. J., Yanez-Ruiz, D. R., Duval, S. M., McEwan, N. R., and Newbold, C. J. (2008). Plant extracts to manipulate rumen fermentation. **Anim. Feed Sci. Technol.** 147: 8-35.

Hosoda, K., Kuramoto, K., Eruden, B., Nishida, T., and Shioya, S. (2006). The effects of three herbs as feed supplements on blood metabolites, hormones, antioxidant activity, IgG concentration, and ruminal fermentation in Holstein steers. **Asian-Aust. J. Anim. Sci.** 19: 35-41.

Klevenhusen, K., Zeitz, J. O., Duval, S., Kreuzer, M., and Soliva, C. R. (2011). Garlic oil and principal component diallyl disulfide fail to mitigate methane, but improve digestibility in sheep. **Anim. Feed Sci. Technol.** 166: 356-363.

Kongmun, P., Wanapat, M., Pakdee, P., and Navanukraw, C. (2010). Effect of coconut oil and garlic powder on *in vitro* fermentation using gas production technique. **Livest. Sci.** 127: 38-44.

Li, Y. L., He, M. L., Li, C., Forster, R., Beauchemin, K. A., and Yang, W. Z. (2012). Effects of wheat dried distillers' grains with solubles and cinnamaldehyde on *in vitro* fermentation and protein degradation using the Rusitec technique. **Arch. Anim. Nutr.** 66: 131-148.

Lin, B., Lu, Y., Wang, J. H., Liang, Q., and Liu, J. X. (2012). The effects of combined essential oils along with fumarate on rumen fermentation and methane production *in vitro*. **J. Anim. Feed. Sci.** 21: 198-210.

Mauricio, R. M., Mould, F. L., Dhanoa, M. S., Owen, E., Channa, K. S., and Theodorou, M. K. (1999). A semi-automated *in vitro* gas production technique for ruminant feedstuff evaluation. **Anim. Feed Sci. Technol.** 79: 321-330.

McIntosh, F. M., Williams, P., Losa, R., Wallace, R. J., Beever, D. A., and Newbold, C. J. (2003). Effect of essential oil on ruminal metabolism and their protein metabolism. **Appl. Environ. Microbiol.** 69: 5011-5014.

Olfert, E. D., Cross, B. M., and McWilliam, A. A. (1993). **Guide to the Care and Use of Experimental Animals** (Vol. 1) (2nd ed.). Ottawa: Canadian Council on Animal Care.

Ramos-Morales, E., Martínez-Fernández, G., Abecia, L., Martin-García, A. I., Molina-Alcaide, E., and Yáñez-Ruiz, D. R. (2013). Garlic derived compounds modify ruminal fatty acid biohydrogenation and induce shifts in the *Butyrivibrio* community in continuous-culture fermenters. **Anim. Feed Sci. Technol.** 184: 38-48.

Rhine, E. D., Sims, G. K., Mulvaney, R. L., and Pratt, E. J. (1998). Improving the berthelot reaction for determining ammonia in soil extracts and water. **Soil Sci. Soc. Am. J.** 62: 473-480.

Valero, M., and Salmeroìn, M. C. (2003). Antibacterial activity of 11 essential oils against Bacillus cereus in tyndallized carrot broth. **Int. J. Food Microbiol.** 85: 73-81.

Van Soest, P. J., Robertson, J. B., and Lewis, B. A. (1991). Methods for dietary fiber, neutral fiber and nonstarch polysaccharides in relation to animal nutrition. **J. Dairy Sci.** 74: 3588-3595.

Wanapat, M., Cherdthong, A., Pakdee, P., and Wanapat, S. (2008). Manipulation of rumen ecology by dietary lemongrass (Cymbopogon citratus Stapf.) powder supplementation. **J. Anim. Sci.** 86: 3497-3503.

Yang, W. Z, Benchaar C, Ametaj, B. N., and Beauchemin K. A. (2010). Dose response to eugenol supplementation in growing beef cattle : Ruminal fermentation and intestinal digestion. **Anim. Feed Sci. Technol.** 158: 57-64.

Yang, W. Z., Benchaar, C., Ametaj, B. N., Chaves, A. V., He, M. L., and McAlister, T. A. (2007). Effects of garlic and juniper berry essential oils on ruminal fermentation and on the site and extent of digestion in lactating cows. **J. Dairy Sci.** 90: 5671-5681.

CHAPTER IV

EFFECTS OF ESSENTIAL OILS SUPPLEMENTATION ON *IN TRO* AND *IN SITU* FEED DIGESTON IN BEEF CATTLE

4.1 Abstract

The objective of this study was to investigate the effect of lemongrass oil (LEM) and a mixture of garlic and ginger oil (CEO) on gas production (GP) and feed digestibility using the batch culture and *in situ* ruminal technique. Four feeds: wheat distillers dried grains with solubles (DDGS), barley grain, grass hay, and a total mixed ration (TMR) were tested with varying essential oil (EO) dosages. The TMR consisted of 35% grass hay, 15% alfalfa hay, 20% barley grain, 10% corn DDGS, 10% wheat DDGS, 5% canola meal, and 5% vitamin and mineral supplement. The *in vitro* study was a complete randomized design with 2 × 4 factorial arrangement of two EO (LEM and CEO) combined with four dosages of EO (*i.e.*, 0, 100, 200, and 300 mg/kg substrate DM). Digestibilities of DM (DMD) and neutral detergent fiber (NDFD) were measured at 24 h and 48 h post incubation, while GP was read at 3, 6, 12, 24, 36, and 48 h post incubation. *In situ* ruminal digestibility was measured using three ruminally fistulated beef heifers with incubation time of 4, 12, 24 or 48 h. There was no interaction on *in vitro* DMD and NDFD between EO source and its dose. The DMD and NDFD were greater with CEO compared to LEM for wheat DDGS

(P<0.01; 48 h) and barley grain (P<0.01; 24 h), but lower for TMR (P<0.05; 24 or 48 h). Increasing the dosage of EO linearly (P<0.01) increased the DMD of wheat DDGS and barley grain at 24 h post incubation, and linearly (P<0.01) and quadratically (P<0.05) improved *in vitro* DMD and NDFD of grass hay and TMR with addition of LEM and CEO at 24 or 48 h post incubation. The cumulative GP was overall affected (P<0.01) by both LEM and CEO in quadratic manner after 24, 36 or 48 h of incubation. *In situ* ruminal DMD of wheat DDGS and barley grain were higher (P<0.05) at 4 or 24 h of incubation with CEO than with control or LEM which had no differences in DMD. However, the *in situ* DMD of grass hay and TMR were improved by both LEM and CEO supplementation after 24 h (P<0.01) or 48 h (P<0.05) post incubation. The dose of 200 mg/kg DM was likely cost-effective to improve DMD for both LEM and CEO. The LEM and CEO appeared to be more effective to improve the DMD of fibrous feeds since the *in vitro* DMD and NDFD of grass hay and TMR were consistently improved at 24 h or 48 h post incubation. These results suggested that the LEM and CEO used in the present study could be potentially developed as rumen modifier to improve feed digestibility in the rumen.

4.2 Introduction

Nutritionists have been searching for alternative additives for reducing the need for in-feed antibiotics such as ionophores. Using antibiotics in animal feed is facing reduced social acceptance due to the appearance of residues and resistant strain bacteria. Plant essential oil (EO) from variety of sources have been intensively studied during the last decades by ruminant scientists aiming to develop rumen modifiers for manipulating rumen fermentation as documented by several review papers

(Calsamiglia et al., 2007; Hart et al., 2008; Benchaar and Greathead, 2011). Many studies focused on cinnamon oil (cinnamaldehyde) or clove oil (eugenol) to evaluate the effects on rumen fermentation characteristics in particular (Busquet et al., 2006; Cardozo et al., 2005; Cardozo et al., 2006; Fraser et al., 2007). The researches demonstrated the ability of using EO to alter rumen fermentation and nutrient utilization in ruminants. Garlic, ginger, and lemongrass are plant extracts and herb of interest. These herbs are widely used in tropical countries as for one of human food composition. Lemongrass has been shown antibacterial (Valero and Salmeroìn, 2003), antioxidant (Cheel et al., 2005), and antihyper-NH_3-producing ruminal bacterial (McIntosh et al., 2003) activities as well as the effects on changes of blood metabolites and rumen fermentation in Holstein steers (Hosoda et al., 2006). However, lemongrass EO (LEM) was little evaluated on its effects on ruminal fermentation. In addition, garlic oils and garlic oil compounds have been explored as an alternative to antibiotics to manipulate rumen fermentation due to their well-known antimicrobial effects (Ramos-Morales et al., 2013). Garlic oil and garlic derived compounds have been demonstrated to have antimethanogenic property with mixed effects on rumen fermentation (Busquet et al., 2005a; Chaves et al., 2008). Ginger oil has also been detected to have inhibitory effects for 10 different micro-organisms (Hammer et al., 1999) but limited studies showed no effect on rumen VFA concentration in a continuous culture (Busquet et al., 2005b). Additive, antagonistic, and synergistic effects have occurred between components of EO (Burt, 2004), suggesting that combinations of EO of different composition, or specific combinations of EO secondary metabolites, may result in additive and/or synergetic effects which may enhance efficiency of rumen microbial fermentation. Finally, the responses of

rumen fermentation and feed digestion to EO supplementation depended on type of substrates or the composition of diets fed to animals (Hart et al., 2008).

4.3 Objectives

The objective of this study was to determine the effect of EO supplementation on gas production (GP) and rumen digestion of individual feed ingredient including wheat dried distillers grain with solubles (DDGS), barley grain, grass hay, and total mixed rations (TMR) using batch culture and in situ technique. Wheat DDGS is a by-product of ethanol plant and is commonly fed to livestock animals in western Canada and elsewhere in the world due to rapidly increased availability.

4.4 Materials and methods

Experiment 4.1 (Exp. 4.1) was a complete randomized design with 2 × 4 factorial arrangement of treatment. The EO were LEM and a combination of garlic oil and ginger oil at ratio of 1 : 1 (CEO), and were purchased commercially (purity >99%; Phodé S.A., Albi, France). The dosages of EO were 0, 100, 200, and 300 mg/kg substrate DM. The substrates included wheat DDGS, barley grain, grass hay, and TMR which consisted of 35% grass hay, 15% alfalfa hay, 20% barley grain, 10% corn DDGS, 10% wheat DDGS, 5% canola meal, and 5% vitamin and mineral supplement (Table 4.1). The substrates were ground through 1 mm screen (standard model 4 Wiley Mill; Arthur Thomas Co., Philadelphia, PA, USA), and mixed with EO before weighing into a test bag. A 0.5 g (DM basis) of substrate was weighed into a ANKOM F57 filter bag (pore size of 50 µm, Ankom Technology Corp., Macedon, NY, USA), and sealed for *in vitro* incubation.

Table 4.1 Ingredient and chemical composition of the diet.

Chemical composition (%)	Wheat DDGS	Barley grain	Grass hay	TMR
Organic matter	92.0	98.0	92.0	94.0
Neutral detergent fiber	23.7	22.1	63.1	39.4
Acid detergent fiber	14.7	10.0	37.3	23.6
Crude protein	38.8	12.7	6.2	16.1
Ether extract	4.1			
Starch	1.3	58.3		

Source of rumen fluid for *in vitro* incubations

Inoculum for the batch culture was obtained from three ruminally fistulated beef heifers (Spayed beef heifer) fed a diet consisting of 64% barley silage, 6% grass hay, 27% dry-rolled barley grain, and 3% vitamin and mineral supplement. Rumen fluid was collected from different sites within the rumen approximately 2 h after the morning feeding, pooled, and squeezed through PeCAP® polyester screen (pore size 355 µm; B & S Thompson, Ville Mont-Royal, QC, Canada) into an insulated thermos, and transported immediately to the laboratory. All animal procedures were in accordance with the guidelines of the Canadian Council on Animal Care (CCAC 1993).

Rumen fluid was added to medium in a ratio of 1 : 3 (rumen fluid : medium). Anaerobic buffer medium (45 mL; Goering and Van Soest, 1970) contained tryptone, buffer, macro and micro mineral solution, resazurin and water. Forty-five milliliters of prewarmed media and 15 mL of inoculum were added anaerobically to the 100 mL bottles by flushing with oxygen free CO_2. Bottles were sealed immediately with a 14 mm butyl rubber stopper plus aluminum crimp cap and incubated at 39 ^{o}C for 24 or 48 h. The incubation was repeated with two runs. Needle was inserted through rubber

stopper of each vial for about 5 seconds to release small amount of gas that might have built up and create starting point for incubation. All vials were returned to the incubator. Rotary shaker was turned on (~120 rpm). Negative control (rumen fluid plus anaerobic buffer medium) and blanks (filter bags plus anaerobic buffer medium and rumen fluid) were also incubated using 4 replications for correction of gas production and disappearance, respectively.

Sample collection and processing

At pre-determined time points, headspace gas production (GP) was measured at 3, 6, 12, 24, 36, and 48 h post incubation by inserting a 23 gauge (0.6 mm) needle attached to a pressure transducer (model PX4200-015GI, Omega Engineering, Inc., Laval, QC., Canada), connected to a visual display (Data Track, Christchurch, UK). A volume of 15 mL gas was sampled using a syringe and transferred into 6.8 mL Exetainer vials (Labco Ltd., Wycombe, Bucks, UK) for immediate measurement of CH_4. Methane concentration was determined using a gas chromatography (Varian 4900 GC; Agilent Technologies Canada Inc., Mississauga, ON, Canada). Pressure values, corrected by the amount of substrate OM incubated and the gas released from negative controls, were used to generate volume using the equation of Mauricio et al. (1999) as :

$$\text{Gas volume} = 0.18 + (3.697 \times \text{gas pressure}) + (0.0824 \times \text{gas pressure}^2)$$

The kinetic parameters of GP were calculated using the equation of France et al. (2000) as:

$$A = b \times (1 - e^{-c(t-L)})$$

Where A is the volume of GP at time *t*; b is the asymptotic GP (mL/g DM); *c* is the rate of GP (/h), and *L* (h) is the discrete lag time prior to gas produced.

After 24 h and 48 h of incubation, the bags were removed from the vials and washed under stream of cold water until the water runs clear. The bags were dried in an oven at 55°C for 48 h to determine DM digestibility. The NDF concentration in the residue was determined as described by Van Soest et al. (1991) using heat-stable α-amylase (Termamyl 120 L, Novo Nordisk Biochem, Franklinton, NC, USA) and sodium sulfite. Procedures to analyze NDF and ADF were adapted for use in an ANKOM200 fiber analyzer (Ankom Technology Corp., Macedon, NY).

Experiment 4.2 (Exp. 4.2), the study was approved by institutional Animal Care Committee of the Agriculture and Agri-Food Canada Research Centre, Lethbridge, Canada, and was conducted in accordance with the guidelines of the Canadian Council on Animal Care (1993). Three ruminally fistulated beef heifers were used and animals were fed *ad libitum* with a TMR that consisted of 60% barley silage, 37% dry rolled barley, and 3% vitamin and mineral supplement. The EO were the same as used in the Exp. 4.1, only one dosage (200 mg/kg DM) was tested. The dosage of the EO was selected based on the results from the batch culture (Exp. 4.1; *i.e.* digestibility of DM and NDF). The feeds used were the same feedstuffs as in Exp. 4.1 (*i.e.*, wheat DDGS, barley grain, grass hay, and TMR) and ground through 4 mm screen (standard model 4 Wiley Mill; Arthur Thomas Co., Philadelphia, PA, USA), mixed with EO before weighing into nylon bag. Five gram (DM basis) sample was weighed into nylon bag (10 × 20 cm; pore size of 50 µm), and sealed with the heater.

The bags were introduced into the rumen through the rumen cannula and incubated for 4, 12, and 24 h for wheat DDGS and barley grain, but only 24 and 48 h for grass hay and the TMR. Three bags were incubated for each time point and each heifer. After removal from the rumen, the bags were washed under running tap water until the effluent was clear and oven-dried at 55 °C for 48 h. The bags and contents

were weighed for calculation of DM digestibility. The residues from triplicates bags belonging to the same treatment and incubated in the same animal were pooled and ground through 1 mm and analyzed for NDF and total nitrogen (N).

Ruminal microbial attachment on the residues in bags incubated in the rumen was measured using ^{15}N as microbial marker. An amount of 3.5 g/d ammonia ^{15}N ($[^{15}NH_4]_2SO_4$) (Sigma Chemical Co., St. Louis, MO, USA) was dissolved in water, mixed with rolled barley (100 g/head per day) as a carrier, and manually mixed into the diet at daily feeding time on 7 days prior to *in situ* incubation and until the end of the experiment. The bags that had wheat DDGS and grass hay were incubated in the rumen for 3 or 6 h in 3 heifers. After the incubation, the bags were removed and washed gently with warmed (~39 °C) 0.9% NaCl water until the water clear to minimize detachment of bacteria from residues. The bags were dried in an oven at 55 °C for 48 h for determining ^{15}N content on the residues.

Table 4.2 Effects of essential oil (EO) source and EO dosage on *in vitro* digestibility (%) of dry matter (DMD) and NDF (NDFD) of individual feed and total mixed rations (TMR) after 24 or 48 h of batch culture.

Feeds	LEM[1/] (mg/kg)				CEO[1/] (mg/kg)				SEM[2/]	P-value			
	0	100	200	300	0	100	200	300		EO	$Dose_L$	$Dose_Q$	EO x Dose
Wheat DDGS													
DMD – 24h	46.9^{bc}	46.2^{c}	49.1^{a}	48.3^{ab}	47.4^{bc}	47.9^{ab}	49.5^{a}	48.3^{ab}	0.006	0.06	0.01	0.17	0.33
DMD – 48h	55.0^{bc}	53.5^{c}	57.3^{a}	55.0^{ab}	56.2^{ab}	57.0^{a}	57.4^{a}	56.8^{a}	0.007	0.01	0.10	0.17	0.06
NDFD – 24h	16.6^{c}	16.6^{c}	17.0^{b}	16.9^{bc}	17.1^{b}	17.1^{b}	17.2^{a}	17.0^{b}	0.002	0.01	0.09	0.08	0.19
NDFD – 48h	27.9	27.6	28.6	28.1	28.4	28.2	28.9	28.6	0.005	0.09	0.21	0.47	0.99
Barley grain													
DMD – 24h	60.1^{cd}	5.91^{d}	61.1^{abc}	60.8^{bc}	60.8^{bc}	60.1^{cd}	62.2^{a}	62.1^{ab}	0.006	0.01	0.01	0.32	0.89
DMD – 48h	70.7	6.98	71.9	70.6	71.3	70.9	71.4	70.7	0.005	0.26	0.92	0.56	0.25
NDFD – 24h	24.7	24.6	24.8	25.0	23.9	24.0	24.7	23.9	0.005	0.06	0.42	0.62	0.61
NDFD – 48h	30.5	30.4	30.8	30.5	30.3	30.3	31.1	30.8	0.003	0.54	0.06	0.52	0.57
Grass hay													
DMD – 24h	30.9^{bc}	30.9^{b}	32.6^{a}	31.8^{ab}	29.6^{c}	30.9^{bc}	32.8^{a}	30.8^{bc}	0.007	0.17	0.01	0.02	0.41
DMD – 48h	40.1^{c}	39.8^{c}	42.0^{ab}	41.2^{b}	40.2^{c}	40.1^{c}	42.2^{a}	40.7^{bc}	0.004	0.99	0.01	0.06	0.58
NDFD – 24h	24.8^{b}	24.2^{b}	27.9^{a}	24.5^{b}	24.2^{b}	24.6^{b}	27.3^{a}	25.0^{b}	0.008	0.82	0.06	0.01	0.61
NDFD – 48h	32.5^{cd}	33.5^{bc}	35.1^{ab}	34.1^{bc}	32.6^{cd}	32.3^{cd}	35.6^{a}	32.2^{d}	0.006	0.23	0.03	0.01	0.08

Table 4.2 Effects of essential oil (EO) source and EO dosage on *in vitro* digestibility (%) of dry matter (DMD) and NDF (NDFD) of individual feed and total mixed rations (TMR) after 24 or 48 h of batch culture (Continued).

Feeds	LEM[1/] (mg/kg)				CEO[1/] (mg/kg)				SEM[2/]	P-value			
	0	100	200	300	0	100	200	300		EO	$Dose_L$	$Dose_Q$	EO x Dose
TMR													
DMD – 24h	40.8^{cd}	40.4^{cd}	43.5^{a}	41.6^{bc}	39.6^{d}	39.5^{d}	42.7^{ab}	40.7^{cd}	0.007	0.03	0.01	0.03	0.97
DMD – 48h	52.5^{c}	52.6^{c}	55.4^{a}	52.8^{bc}	52.9^{bc}	53.3^{b}	55.9^{a}	53.6^{b}	0.003	0.01	0.01	0.01	0.78
NDFD – 24h	17.5^{b}	17.6^{b}	19.8^{a}	18.4^{ab}	17.2^{b}	17.1^{b}	19.7^{a}	19.2^{a}	0.006	0.99	0.01	0.18	0.55
NDFD – 48h	31.0^{c}	31.3^{c}	33.6^{a}	31.0^{c}	31.2^{c}	31.6^{c}	33.8^{a}	32.5^{b}	0.004	0.02	0.01	0.01	0.14

[a-d]Within a row means without a common superscript letter differ.

[1/]LEM = Lemongrass EO; CEO = Equally blend of garlic and ginger EO.

[2/]SEM = standard error of the mean.

4.5 Statistical analysis

In Exp. 5.1, data were analyzed using the mixed model procedure of SAS (SAS Inst. Inc., Cary, NC) to account for the fixed effect of EO source, EO dosage, interaction between EO and dosage, and run was random effect (experimental unit). The effect of increasing levels of EO from 0, 100, 200 to 300 mg/kg DM in the substrate was examined through linear and quadratic orthogonal contrasts using the CONTRAST statement of SAS. Differences were declared significant at $P \leq 0.05$. Trends were discussed at $0.05 < P \leq 0.10$ unless otherwise stated.

In Exp. 5.2, data were analyzed using the mixed model procedures of SAS (SAS Institute, Inc., Cary, NC) to account for the fixed effects of treatment and the random effect of animal. Results were reported as least squares means. Differences between treatments were declared significant at $P \leq 0.05$ and means were compared using the Tukey correction for multiple comparisons. Trends were discussed at $0.05 < P \leq 0.10$ unless otherwise stated.

4.6 Experimental site

The experiment was conducted at Research Centre, Agriculture and Agri-Food Canada, Lethbridge, Alberta, Canada.

4.7 Duration

The duration of this trial was from January to March 2013.

4.8 Results

4.8.1 Exp. 1 – Feed digestion and gas production

There was no interaction overall on DMD and NDF disappearance (NDFD) between EO source and the dose at either 24 or 48 h of incubation (Table 4.2). The digestibility of DM and NDF were overall improved (P<0.05) by CEO compared with LEM for wheat DDGS, barley grain (only at 24 h post incubation) and TMR except for grass hay which was not affected by the EO source. Increasing the dosage of EO linearly (P<0.01) increased DMD of wheat DDGS and barley grain at 24 h of incubation, and overall linearly (P<0.05) and quadratically (P<0.05) increased the DMD and NDFD of grass hay and TMR at either 24 or 48 h post incubation.

Gas production kinetics and cumulative GP as affected by EO sources and the dosages of EO were shown in Tables 4.3 through 4.6, respectively, for wheat DDGS, barley grain, grass hay, and TMR. There were no interactions between EO sources and dosages of EO on GP kinetic parameters and cumulative GP. The GP kinetic parameters and cumulative GP of wheat DDGS and barley grain were not affected by EO supplementation except that a few trend (P<0.10) effects were observed. For the substrate of grass hay, the maximum GP was affected neither by EO source nor with increasing EO dosages; whereas, rate constant of GP tended (P<0.09) to linearly decrease with increasing the dose of LEM. Furthermore, cumulative GP of grass hay linearly increased with addition of LEM after 36 h (P<0.05) or 48 h (P<0.05) of incubation. There were also quadratic changes of GP with CEO after 48 h (P<0.05) of incubation. Finally for the substrate of TMR, adding LEM did not affect the GP kinetics, whereas adding CEO tended (P<0.07) to increase the maximum GP without

affecting the rate constant of GP. The cumulative GP was generally affected (P<0.01) by both LEM and CEO in quadratic manner after 24, 36 or 48 h of incubation.

Methane production was not affected by treatments with wheat DDGS, barley grain, and TMR at 24 or 48 h (Table 4.7). There was interaction between EO source and the dosage of EO on methane production of grass hay after 24 h of incubation; methane production was quadratically (P<0.01) changed with increasing LEM but not with increasing CEO supplementation.

4.8.2 Exp. 2 – *In situ* ruminal digestion and microbial attachment

In situ ruminal DMD of wheat DDGS and barley grain were higher (P<0.05) at 4 or 24 h post incubation with CEO than with control or LEM which had no differences in DMD (Table 4.8). However, *in situ* DMD of grass hay and TMR were improved by addition of both LEM and CEO after 24 h (P<0.01) or 48 h (P<0.05) of incubation. Similarly, *in situ* ruminal digestibility of NDF of grass hay and TMR were improved (P<0.01) by LEM and CEO addition, but that of wheat DDGS and barley grain was not affected by LEM and CEO supplementation. Ruminal microbial attachment measured as ^{15}N concentration on the residues of wheat DDGS after 3 or 6 h of incubation was not affected with EO addition (Table 4.9). However, microbial attachment on the residues of grass hay was increased (P<0.01) by LEM or CEO after 6 h of incubation in the rumen.

Table 4.3 Effect of essential oil (EO) source and dosages on gas production (GP) kinetics and cumulative GP of wheat dried distillers grain with solubles after 48 h of batch culture.

	Parameters[1/]			***In vitro* GP (ml/g DM)**						
	b	**c**	**L**	**GP_3**	**GP_6**	**GP_9**	**GP_{12}**	**GP_{24}**	**GP_{36}**	**GP_{48}**
LEM[2/] **(mg/kg)**										
0	94.6	0.075	0.12	17.8	33.9	45.6	56.2	74.6	83.9	94.1
100	94.7	0.073	0.05	18.1	34.9	44.6	56.0	74.0	84.4	94.5
200	93.2	0.083	0.04	20.5	37.5	47.7	58.6	75.3	84.1	94.6
300	95.3	0.077	0.04	19.8	36.1	45.6	57.1	74.5	84.6	94.9
Linear	0.93	0.55	0.19	0.09	0.36	0.66	0.53	0.91	0.71	0.60
Quadratic	0.46	0.78	0.30	0.64	0.59	0.75	0.71	0.96	0.96	0.98
CEO[2/] **(mg/kg)**										
0	94.9	0.075	0.06	20.3	35.3	44.4	56.6	74.1	83.5	94.0
100	94.5	0.070	0.08	19.4	35.2	45.5	57.8	74.9	84.3	94.6
200	94.2	0.080	0.02	21.0	37.0	46.7	58.4	75.5	85.1	95.4
300	94.2	0.079	0.05	20.2	36.4	45.5	57.8	74.5	84.0	94.5
Linear	0.68	0.44	0.49	0.76	0.61	0.53	0.59	0.82	0.67	0.62
Quadratic	0.90	0.72	0.44	0.95	0.92	0.46	0.63	0.61	0.42	0.50
SEM[3/]	1.86	0.0084	0.35	1.51	3.21	2.22	2.59	2.50	1.64	1.56

Table 4.3 Effect of essential oil (EO) source and dosages on gas production (GP) kinetics and cumulative GP of wheat dried distillers grain with solubles after 48 h of batch culture (Continued).

	Parameters[1/]			***In vitro* GP (ml/g DM)**						
	b	**c**	**L**	**GP_3**	**GP_6**	**GP_9**	**GP_{12}**	**GP_{24}**	**GP_{36}**	**GP_{48}**
P-value										
EO	0.99	0.82	0.78	0.12	0.82	0.75	0.61	0.92	0.97	0.90
Dose	0.83	0.44	0.51	0.24	0.64	0.46	0.69	0.93	0.87	0.85
EO × dose	0.87	0.97	0.49	0.78	0.98	0.91	0.96	0.98	0.92	0.96

[1/]Parameters : b is the theoretical maximum GP (ml/g DM); c is the rate constant of GP (/h); Lag is the initial delay before GP begins (h).

[2/]LEM = Lemongrass EO; CEO = Equally blend of garlic and ginger EO.

[3/]SEM = standard error of the mean.

Table 4.4 Effect of essential oil (EO) source and dosages on gas production (GP) kinetics and cumulative GP of barley grain after 48 h of batch culture.

	Parameters[1/]			*In vitro* GP (ml/g DM)						
	b	**c**	**L**	**GP_3**	**GP_6**	**GP_9**	**GP_{12}**	**GP_{24}**	**GP_{36}**	**GP_{48}**
LEM[2/] (mg/kg)										
0	98.5	0.079	0.01	15.8	32.2	36.9	45.1	69.5	82.1	107.5
100	98.6	0.076	0.01	16.0	32.4	37.5	45.7	68.9	85.5	107.5
200	101.4	0.078	0.06	15.6	33.0	38.9	47.7	72.7	86.8	111.3
300	96.8	0.081	0.05	16.7	33.0	38.4	46.9	71.2	84.7	108.1
Linear	0.71	0.64	0.14	0.81	0.89	0.75	0.70	0.59	0.19	0.39
Quadratic	0.13	0.56	0.94	0.80	0.98	0.89	0.86	0.91	0.07	0.26
CEO[2/] (mg/kg)										
0	98.3	0.080	0.16	15.2	31.0	36.3	45.0	68.9	82.9	107.9
100	99.9	0.079	0.10	15.7	31.9	37.2	45.2	69.0	83.8	106.6
200	101.4	0.072	0.19	15.3	31.6	37.1	45.3	71.0	86.8	112.2
300	98.8	0.068	0.21	15.2	31.1	35.9	43.6	69.7	82.6	108.2
Linear	0.64	0.06	0.44	0.97	0.99	0.94	0.82	0.80	0.76	0.32
Quadratic	0.18	0.73	0.37	0.88	0.87	0.79	0.81	0.85	0.09	0.35
SEM[3/]	1.96	0.0060	0.079	2.81	6.29	5.68	5.78	5.25	2.14	2.04

Table 4.4 Effect of essential oil (EO) source and dosages on gas production (GP) kinetics and cumulative GP of barley grain after 48 h of batch culture (Continued).

	Parameters[1/]			*In vitro* GP (ml/g DM)						
	b	**c**	**L**	**GP_3**	**GP_6**	**GP_9**	**GP_{12}**	**GP_{24}**	**GP_{36}**	**GP_{48}**
P-value										
EO	0.45	0.26	0.32	0.62	0.69	0.65	0.59	0.72	0.48	0.94
Dose	0.14	0.70	0.65	0.99	0.99	0.99	0.98	0.86	0.07	0.07
EO x dose	0.83	0.31	0.81	0.99	0.99	0.99	0.98	0.99	0.75	0.93

[1/]Parameters : b is the theoretical maximum GP (ml/g DM); c is the rate constant of GP (/h); Lag is the initial delay before GP begins (h).

[2/]LEM = Lemongrass EO; CEO = Equally blend of garlic and ginger EO.

[3/]SEM = standard error of the mean.

Table 4.5 Effect of essential oil (EO) source and dosages on gas production (GP) kinetics and cumulative GP of grass hay after 48 h of batch culture.

	Parameters[1/]			*In vitro* GP (ml/g DM)						
	b	**c**	**L**	**GP_3**	**GP_6**	**GP_9**	**GP_{12}**	**GP_{24}**	**GP_{36}**	**GP_{48}**
LEM[2/] (mg/kg)										
0	91.5	0.041	0.06	11.4	19.0	24.2	33.1	51.8^{b}	61.9^{c}	74.0^{b}
100	91.7	0.039	0.12	10.7	18.7	23.5	33.8	52.0^{ab}	62.2^{c}	74.9^{b}
200	100.3	0.038	0.24	10.4	18.6	24.7	35.6	56.0^{a}	67.2^{ab}	80.7^{a}
300	96.0	0.033	0.21	9.7	17.5	22.6	32.0	51.8^{b}	64.1^{bc}	76.9^{b}
Linear	0.24	0.09	0.06	0.23	0.39	0.51	0.82	0.57	0.04	0.02
Quadratic	0.58	0.54	0.22	0.99	0.78	0.53	0.16	0.16	0.18	0.08
CEO[2/] (mg/kg)										
0	99.1	0.034	0.11	10.5	17.8	22.6	32.0	51.5^{b}	63.7^{c}	76.1^{b}
100	98.5	0.034	0.19	9.3	17.2	22.5	32.0	51.8^{b}	63.2^{c}	75.8^{b}
200	100.2	0.038	0.15	11.6	20.3	25.8	36.1	56.2^{a}	68.9^{a}	82.2^{a}
300	90.9	0.039	0.10	11.1	18.9	23.6	34.0	53.4^{ab}	63.9^{bc}	75.8^{b}
Linear	0.22	0.15	0.09	0.36	0.23	0.25	0.14	0.15	0.28	0.36
Quadratic	0.29	0.87	0.45	0.73	0.76	0.39	0.52	0.32	0.08	0.02
SEM[3/]	5.41	0.0038	0.089	1.41	1.68	1.69	2.16	2.20	1.80	1.87

Table 4.5 Effect of essential oil (EO) source and dosages on gas production (GP) kinetics and cumulative GP of grass hay after 48 h of batch culture (Continued).

	Parameters[1/]			*In vitro* GP (ml/g DM)						
	b	**c**	**L**	**GP_3**	**GP_6**	**GP_9**	**GP_{12}**	**GP_{24}**	**GP_{36}**	**GP_{48}**
P-value										
EO	0.42	0.39	0.38	0.95	0.93	0.90	0.92	0.74	0.24	0.37
Dose	0.39	0.93	0.83	0.73	0.62	0.21	0.12	0.02	0.01	0.01
EO x dose	0.37	0.17	0.20	0.37	0.39	0.59	0.60	0.92	0.85	0.64

[a-c]Within a column means without a common superscript letter differ.

[1/]Parameters : b is the theoretical maximum GP (ml/g DM); c is the rate constant of GP (/h); Lag is the initial delay before GP begins (h).

[2/]LEM = Lemongrass EO; CEO = Equally blend of garlic and ginger EO.

[3/]SEM = standard error of the mean.

Table 4.6 Effect of essential oil (EO) source and dosage on gas production (GP) kinetics and cumulative GP of total mixed ration (TMR) after 48 h of batch culture.

	Parameters[1/]			***In vitro* GP (ml/g DM)**						
	b	**C**	**L**	**GP_3**	**GP_6**	**GP_9**	**GP_{12}**	**GP_{24}**	**GP_{36}**	**GP_{48}**
LEM[2/] **(mg/kg)**										
0	123.9	0.039	0.14	15.0	28.6	38.6	48.1	73.1^{bc}	91.8^{c}	109.4^{b}
100	123.3	0.042	0.13	17.4	31.4	39.5	49.6	74.8^{bc}	95.0^{bc}	111.1^{b}
200	127.9	0.040	0.13	18.6	32.7	41.0	51.0	78.2^{a}	99.1^{a}	117.7^{a}
300	123.5	0.043	0.13	18.1	31.6	39.8	48.3	73.1^{bc}	92.9^{c}	109.0^{b}
Linear	0.67	0.45	0.57	0.04	0.12	0.31	0.74	0.56	0.23	0.36
Quadratic	0.28	0.83	0.61	0.21	0.19	0.35	0.09	0.01	0.01	0.01
CEO[2/] **(mg/kg)**										
0	124.8	0.039	0.13	18.0	31.3	38.5	47.9	71.2^{c}	90.9^{c}	110.7^{b}
100	126.4	0.038	0.14	17.4	31.0	38.1	47.1	71.5^{c}	92.8^{c}	110.4^{b}
200	130.7	0.039	0.14	19.1	33.2	40.7	49.9	76.2^{ab}	97.4^{ab}	118.1^{a}
300	128.5	0.041	0.14	18.2	31.9	38.8	48.8	73.0^{bc}	91.8^{c}	111.1^{b}
Linear	0.07	0.65	0.06	0.63	0.54	0.47	0.33	0.09	0.24	0.12
Quadratic	0.28	0.53	0.21	0.91	0.73	0.51	0.92	0.19	0.01	0.01
SEM[3/]	2.32	0.0036	0.032	1.58	2.11	1.57	1.75	1.88	1.94	1.81

Table 4.6 Effect of essential oil (EO) source and dosage on gas production (GP) kinetics and cumulative GP of total mixed ration (TMR) after 48 h of batch culture (Continued).

	Parameters[1/]			*In vitro* GP (ml/g DM)						
	b	**C**	**L**	**GP_3**	**GP_6**	**GP_9**	**GP_{12}**	**GP_{24}**	**GP_{36}**	**GP_{48}**
P-value										
EO	0.07	0.35	0.07	0.24	0.47	0.33	0.34	0.06	0.14	0.38
Dose	0.07	0.65	0.11	0.19	0.24	0.17	0.21	0.01	0.01	0.01
EO x dose	0.66	0.91	0.17	0.51	0.74	0.93	0.64	0.9	0.96	0.73

[a-c]Within a column means without a common superscript letter differ.

[1/]Parameters : b is the theoretical maximum GP (ml/g DM); c is the rate constant of GP (/h); Lag is the initial delay before GP begins (h).

[2/]LEM = Lemongrass EO; CEO = Equally blend of garlic and ginger EO.

[3/]SEM = standard error of the mean.

Table 4.7 Effect of essential oil (EO) source and dosage on methane (CH_4, ml/g DM) production of individual feed and total mixed ration (TMR) after 24 or 48 h of batch culture.

Feeds	LEM[1/] (mg/kg)				CEO[1/] (mg/kg)				SEM[2/]	P-value			
	0	100	200	300	0	100	200	300		EO	$Dose_L$	$Dose_Q$	EO x Dose
Wheat DDGS													
24 h	10.1	9.6	10.3	10.0	10.0	10.2	10.3	10.3	0.38	0.42	0.49	0.99	0.61
48 h	11.0	11.0	11.2	11.0	11.1	11.2	11.3	11.0	0.37	0.60	0.98	0.43	0.95
Barley grain													
24 h	10.8	10.7	10.7	10.8	10.4	10.2	10.2	10.6	0.30	0.06	0.64	0.19	0.94
48 h	11.9	11.6	12.2	11.8	11.7	11.9	12.5	11.6	0.27	0.69	0.68	0.06	0.43
Grass hay													
24 h	6.8^{ab}	7.0^{a}	7.0^{a}	6.2^{c}	6.8^{ab}	6.6^{b}	6.8^{ab}	6.6^{b}	0.16	0.04	0.84	0.01	0.01
48 h	8.7	8.8	9.4	9.0	9.0	8.9	9.4	8.9	0.21	0.96	0.12	0.12	0.50
TMR													
24 h	9.7	9.9	10.0	9.9	9.7	9.5	10.0	9.8	0.35	0.47	0.35	0.65	0.81
48 h	14.5	14.2	14.2	14.5	14.3	14.5	15.8	14.8	0.32	0.66	0.81	0.61	0.50

[a-c]Within a column means without a common superscript letter differ.

[1/]LEM = Lemongrass EO; CEO = Equally blend of garlic and ginger EO.

[2/]SEM = standard error of the mean.

Table 4.8 Effect of essential oil (EO) supplementation (200 mg/kg) on *in situ* ruminal digestibility of dry matter (DMD, g/kg), neutral detergent fiber (NDFD) and crude protein (CPD).

Feeds	DMD			SEM[2/]	P<	NDFD			SEM[2/]	P<	CPD			SEM[2/]	P<
	Control	LEM[1/]	CEO[a]			Control	LEM[1/]	CEO[a]			Control	LEM[1/]	CEO[a]		
Wheat DDGS															
4 h	44.7[b]	44.7[b]	45.8[a]	0.003	0.05	7.2	7.6	7.3	0.002	0.22	36.1	35.9	36.5	0.003	0.26
12 h	56.1	56.4	56.4	0.007	0.87	11.9	11.9	11.9	0.001	0.95	46.0	45.8	46.4	0.003	0.23
24 h	64.8[b]	65.0[b]	66.0[a]	0.003	0.02	17.0	17.0	17.0	0.001	0.94	56.1	56.6	55.7	0.004	0.16
Barley grain															
4 h	48.5[b]	48.3[b]	49.6[a]	0.003	0.01	7.3	7.5	7.8	0.002	0.21	35.8	35.9	35.8	0.007	0.97
12 h	61.4	61.4	62.1	0.005	0.37	13.8	14.2	14.2	0.003	0.23	55.7	56.0	55.9	0.003	0.55
24 h	71.0[b]	70.9[b]	71.7[a]	0.002	0.03	18.2	18.3	18.4	0.001	0.17	68.8	68.8	69.4	0.002	0.06
Grass hay															
24 h	32.9[b]	35.3[a]	35.8[a]	0.005	0.01	24.3[b]	26.9[a]	26.9[a]	0.002	0.01	37.3	37.7	37.3	0.002	0.08
48 h	43.5[b]	46.2[a]	46.7[a]	0.007	0.02	34.7[b]	38.1[a]	38.1[a]	0.003	0.01	51.1	51.5	51.4	0.003	0.47
TMR															
24 h	39.2[c]	41.4[b]	42.2[a]	0.002	0.01	18.3[b]	21.2[a]	21.5[a]	0.001	0.01	41.3	41.4	41.3	0.001	0.08
48 h	49.8[b]	52.6[a]	51.7[a]	0.003	0.01	30.1[b]	34.1[a]	34.2[a]	0.001	0.01	55.5	55.6	55.5	0.001	0.76

[a-c]Within a column means without a common superscript letter differ.

[1/]LEM = Lemongrass EO; CEO = Equally blend of garlic and ginger EO. [2/]SEM = standard error of the mean.

Table 4.9 Effect of essential oils (EO) supplementation (200 mg/kg) on microbial attachment (mg ^{15}N/kg DM) on *in situ* residues.

Feeds	Microbial attachment			SEM[2/]	P <
	Control	LEM[1/]	CEO[1/]		
Wheat DDGS					
3 h	51.8	52.2	51.3	0.60	0.41
6 h	114.1	114.1	110.9	1.20	0.12
Grass hay					
3 h	20.0	19.8	19.8	0.35	0.80
6 h	40.7[b]	42.9[a]	43.0[a]	0.08	0.01

[a-b]Within a column means without a common superscript letter differ.

[1/]LEM = Lemongrass EO; CEO = Equally blend of garlic and ginger EO.

[2/]SEM = standard error of the mean.

4.9 Discussion

LEM

There is limited information on the effects of LEM supplementation on *in vitro* or *in vivo* rumen fermentation and feed digestion. Lin et al. (2012) reported the inhibited rumen fermentation and reduced population of rumen microbes with adding mixtures of thyme, oregano, cinnamon and lemon that varied in ratios. Hosada et al. (2006) fed 50 g/kg lemongrass (DM basis) to steers and found no difference in rumen VFA concentration and the molar proportion of individual VFA, but rumen ammonia concentration was increased compared to control group. In contrast, Wanapat et al. (2008) reported that beef cattle fed increased levels of lemongrass powder from 0, 100, 200 to 300 g/d had linear reduction in rumen ammonia concentration without

changing VFA concentration, whereas, DM digestibility was quadratically changed with highest DM digestibility at 100 g/d. The linearly increased *in vitro* DMD of grass hay and TMR with increasing LEM addition is consistent with the findings of Wanapat et al. (2008). Results suggested that the effect of LEM on rumen digestibility is in dose-depending manner. The increased *in vitro* DMD with LEM supplementation was due at least partly to the increased NDF digestion for high-fiber feed such as grass hay or high-forage TMR. This suggestion is confirmed by our *in situ* results of consistently improved ruminal digestibility of DM and NDF of grass hay and TMR with EO addition. In contrast, the improved *in vitro* or *in situ* DM digestibility of wheat DDGS or barley grain (concentrate feed) was overall not followed by an improved NDF digestion. Similarly, Wanapat et al. (2008) reported the quadratic change of the total DM and NDF digestibility without altering the digestibility of CP and ADF with increasing lemongrass in the high-forage (73% diet DM) diets fed to steers. It suggests that the LEM may particularly be effective to improve fiber digestion of roughage, thus improve DM digestion.

The effect of LEM on feed digestion likely varied with substrates targeted, fibrous feed appeared to be affected more versus concentrate feed. Using dairy type diet (>50% forage), Busquet et al. (2005c) reported that adding cinnamaldehyde (CIN) at the doses of 31.2 or 312 mg/L had no effects on DM and NDF digestibility in continuous culture. However, the decreased disappearances of DM, CP, NDF and starch by CIN supplementation (300 mg/L) were observed by Li et al. (2012) in continuous culture using a high-grain diet (90%). The discrepancy between those two studies may be primarily attributed to the difference in substrate composition since the dose of CIN (300 vs. 312 mg/L) and the culture techniques used were similar. The biological properties of EO are mainly determined by their principal active

components and vary with the chemical structure of the main component (Castillejos et al., 2006). The LEM and cinnamon oil are aldehyde-based oils, suggesting similar mode of action in the rumen. The present results indicated the more effective for fibrous feed than concentrate feed by LEM. The present *in situ* results suggested that effect of LEM on rumen N metabolism was minimal. Busquet et al. (2006) demonstrated that some EO (e.g., anise oil, cinnamon oil, clove bud oil, garlic oil, ginger oil, oregano, oil, and tea tree oil) and their main components inhibited NH_3–N concentration at high concentrations (*i.e.*, 3000 mg/L), but effects were marginal at moderate doses (*i.e.*, 300 mg/L).

Although GP kinetics were generally not affected by LEM addition, there was great consistency between cumulative GP and DMD; the cumulative GP was more affected by LEM supplementation for grass hay and TMR than for wheat DDGS and barley grain. The results confirmed that GP is a reliable indicator of feed fermentation in the batch culture. The similar effect of LEM on the digestibility of DM and NDF between *in vitro* and *in situ* measurements suggested that LEM was effective to improve feed digestion. These results were also supported by increasing microbial attachment at 6 h of *in situ* incubation since microbial colonization on feed particles is a necessary step to make feed digested (McAllister et al., 1994). Consistently, Wanapat et al. (2008) observed that adding 100 g/d of lemongrass powder increased DM digestibility and viable total bacterial count.

Failure to have the effect of LEM supplementation on methane concentration in fermentation media is in consistency with previous studies. Hosoda et al. (2006) and Wanapat et al. (2008) reported that although methane production was not measured, rumen propionate concentration and acetate to propionate ratio were not affected with lemongrass supplementation in beef cattle, suggesting that methane

production was not reduced. In fact, the reduced methane production would result in an increase of propionate as the H^+ must have a recipient. With our best knowledge, there is no report on the effect of LEM on rumen methane concentration. The effects of EO on rumen methane production are actually not consistent (Benchaar and Greathead, 2011), depending on the number of factors such as EO source, dose, substrates used, etc.

CEO

Garlic oil is a complex mix of many different compounds present in the plant or derived from processing. It has antimicrobial activity against a wide spectrum of gram-positive and gram-negative bacteria and its potential effect on modifying rumen microbial fermentation has been studied recently (Calsamiglia et al., 2007; Chaves et al., 2008; Kongmun et al., 2010). The garlic oil and 4 purified active components (allicin, diallyl sulfide, diallyl disulfide, and allyl mercaptan) thought to play a major role in its antimicrobial activity, were tested *in vitro* to determine their effect on rumen microbial fermentation (Busquet et al., 2005a). Ginger (*Zingiber officinale*) EO consists of various chemical constituents such as a-pinene, camphene, b-pinene, linalool, borneol, y-terpineol, nerol, geraniol, geranial, etc., and showed inhibitory effects for 10 different micro-organisms (Hammer et al., 1999), but limited study was conducted to investigate its effect on rumen fermentation (Busquet et al., 2005b). Further, as additive, synergistic, and antagonistic effects of combined EO have been reported previously (Burt, 2004), it is suggested that combinations of EO of different composition, or specific combinations of EO secondary metabolites, may result in additive and synergetic effects which may enhance the efficiency of rumen microbial fermentation. In the present study, linearly increased DMD of wheat DDGS, barley grain, grass hay and TMR suggested a dose-depending manner of the CEO on feed

digestion. The trend of decrease in DMD with increasing the dose of 200 to 300 mg/kg DM suggested that the optimum dose of CEO would be at 200 mg/kg feed DM under current *in vitro* conditions, which was tested with *in situ* rumen DMD.

Apparently only one *in vitro* study was published to assess the effect of ginger oil on rumen fermentation (Busquet et al., 2006); the study demonstrated no effect on ammonia, total VFA and molar proportion of individual VFA at the dose of 2.2 mg/L. The effects of adding garlic oil and its main active components on rumen fermentation were not consistent and varied with active components of garlic oil (Busquet et al., 2005a), fermentation pH (Cardozo et al., 2005) or substrates incubated (Calsamiglia et al., 2007). For example, Cardozo et al. (2005) reported *in vitro* at pH 7.0 that garlic oil resulted in lower ammonia N and total VFA concentrations, whereas at pH 5.5 the ammonia N concentration was also reduced, but the total VFA and propionate concentration increased compared with control (no garlic oil), suggesting a shift in rumen microbial fermentation by changing rumen pH. In the present study, the fermentation pH was relatively stable due to high buffering capacity of buffer solution, thus the effect of pH on fermentation characteristics would be minimal. However, the effects of CEO on *in vitro* and *in situ* DMD varied with substrates; there were more effects on fibrous feeds than the grain feeds, similar effects as observed with LEM. The improved DMD of feeds with CEO containing garlic oil contrasted to the reports by Busquet et al. (2005a, 2005c), in those *in vitro* studies although digestibility of DM and NDF was not affected, rumen VFA concentration and acetate to propionate ratio were affected and methane production was reduced at high concentration (*i.e.,* >300 mg/L). The concentration of CEO used in the present study was only 2.5 mg/L. It suggested that the blend of garlic and ginger oils may improve the EO activity in the rumen. Lin et al. (2012) have evaluated five mixtures of EO that

were blended at various ratios using EO from thyme, oregano, cinnamon, and lemon, and showed that the effects of EO mixtures differed depending on the variables measured, indicating that each mixture of EO may have specific mode of action. The improved DMD by CEO is consistent with some *in vivo* reports using garlic oil. Yang et al. (2007) showed that adding garlic oil at 5 g/d increased DM digestibility without effect on NDF digestibility in the rumen of lactating dairy cows. Klevenhusen et al. (2011) reported that although garlic oil supplementation had no effect on feed digestion, its principal organosulfur compound improved feed digestion in sheep.

4.10 Conclusions

Supplementing LEM or CEO overall linearly or quadratically increased *in vitro* DMD of feed ingredient and TMR with increasing EO from 0, 100, 200 to 300 mg/kg feed DM, indicating that the EO used in the present study affected feed digestion in a dose-dependent manner. The results suggested that the dose of 200 mg/kg DM was cost-effective for both LEM and CEO, which is consistent with *in situ* rumen DMD. The LEM and CEO appeared to be more effective to improve the DMD of fibrous feeds such as grass hay and TMR containing 50% of roughage, and the improvement of DMD was partly attributed to the improved NDF digestibility. However, the effect of EO on methane production was apparently negligible. These results suggested that the EO used in the present study could be potentially developed as rumen modifier to improve feed digestion, especially roughage feeds in ruminant animals.

4.11 References

Association of Official Analytical Chemists. (1990). **Official Methods of Analysis**. USA.

Benchaar, C., and Greathead, H. (2011). Essential oils and opportunities to mitigate enteric methane emissions from ruminants. **Anim. Feed Sci. Technol.** 166: 338-355.

Burt, S. (2004). Essential oils : Their antibacterial properties and potential applications in foods – A review. **Int. J. Food Microbiol.** 94: 223-253.

Busquet, M., Calsamiglia, S., Ferret, A., and Kamel, C. (2005b). Screening for effects of plants on dairy cattle rumen microbial fermentation in a continuous culture system. **Anim. Feed Sci. Technol.** 123: 597-613.

Busquet, M., Calsamiglia, S., Ferret, A., and Kamel, C. (2006). Plant extracts affect *in vitro* rumen microbial fermentation. **J. Dairy Sci.** 89: 761-771.

Busquet, M., Calsamiglia, S., Ferret, A., Cardozo, P. W., and Kamel, C. (2005c). Effects of cinnamaldehyde and garlic oil on rumen microbial fermentation in a dual flow continuous culture. **J. Dairy Sci.** 88: 2508-2516.

Busquet, M., Calsamiglia, S., Ferret, A., Carro, M. D., and Kamel, C. (2005a). Effect of garlic oil and four of its compounds on rumen microbial fermentation. **J. Dairy Sci.** 88: 4393-4404.

Calsamiglia, S., Busquet, M., Cardozo, P. W., Castillejos, L., and Ferret, A. (2007). Invited review : Essential oils as modifiers of rumen microbial fermentation. **J. Dairy Sci.** 90: 2580-2595.

Cardozo, P. W., Calsamiglia, S., Ferret, A., and Kamel, C. (2006). Effects of alfalfa extract, anise, capsicum, and a mixture of cinnamaldehyde and eugenol on

ruminal fermentation and protein degradation in beef cattle heifers fed a high-concentrate diet. **J. Anim. Sci.** 84: 2801-2808.

Cardozo, P. W., Calsamiglia, S., Ferret, A., and Kamel, C. (2005). Screening for the effects of natural plant extracts at different pH on *in vitro* rumenmicrobial fermentation of a high-concentrate diet for beef cattle. **J. Anim. Sci.** 83: 2572-2579.

Castillejos, L., Calsamiglia, S., and Ferret, A. (2006). Effect of essential oil active compounds on rumen microbial fermentation and nutrient flow in *in vitro* systems. **J. Dairy Sci.** 89: 2649-2658.

Chaves, A.V., He, M. L., Yang, W. Z., Hristov, A. N., McAllister, T. A., and Benchaar, C. (2008). Effects of essential oils on proteolytic, deaminative and methanogenic activities of mixed ruminal bacteria. **Can. J. Anim. Sci.** 88: 117-122.

Cheel, J., Theoduloz, C., Rodriìguez, J., and Schmeda-Hirschmann, G. (2005). Free radical scavengers and antioxidants from lemongrass (Cymbopogon citratus (DC.) Stapf.). **J. Agric. Food Chem.** 53: 2511-2517.

France, J., Dijkstra, J., Dhanoa, M. S., Lopez, S., and Bannink, A. (2000). Estimating the extent of degradation of ruminant feeds from a description of their gas production profiles observed *in vitro* : Derivation of models and other mathematical considerations. **Br. J. Nutr.** 83: 143-150.

Fraser, G.R., Chaves, A. V., Wang, Y., McAllister, T. A., Beauchemin, K. A., and Benchaar, C. (2007). Assessment of the effects of cinnamon leaf oil on rumen microbial fermentation using two continuous culture systems. **J. Dairy Sci.** 90: 2315-2328.

Goering, H. H., and Van Soest, P. J. (1970). **Forage fiber analysis (apparatus, reagents, procedures and some applications)**. USA: U.S. Agricultural Research Service.

Hall, M. B., Pell, A. N., and Chase, L. E. (1998). Characteristics of neutral detergent-soluble fiber fermentation by mixed ruminal microbes. **Anim. Feed Sci. Technol.** 70: 23-39.

Hammer, K. A., Carson, C. F., and Riley, T. V. (1999). Antimicrobial activity of essential oils and other plant extracts. **J. Appl. Microbiol.** 86: 985-990.

Hart, K. J., Yanez-Ruiz, D. R., Duval, S. M., McEwan, N. R., and Newbold, C. J. (2008). Plant extracts to manipulate rumen fermentation. **Anim. Feed Sci. Technol.** 147: 8-35.

Hosoda, K., Kuramoto, K., Eruden, B., Nishida, T., and Shioya, S. (2006). The effects of three herbs as feed supplements on blood metabolites, hormones, antioxidant activity, IgG concentration, and ruminal fermentation in Holstein steers. **Asian-Aust. J. Anim. Sci.** 19: 35-41.

Klevenhusen, K., Zeitz, J. O., Duval, S., Kreuzer, M., and Soliva, C. R. (2011). Garlic oil and principal component diallyl disulfide fail to mitigate methane, but improve digestibility in sheep. **Anim. Feed Sci. Technol.** 166: 356-363.

Kongmun, P., Wanapat, M., Pakdee, P., and Navanukraw, C. (2010). Effect of coconut oil and garlic powder on *in vitro* fermentation using gas production technique. **Livest. Sci.** 127: 38-44.

Li, Y. L., He, M. L., Li, C., Forster, R., Beauchemin, K. A., and Yang, W. Z. (2012). Effects of wheat dried distillers' grains with solubles and cinnamaldehyde on *in vitro* fermentation and protein degradation using the Rusitec technique. **Arch. Anim. Nutr.** 66: 131-148.

Lin, B., Lu, Y., Wang, J. H., Liang, Q., and Liu, J. X. (2012). The effects of combined essential oils along with fumarate on rumen fermentation and methane production *in vitro*. **J. Anim. Feed. Sci.** 21: 198-210.

Mauricio, R. M., Mould, F. L., Dhanoa, M. S., Owen, E., Channa, K. S., and Theodorou, M. K. (1999). A semi-automated *in vitro* gas production technique for ruminant feedstuff evaluation. **Anim. Feed Sci. Technol.** 79: 321-330.

McAllister, T. A., Bae, H. D., Jones, G. A., and Cheng, K. J., (1994). Microbial attachment and feed digestion in the rumen. **J. Anim. Sci.** 72: 3004-3018.

McIntosh, F. M., Williams, P., Losa, R., Wallace, R. J., Beever, D. A., and Newbold, C. J. (2003). Effect of essential oil on ruminal metabolism and their protein metabolism. **Appl. Environ. Microbiol.** 69: 5011-5014.

Olfert, E. D., Cross, B. M., and McWilliam, A. A. (1993). **Guide to the Care and Use of Experimental Animals** (Vol. 1) (2nd ed.). Ottawa: Canadian Council on Animal Care.

Ramos-Morales, E., Martínez-Fernández, G., Abecia, L., Martin-García, A. I., Molina-Alcaide, E., and Yáñez-Ruiz, D. R. (2013). Garlic derived compounds modify ruminal fatty acid biohydrogenation and induce shifts in the *Butyrivibrio* community in continuous-culture fermenters. **Anim. Feed Sci. Technol.** 184: 38-48.

Valero, M., and Salmeroìn, M. C. (2003). Antibacterial activity of 11 essential oils against Bacillus cereus in tyndallized carrot broth. **Int. J. Food Microbiol.** 85: 73-81.

Van Soest, P. J., Robertson, J. B., and Lewis, B. A. (1991). Methods for dietary fiber, neutral fiber and nonstarch polysaccharides in relation to animal nutrition. **J. Dairy Sci.** 74: 3588-3595.

Wanapat, M., Cherdthong, A., Pakdee, P., and Wanapat, S. (2008). Manipulation of rumen ecology by dietary lemongrass (Cymbopogon citratus Stapf.) powder supplementation. **J. Anim. Sci.** 86: 3497-3503.

Yang, W. Z., Benchaar, C., Ametaj, B. N., Chaves, A. V., He, M. L., and McAlister, T. A. (2007). Effects of garlic and juniper berry essential oils on ruminal fermentation and on the site and extent of digestion in lactating cows. **J. Dairy Sci.** 90: 5671-5681.

CHAPTER V

USE OF LEMONGRASS OIL FOR MANIPULATION OF RUMINAL FERMENTATION USING RUSITEC TECHNIQUE

5.1 Abstract

A study using Rusitec was conducted to investigate the effect of increasing lemongrass oil (LEM) supplementation on fermentation characteristics of a dairy cow diet. Increasing LEM from 0, 100 to 200 mg/kg dry matter did not affect volatile fatty acid concentration, whereas linearly increased large and small peptide N and reduced ammonia N concentration. Increasing LEM addition also linearly increased the microbial N production without significantly improving the efficiency of microbial protein synthesis. However, feed digestibility was not affected by the LEM supplementation. These results suggest that the addition of LEM may particularly inhibit deamination in the rumen.

5.2 Introduction

Nutritionists have been searching for alternative additives for reducing the need for in-feed antibiotics such as ionophores in animal production because using antibiotics in animal feed is facing reduced social acceptance due to the appearance of residues and resistant strain bacteria. Plant extracts contain secondary metabolites,

such as essential oils (EO) that have antimicrobial properties make them potential alternatives to antibiotics to manipulate microbial activity in the rumen. Various plant EO have been intensively studied during last decades for manipulating ruminal fermentation as documented by several review papers (Calsamiglia et al., 2007; Hert et al., 2008). Many studies were focused on cinnamon oil (cinnamaldehyde) or clove oil (eugenol) to evaluate the effects on particular rumen fermentation characteristics (Busquet et al., 2005a; Busquet et al., 2005b). The researches demonstrated the ability of using EO to alter rumen fermentation and nutrient utilization in ruminants. Lemongrass (*Cymbopogon citratus*) is an herb that widely used in tropical countries for food composition and antibacterial agents. Citral is a key component of the EO extracted from lemongrass that is necessary for vitamin A synthesis (Wanapat et al., 2008). Supplementation of lemongrass products could reduce ammonia N concentration, methane production, and protozoa in beef cattle (Wanapat et al., 2008; Wanapat et al., 2013). The lemongrass has been showed antibacterial, antioxidant (Cheel et al., 2005), and antihyper-NH3-producing ruminal bacterial (McIntosh et al., 2003) activities as well as the effects on changes of blood metabolites and rumen fermentation in Holstein steers (Hosoda et al., 2006). However, the lemongrass EO (LEM) was little evaluated for its ruminal fermentation. Additionally, although monensin (MON) is commonly fed to finishing cattle or dairy cows in North America, and generally improves feed efficiency, there is indication that high energy density diets such as diets that contain highly processed grain or high energy feed ingredient such as dried distillers grains with solubles (DDGS) are less responsive to MON addition (DiLorenzo and Galyean 2010).

5.3 Objectives

We hypothesised that supplementation of LEM in a dairy cow diet may alter the ruminal fermentation pattern in a desirable manner and increase ruminal by-pass protein by reducing NH_3-N concentration as a result of inhibiting proteolytic activity.

5.4 Materials and methods

The experiment was designed to evaluate the effect of increasing LEM levels of supplementation on in vitro fermentation characteristics and the protein degradation. The addition of MON was used as positive control. The experiment was completely randomized design with four treatments and four replicates of each treatment. Substrate was a dairy ration consisting of 50% forage and 50% concentrate (Table 5.1). Treatments were control (no LEM and no MON), Low LEM (L-LEM; 100 mg/kg diet dry matter [DM]), High LEM (H-LEM; 200 mg/kg diet DM; purity >99%; Phode S.A., Albi, France); and MON (30 mg/kg diet DM). The dosages of LEM were chosen based on our screening trial using batch culture. The substrate was ground through a 4 mm screen (Arthur Thomas Co., Philadelphia, PA), mixed with MON or LEM before weighing into nylon bag. A 10 g (DM basis) sample of substrate was weighed into individual nylon bag (90 × 160 nm; pore size of 50 Im, B. & S. H. Thompson, Ville Mont-Royal, QC), sealed with the heater and tied with rubber band.

Table 5.1 Ingredients and chemical composition of the experimental substrate.

Ingredients (%)	
Grass hay	35.0
Alfalfa hay	15.0
Barley grain	25.0
Wheat DDGS	20.0
Vitamin and Mineral supplement[1/]	5.0
Chemical composition (%)	
Dry matter	93.6
Neutral detergent fiber	44.2
Acid detergent fiber	23.6
Starch	16.1
Crude protein	18.2

[1/]Supplied per kilogram of dietary DM : 15 mg of Cu, 65 mg of Zn, 28 mg of Mn, 0.7 mg of I, 0.2 mg of Co, 0.3 mg of Se, 6000 IU of vitamin A, 600 IU of vitamin D, and 47 IU of vitamin E.

Two Rusitec apparatuses were used, each equipped with eight 920 mL volume anaerobic fermenters. Each fermenter was outfitted with a site for buffer input, and effluent output. Collectively, the fermenters were immersed in a water bath maintained at a constant 39 ^{o}C. To begin, each fermenter was filled with 200 mL of McDougall's buffer, 700 mL of strained rumen fluid, two nylon bags; one containing 10 g of solid ruminal digesta, and one containing 10 g (DM) of substrate. After 24 h, the bag containing the solid rumen digesta was removed and a bag containing 10 g of diet substrate was added. Thereafter, one bag was replaced daily in the morning so that each bag remains in the fermenter for 48 h. Artificial saliva (McDougall, 1948) was continuously infused into the fermenters at a dilution rate of 2.9% per h.

Effluent accumulation was collected in a 1 L volumetric flask preserved with sodium azide (0.1% final concentration, wt/vol). Fermentation gas was collected into reusable 2 L bags attached to each fermenter. The experimental period consisted of 17 d with 10 d of adaptation and 7 d of sampling and data collection. The buffer was modified according to McDougall (1948) with pH 6.58, using 0.82% (wt/vol) $NaH_2PO_4 \cdot H_2O$ and 0.63% (wt/vol) $NaHCO_3$, containing 0.03% (wt/vol) of $(NH_4)_2SO_4$.

Inoculum was obtained from three ruminally fistulated Holstein friesian fed 75% barley silage and 25% barley-based concentrate. Rumen fluid was collected, pooled, and filtered through two layers of cheesecloth into an insulated thermos and transported immediately to the laboratory. Approximately 320 g of ruminal solid content was also collected for initial inoculation of the fermenters. All procedures with the animals were performed in according with the guidelines of the Canadian Council on Animal Care (CCAC, 1993).

Dry matter disappearance (DMD) from feed bags at 48 h were determined from days 1 to 17 of the sampling period. Feed bags were withdrawn from each vessel and washed under cold running water until the water running off is clear. The bags were dried at 55 ^{o}C for 48 h, and weighed to determine the DMD. The residues were pooled over the 5 d, ground through a 1 mm screen and analyzed for neutral detergent fiber (NDF; Van Soest et al. 1991) with α-amylase but without sodium sulfite used in the NDF procedure, acid detergent fiber (ADF; AOAC 1995; method 973.18), total N using flash combustion (Carlo Erba Instruments, Milan, Italy). Starch was determined using an enzymatic method by hydrolyzing starch to glucose using amylase, and then free glucose was reacted with glucose oxidase/peroxidase (No. P7119, Sigma, St.

Louis, MO) and dianisidine dihydrochloride, and absorbance was measured using a plate reader (SpectraMax 190, Molecular Devices Corp., Sunnyvale, CA).

Fermenter pH, volume of the effluent, and gas volume were measured daily during sampling period at the time of feed bag exchange. A volume of 20 mL gas was sampled using a syringe and passed it into 6.8 mL exetainer vials (evacuated in advanced) for immediate measuring of methane concentration.

The volatile fatty acid (VFA) and ammonia N (NH_3-N) analysis were conducted from days 11 to 15 at the time of morning feeding. Two 5 mL samples was taken from the fermenter liquid directly at the time of feed bag exchange, and placed in screw-capped vials preserved with 1 mL of 25% (wt/wt) metaphosphoric solution, or with 1 mL of 1% H_2SO_4, and immediately frozen at -20 °C for VFA and NH3-N analysis, respectively. Concentation of VFA was quantified using gas chromatograph (model 5890, Hewkett-Packard Lab, Palo Alto, CA) with a capillary column (30 m × 0.32 mmi.d., 1 Im phase thickness, Zeborn ZB-FAAP, Phenomenex, Torrance, CA), and flame ionization detection, and crotonic acid (trans-2-butenoic acid) was used as the internal standard. The NH_3-N was determined as described by Rhine et al. (1998). Five milliliter of samples from effluent was added to 1.2 mL of 10% (wt/vol) sodium tungstate and 1.2 mL of 1.07N sulfuric acid. After allowing the tubes to stand at 5°C for 4 h, they were centrifuged at 9000 × g for 15 min, and the supernatant was frozen until analyzed for TA soluble N. To determine TCA soluble N, 1 mL of 50% (wt/vol) TCA solution was added to 5 mL samples from effluent. After 4 h at 5 °C, tubes were centrifuged at 9000 × g for 15 min. The supernatant was frozen until analyzed for TCA soluble N. The results of those analysis were used to calculate large peptides (LPep N = TCA-N – TA-N), small peptides plus amino acid (SPep+AA-N = TA-N – NH_3-N).

Bacteria in the fermenters were labeled using ^{15}N (Li et al., 2012). The total microbial protein synthesis was quantified as the sum of liquid-associated bacteria (LAB) in effluent and solid-associated bacteria (SAB) in bag residues. Efficiency of bacterial protein synthesis (EMPS) was defined as g of bacterial protein per kilogram of OM disappeared. The preparation of bacterial pellets, bacterial ^{15}N determination and calculation of microbial protein synthesis were followed the procedures of Li et al. (2012).

5.5 Statistical analysis

Data were analyzed using the mixed model procedures of SAS (SAS Institute, Inc., Cary, NC) to account for the repeated measures (sampling day), the fixed effects of treatment, and the random effect of the replicate. The fermenter was the experimental unit for sampling and data collection. For the repeated measures, various covariance structures were assessed with the final choice depending on lowest values for the Akaike's information criteria. Contrasts were generated to compare the control and MON diets. The effect of increasing levels of LEM was examined through linear and quadratic orthogonal contrasts using the CONTRAST statement of SAS. Differences between treatments were declared significant at $P \leq 0.5$. Trends were discussed at $0.05 < P < 0.10$ unless otherwise stated.

5.6 Experimental site

The experiment was conducted at Research Centre, Agriculture and Agri-Food Canada, Lethbridge, Alberta, Canada.

5.7 Duration

The duration of this trial was from December 2012 to February 2013

5.8 Results

Supplementing LEM or MON had no effect on fermentation characteristics, gas production, and methane production (Table 5.2). Supplementation with LEM linearly ($P<0.01$) increased LPep N and SPep+AA N concentration but linearly ($P<0.01$) decreased NH_3-N concentration (Table 5.2). However, supplementing MON did not affect N fraction concentration (Table5.2). Nutrient digestibility were unaffected by treatments (Table 4.3). Addition of H-LEM linearly ($P<0.05$) increased total bacteria N flow and LAB without effect on SAB and EMPS (Table 5.3).

Table 5.2 Effect of lemongrass oil (LEM) and monensin (MON) on fermentation characteristics, gas production (GP) and N fractions in Rusitec.

	Treatment[1/]				SEM[2/]	P-value[3/]		
	Control	**L-LEM**	**H-LEM**	**MON**		**LEM_L**	**LEM_Q**	**Ctrl vs. MON**
pH	6.95	6.94	6.94	6.95	0.01	0.23	0.47	0.74
Total VFA (m*M*)	25.6	26.0	26.5	23.7	1.39	0.65	0.95	0.34
VFA (mol/100 mol)								
Acetate (A)[4/]	48.7	49.6	48.8	49.1	0.35	0.84	0.09	0.54
Propionate (P)[4/]	22.5	23.8	23.6	23.1	0.52	0.23	0.18	0.36
Butyrate (B)[4/]	16.9^{a}	15.5^{b}	16.2^{ab}	16.2^{ab}	0.38	0.22	0.05	0.18
A : P	2.17	2.09	2.10	2.13	0.05	0.29	0.46	0.51
A + B/P	2.93	2.75	2.80	2.83	0.07	0.22	0.21	0.34
Gas production (mL/d)	999	1210	1106	1040	88.3	0.36	0.14	0.72
CH_4 (mL/d)	17.5	17.0	17.6	15.9	1.39	0.95	0.75	0.37

Table 5.2 Effect of lemongrass oil (LEM) and monensin (MON) on fermentation characteristics, gas production (GP) and N fractions in Rusitec (Continued).

	Treatment[1/]				SEM[2/]	P-value[3/]		
	Control	L-LEM	H-LEM	MON		LEM_L	LEM_Q	Ctrl vs. MON
N fraction (mg/100 ml)								
LPep N[5/]	2.6^{b}	3.1^{ab}	3.3^{a}	2.7^{b}	0.16	0.01	0.42	0.48
SPep + AA N[5/]	3.3^{c}	4.1^{ab}	4.5^{a}	3.6^{bc}	0.19	0.01	0.44	0.34
Ammonia N	8.8^{a}	8.3^{bc}	7.9^{c}	8.6^{ab}	0.16	0.01	0.69	0.39

[a-c]Within a row means without a common superscript letter differ.

[1/]Control = no LEM and no MON; L-LEM = low LEM (100 mg/kg diet DM); H-LEM = High LEM (200 mg/kg diet DM); and MON = monensin (30 mg/kg diet DM).

[2/]SEM = standard error of the mean.

[3/]LEM_L, LEM_Q = linear or quadratic effects of increasing LEM supplementation; Ctrl vs. MON = control vs. MON.

[4/]The proportion of individual volatile fatty acids did not include isobutyrate, isovalerate, valerate, and caproate.

[5/]LPep = large peptides; SPep + AA = small peptides plus AA.

Table 5.3 Effect of lemongrass oil (LEM) and monensin (MON) on nutrient digestibility and bacterial protein synthesis in Rusitec.

	Treatment[1/]				SEM[2/]	P-value[3/]		
	Control	L-LEM	H-LEM	MON		LEM_L	LEM_Q	Ctrl vs. MON
Digestibility (%)								
DM	52.6	52.1	53.8	51.2	0.88	0.39	0.34	0.26
NDF	41.4	41.9	42.4	41.0	0.63	0.27	0.98	0.74
ADF	21.8	21.8	22.5	20.8	0.96	0.64	0.74	0.45
Starch	76.5	76.9	76.2	76.4	0.68	0.67	0.37	0.86
CP	58.5	58.6	60.0	58.8	0.63	0.13	0.44	0.76
Bacterial N[4/]								
Total (mg/d)	66.3^{b}	66.2^{b}	70.1^{a}	65.4^{b}	0.92	0.02	0.10	0.48
LAB (mg/d)	33.3^{b}	33.2^{ab}	36.5^{a}	32.5^{b}	0.73	0.01	0.08	0.50
SAB (mg/d)	33.1	33.0	33.6	32.8	0.83	0.67	0.77	0.85
EMPS	12.8	12.9	13.3	12.9	0.21	0.11	0.48	0.77

[a-b]Within a row means without a common superscript letter differ.

[1/]Control = no LEM and no MON; L-LEM = low LEM (100 mg/kg diet DM); H-LEM = High LEM (200 mg/kg diet DM); and MON = monensin (30 mg/kg diet DM). [2/]SEM = standard error of the mean.

[3/]LEM_L, LEM_Q = linear or quadratic effects of increasing LEM supplementation; Ctrl vs. MON = control vs. MON.

[4/]LAB = liquid associated bacteria; SAB = solid associated bacteria; EMPS = efficiency of bacterial protein synthesis (g of bacteria protein/kg of organic matter disappeared).

5.9 Discussion

Fermentation pH, VFA concentration and molar proportion of individual VFA, gas production (GP), and methane production were not affected with LEM supplementation. Information on the effect of LEM supplementation on the ruminal fermentation is scarce. Hristov et al. (2008) screened forty different EO in batch culture and found no effect of LEM supplementation of 10 and 100 mg/L on total and individual VFA concentrations. From *in vivo* study, Wanapat et al. (2008) also reported no effect of lemongrass powder addition on the total VFA concentrations and individual VFA proportion in beef cattle fed a rice straw-based diet. In a recent study, Wanapat et al. (2013) also showed overall no differences in total VFA and the molar proportion of individual VFA between control and lemongrass meal supplemented cattle. However, a decrease in the molar proportion of acetate and an increase in the propionate, consequently a decrease in ratio of acetate to propionate were observed when a mixture of lemongrass meal, peppermint powder, and garlic powder was added (Wanapat et al., 2013). These results suggest limited effect on rumen fermentation of LEM or lemongrass powder or meal alone but a potential synergic effect when combined with other plant extracts. The effect of adding blend EO (BEO) on total VFA or individual VFA was inconsistent. Supplementation of a BEO had no effect on *in vitro* fermentation pH and total concentration of VFA but decreased the molar proportion of acetate and butyrate and increased propionate proportion (Kung et al., 2008). Supplementation of BEO resulted in higher total VFA concentration without affecting the proportion of individual VFA in continuous culture (Castillejos et al., 2005). It is well known that the effects of EO on in vitro fermentation vary with substrates, EO source, and dosage of EO (Calsamiglia et al., 2007).

Increasing LEM supplementation increased concentrations of LPep N and SPep + AA N, but decreased the concentration of NH_3-N. The result suggests that the deamination was inhibited by LEM supplementation. The reduction of NH_3-N associated with the increased small peptides plus AA with LEM is consistent with the previous report that the proteolytic bacteria was reduced by adding lemongrass meal or lemongrass powder in beef cattle (Wanapat et al., 2008; Wanapat et al., 2013). McEwan et al. (2002) reported that addition of EO resulted in a reduction in the number and diversity of hyper-NH_3-producing bacteria, thereby decreased rate of NH_3 production from AA. Wallace et al. (2002) suggested two possible mode of action by EO in the rumen : one is to affect the pattern of bacterial colonization of substrates, especially starch rich substrates; and second is to inhibit hyper-NH3-producing bacteria involved in AA deamination.

Supplementation of MON did not affect the fermentation pH, VFA concentration, total gas and methane production. The present result is in agreement with several studies using continuous culture (Busquet et al., 2005a; Lourenco et al., 2008), but contrasted to the fact that MON has known effects on increase of propionate production. In fact, the inconsistent effect of MON among the studies may have resulted from the dosage of MON or substrate used in the study. Busquet et al. (2005b) reported that addition of 1.25 mg/L MON had no effect on total VFA, whereas at the dose 12.5 mg/L of MON reduced total VFA, acetate, and butyrate, and increased propionate. The dose of MON used in the present study was about 0.6 mg/L, lower than 12.5 mg/L. Additionally, Li et al. (2012) reported no response of VFA concentration to MON supplementation and suggested that substrate containing high fat DDGS may respond less to MON because dietary fat and MON have similar effect on ruminal VFA production (Richardson et al., 1976).

Supplementation with LEM or MON did not affect nutrient digestibility in continuous culture. The results are consistent with the no differences in VFA concentration with increasing LEM supplementation. The lack of difference in CP digestibility with the accumulation of peptides and reduction of NH_3-N as a result of LEM supplementation confirm the previous suggestion on the inhibition of deamination by LEM. Wanapat et al. (2008) reported dose-depending manner of adding lemongrass powder on the digestibility of DM in the total digestive tract of beef cattle; the DM digestibility was increased at the dose of 100 g/d, while no difference in the digestibility of DM was observed for cattle supplemented with 200 or 300 g/d compared with control group. Wanapat et al. (2013) also reported no difference in the total digestibility of DM by adding 100 g/d of lemongrass meal compared to control in beef cattle fed forage-based diet. Several *in vitro* studies showed that the digestibility of DM was not affected by adding a BEO in a typical dairy cow diet (Castillejos et al., 2005; Castillejos et al., 2007). The lack of the effect on the digestibility of DM with MON addition is consistent with the results of Busquet et al. (2005b), who observed no change in DM, OM, NDF, and ADF degradation when MON was added at the dose of 1.25 or 12.5 mg/L of culture fluid in continuous culture.

Bacterial protein synthesis increased with increasing LEM supplementation, which was primarily resulted from increased LAB protein since the SAB protein synthesis was not affected. The increased bacterial protein production without increasing DM digestibility with LEM addition suggested a potential higher microbial efficiency. In fact, the EMPS was numerically improved by adding LEM. However, the lack of increased SAB protein synthesis appeared to contrast to the assumption that EO favor microbial colonization of substrates, nevertheless such effect is

especially happen with starch rich substrate (Wallace et al., 2002). Starch content of the present substrate was low.

5.10 Conclusions

The results of this study demonstrate that supplementation of LEM altered profiles of peptide, AA, and NH_3-N in the fermentation media, and increased microbial protein synthesis without changing fermentation VFA and nutrient digestibility. The results suggest that LEM inhibit the deamination process by likely altering microbial populations, in particular proteolytic bacteria.

5.11 References

Association of Official Analytical Chemists. (1990). **Official Methods of Analysis**. USA.

Busquet, M., Calsamiglia, S., Ferret A., and Kamel, C. (2005a). Screening for effects of plants on dairy cattle rumen microbial fermentation in a continuous culture system. **Anim. Feed Sci. Technol.** 123: 597-613.

Busquet, M., Calsamiglia, S., Ferret, A., Cardozo, P. W., and Kamel, C. (2005b). Effects of cinnamaldehyde and garlic oil on rumen microbial fermentation in a dual flow continuous culture. **J. Dairy Sci.** 88: 2508-2516.

Calsamiglia, S., Busquet, M., Cardozo, P. W., Castillejos, L., and Ferret, A. (2007). Invited review: essential oils as modifiers of rumen microbial fermentation. **J. Dairy Sci.** 90: 2580-2595.

Castillejos, L., Calsamiglia, S., Ferret, A., and Losa, R. (2005). Effects of specific blend of essential oil compounds and the type of diet on rumen microbial

fermentation and nutrient flow from a continuous culture system. **Anim. Feed Sci. Technol.** 119: 29-41.

Castillejos, L., Calsamiglia, S., Ferret, A., and Losa, R. (2007). Effects of dose and adaptation time of specific blend of essential oil compounds on rumen fermentation. **Anim. Feed Sci. Technol.** 132: 186-201.

Cheel, J., Theoduloz, C., Rodriìguez, J., and Schmeda-Hirschmann, G. (2005). Free radical scavengers and antioxidants from lemongrass (Cymbopogon citratus (DC.) Stapf.). **J. Agric. Food Chem.** 53: 2511-2517.

DiLorenzo, N., and Galyean, M. L. (2010). Applying technology with newer feed ingredients in feedlot diets : Do the old paradigms apply? **J. Anim. Sci.** 88: 123-132.

Hart, K. J., Yanez-Ruiz, D. R., Duval, S. M., McEwan, N. R., and Newbold, C. J. (2008). Plant extracts to manipulate rumen fermentation. **Anim. Feed Sci. Technol.** 147: 8-35.

Hosoda, K., Kuramoto, K., Eruden, B., Nishida, T., and Shioya, S. (2006). The effects of three herbs as feed supplements on blood metabolites, hormones, antioxidant activity, IgG concentration, and ruminal fermentation in Holstein steers. **Asian-Aust. J. Anim. Sci.** 19: 35-41.

Hristov, A. N., Ropp, J. K., Zaman, S., and Melgar, A. (2008). Effects of essential oils on *in vitro* ruminal fermentation and ammonia release. **Anim. Feed Sci. Technol.** 144: 55-64.

Kung Jr, L., Williams, P., Schmidt, R. J., and Hu, W. (2008). A blend of essential plant oils used as an additive to alter silage fermentation or used as a feed additive for lactating dairy cows. **J. Dairy Sci.** 91: 4793-4800.

Li, Y. L., He, M. L., Li, C., Forster, R., Beauchemin, K. A., and Yang, W. Z. (2012). Effects of wheat dried distillers' grains with solubles and cinnamaldehyde on *in vitro* fermentation and protein degradation using the Rusitec technique. **Arch. Anim. Nutr.** 66: 131-148.

Lourenco, M., Cardozo, P. W., Calsamiglia, S., and Fievez, V. (2008). Effects of saponins, quercetin, eugenol, and cinnamaldehyde on fatty acid biohydrogenation of forage poly unsaturated fatty acids in dual-flow continuous culture fermenters. **J. Anim. Sci.** 86: 3045-3053.

McDougall, E. I. (1948). Studies on ruminant saliva. 1. The composition and output of sheep's saliva. **Biochem. J.** 43: 99-109.

McEwan, N. R., Graham, R. C., Wallace, R. J., Losa, R., Williams, P., and Newbold, C. J. (2002). Effect of essential oils on ammonia production by rumen microbes. **Reprod. Nutr. Dev.** 42(Suppl. 1): S65.

McIntosh, F. M., Williams, P., Losa, R., Wallace, R. J., Beever, D. A., and Newbold, C. J. (2003). Effect of essential oil on ruminal metabolism and their protein metabolism. **Appl. Environ. Microbiol.** 69: 5011-5014.

Olfert, E. D., Cross, B. M., and McWilliam, A. A. (1993). **Guide to the Care and Use of Experimental Animals** (Vol. 1) (2nd ed.). Ottawa: Canadian Council on Animal Care.

Rhine, E. D., Sims, G. K., Mulvaney, R. L., and Pratt, E. J. (1998). Improving the berthelot reaction for determining ammonia in soil extracts and water. **Soil Sci. Soc. Am. J.** 62: 473-480.

Richardson, L. F., Raun, A. P., Potter, E. L., Cooley, C. O., and Rathmacher, R. P. (1976). Effect of monensin on rumen fermentation *in vitro* and *in vivo*. **J. Anim. Sci.** 43: 657-664.

Van Soest, P. J., Robertson, J. B., and Lewis, B. A. (1991). Methods for dietary fiber, neutral fiber and nonstarch polysaccharides in relation to animal nutrition. **J. Dairy Sci.** 74: 3588-3595.

Wallace, R. J., McEwan, N. R., McIntosh, F. M., Teferedegne, B., and Newbold, C. J. (2002). Natural products as manipulators of rumen fermentation. **Asian-Aust. J. Anim. Sci.** 15: 1458-1468.

Wanapat, M., Cherdthong, A., Pakdee, P., and Wanapat, S. (2008). Manipulation of rumen ecology by dietary lemongrass (Cymbopogon citratus Stapf.) powder supplementation. **J. Anim. Sci.** 86: 3497-3503.

Wanapat, W., Kang, S., Khejornsart, P., and Wanapat, S. (2013). Effects of plant herb combination supplementation on rumen fermentation and nutrient digestibility in beef cattle. **Asian-Aust. J. Anim. Sci.** 26: 1127-1136.

CHAPTER VI

EFFECTS OF ESSENTIAL OILS ON FEED DIGESTIBILITY, RUMEN FERMENTAION AND BLOOD METABOLITE IN FISTULATED NON-LACTATING DAIRY COWS

6.1 Abstract

The effects of essential oils (EOs) on ruminal disappearance and rumen fermentation, nutrient digestibility and blood metabolite in fistulated non-lactating dairy cows were studied. Four fistulated non-lactaing dairy cows were used in a 4 × 4 Latin square design; the experiment consisted of 4 periods of 21 d in each period, with the first 14 d for adaptation. Animals were fed 3 kg/d of 21% CP concentrate and *ad libitum* corn silage. Treatment were : 1) control, 2) 2 ml Allicin/cow/d, 3) 2 ml Zingiberene/cow/d, and 4) 2 ml Citral/cow/d. The results demonstrated that EOs increased DM and NDF digestibilities at 48 and 72 h, but had no effect on ADF and CP digestibilities. EOs did not change ruminal pH, NH_3-N, protozoa, VFA concentration and blood glucose but reduced blood urea N at 4 h.

6.2 Introduction

In recent decades, researchers have studies essential oils (EOs) for alternative additive to replace antibiotics such as ionophores in animal because using antibiotics

in animal feed is facing reduced social acceptance due to the appearance of residues and resistant strain bacteria. Many plants EOs have been tested in laboratory and animals in the last decades. At the beginning, cinnamon oil (cinnamaldehyde) and clove oil (eugenol) have been intensively studied to evaluate the effects on particular rumen fermentation characteristics. In recent years, garlic and garlic compound were studied in laboratory and animal by several authors (Ramos-Morales et al., 2013; Klevenhusen et al., 2011; Kongmun et al., 2010). Garlic oil and garlic derived compounds have been demonstrated to have antimethanogenic property with mixed effects on rumen fermentation (Busquet et al., 2005a; Chaves et al., 2008b). Ginger oil has also been detected to have inhibitory effects for 10 different micro-organisms (Hammer et al., 1999). Ginger oil had no effect on rumen VFA concentration in continuous culture (Busquet et al., 2005b) but combination between garlic and ginger oil improved DM and NDF disappearance in batch culture and *in situ* (Nanon et al., 2014a). However, ginger oil was little evaluated on its effects on nutrient digestibility and ruminal fermentation. Lemongrass is one herb that was little evaluated on its effects on feed digestion and ruminal fermentation. Although, lemongrass have antibacterial property (Valero and Salmeroìn, 2003) and antihyper-NH3-producing ruminal bacterial (McIntosh et al., 2003) activities as well as the effects on changes of blood metabolites and rumen fermentation in Holstein steers (Hosoda et al., 2006). Most of previous experiments were studied with garlic, ginger, and lemongrass using the oil not main active component. Therefore, the question about the effects of essential oil that has been shown comes from which active component.

6.3 Objectives

The objective of this study was to determine the effect of main active component of garlic, ginger, and lemongrass oil on feed digestion, rumen fermentation, and blood metabolites in fistulated non-lactating dairy cows.

6.4 Material and methods

6.4.1 Animals and feeding

Four fistulated Crossbred Holstein Friesian non-lactating dairy cows housed in individual pens were assigned to one of four treatments in 4 × 4 Latin squares design. The trial consisted of 4 periods, with 21 d in each period, 14 d for adaptation to diets and 7 d for ruminal sample collection and *in vivo* disappearance trial. Treatments were : 1) control, 2) 2 ml Allicin ($C_6H_{10}OS_2$)/cow/d, 3) 2 ml Zingiberene ($C_{15}H_{24}$)/cow/d, and 4) 2 ml Citral($C_{10}H_{16}O$)/cow/d. Essential oils were purchased commercially (purity >95% g/kg; Power Tech Chemical Industry Co.,Ltd., Bangkok, Thailand).

6.4.2 Measurements and chemical analysis

6.4.2.1 Feed intake

Diets offered as 3 kg/d of concentrate containing 21% CP, divided into 2 equal meals at 0800 and 1600 h together with *ad libitum* corn silage and clean water. Feed offered and feed refused were measured and recorded daily during the experimental periods. Dry matter content (48 h at 65 °C) of the concentrate and corn silage for individual cows was determined daily to calculate DMI.

The samples were ground through a 1 mm screen for chemical analysis. Dry matter (DM) of corn silage and concentrate were determined by oven drying at

105 °C to a constant weight. The samples were analyzed for crude protein (CP), neutral detergent fiber (NDF), and acid detergent fiber acid (ADF) (Van Soest et al., 1991). Concentrate and roughage were ground through a 2 mm screen then mixed together for *in vivo* ruminal disappearance determination. Approximately 5 g of 2 mm ground samples were placed into 8 × 11 cm nylon bags with 47 µm pore size. Samples of rice straw were suspended in the rumen of each fistulated non-lactating dairy cow for 0, 3, 6, 12, 24, 48, and 72 h, and all bags were retrieved and placed in ice water to stop the fermentation. The bags were washed under a gentle stream of water until the water ran clear and the bags were oven-dried at 65 °C for 48 h. After weighing each bag individually, the residues were analyzed for DM, NDF, and ADF content. The disappearance values were determined and expressed as a proportion of DM, NDF, and ADF incubated, respectively.

To evaluate ruminal fermentation, on the last day of each experimental period (d 21), ruminal fluid samples were collected from each fistulated non-lactating dairy cow at 0, 2, 4 and 6 h after the morning feeding. Approximately 200 ml of ruminal fluid was collected and filtered through 4 layers of cheesecloth at 0 (pre feeding), 2, 4, 6 h post feeding. One portion of rumen fluid was immediately analyzed for pH (pH meter model UB-5, Denver Instrument, Germany). Ruminal volatile fatty acids (VFA) and ammonia N were determined in rumen fluid samples by taking 20 ml of rumen fluid and was then combined with 5 ml 6N HCl, kept frozen for analysis of VFA and ammonia N. The samples were later thawed at 4 °C and centrifuged at 3,000 rpm for 15 min. All samples were stored frozen at -20 °C until analysis. The supernatant was analyzed for ammonia N by Kjeldahl and concentrations of VFA were determined by GC (Hewlett Packard GC system HP 6890 A; Hewlett Packard, Avondale, PA) equipped with a 30 m × 0.32 mm × 0.15 µm film fused silica capillary

column (HP_Innowax, AB 002, Agient, USA). Injector and detector temperatures were 250 °C. The column temperature was kept at 80 °C for 5 min, then increased at 10 °C/min to 170 °C and then increased at 30 °C/min to 250 °C and held at 250 °C for 5 min. Protozoa populations were counted by Hematocytometer in rumen fluid samples which preserved with 10% normal saline solution. Blood was taken 10 ml from the jugular vein of each cow using 6 ml tube. The tubes were immediately transferred to the laboratory and the supernatant (i.e., serum) was centrifuged (1800 ×g, 20 min, +4 °C) and harvested for later analyses of glucose and urea N.

6.5 Statistical analysis

All data were analyzed as repeated measurements for a 4 × 4 Latin squares design using ANOVA procedure of SAS (SAS, 1996).

6.6 Experimental site

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

6.7 Duration

The duration of this trial was from February 2014 to July 2014.

6.8 Results

Feed intake and nutrient digestibility were shown in Table 6.1. Total DM feed intakes were 10.6, 10.5, 10.4 and 10.3 kg/d, respectively which were unaffected by supplementation of EOs. Essential oils improved DM and NDF digestibilities at 48

and 72 h (P<0.05) without any effect on ADF and CP. Ruminal pH, VFA concentration, blood glucose, and protozoa population were not influenced by EOs (Table 6.2, 6.3). Unexpectedly, ammonia N concentration was unchanged by treatments (Table 6.3). However, all treatments reduced blood urea N at 4 h (P<0.05).

Table 6.1 Effect of essential oils on feed intake and nutrient digestibility of fistulated non-lactating dairy cows.

Item		Treatments[1/]				SEM[2/]	P-value
		CON	ALC	ZIN	CIT		
DM intake (kg/d)		10.6	10.5	10.4	10.3	0.18	0.75
Digestibility (%)							
DM	3 h	33.5	34.4	34.3	33.7	0.42	0.44
	6 h	39.8	39.5	39.2	39.2	0.76	0.93
	12 h	45.6	45.0	46.9	43.7	1.14	0.34
	24 h	55.8	57.6	57.2	57.6	0.77	0.37
	48 h	65.2^{b}	67.4^{a}	68.0^{a}	67.8^{a}	0.72	0.05
	72 h	72.1^{b}	74.0^{a}	74.5^{a}	74.4^{a}	0.28	0.01
NDF	3 h	7.2	7.2	7.3	7.2	0.21	0.62
	6 h	9.5	9.4	9.5	9.3	0.36	0.43
	12 h	15.4	15.5	15.6	15.3	0.32	0.76
	24 h	24.1	24.0	24.2	23.5	0.85	0.41
	48 h	32.3^{b}	33.6^{a}	33.8^{a}	33.6^{a}	0.48	0.05
	72 h	40.5b	42.1^{a}	42.3^{a}	42.0^{a}	0.62	0.05
ADF	3 h	4.1	4.3	4.2	4.1	0.22	0.36
	6 h	5.8	5.8	5.7	5.6	0.34	0.45
	12 h	7.6	7.5	7.6	7.6	0.19	0.62
	24 h	10.5	10.5	10.6	10.4	0.25	0.87
	48 h	20.5	21.2	21.4	20.6	0.43	0.16
	72 h	27.3	27.3	27.8	27.4	0.53	0.46

Table 6.1 Effect of essential oils on feed intake and nutrient digestibility of fistulated non-lactating dairy cows (Continued).

Item		Treatments[1/]				SEM[2/]	P-value
		CON	ALC	ZIN	CIT		
Digestibility (%)							
CP	3 h	16.3	16.4	16.4	16.4	0.66	0.99
	6 h	25.1	25.6	25.3	25.3	1.08	0.99
	12 h	31.1	31.9	31.3	31.5	1.58	0.98
	24 h	43.3	43.4	43.6	43.4	0.96	0.99
	48 h	57.3	57.8	57.6	57.8	1.26	0.99
	72 h	67.5	67.7	68.1	68.1	1.98	0.99

[a-b] Within a row means without a common superscript letter differ.

[1/]CON = Control; ALC = Allicin; ZIN = Zingiberene; CIT = Citral.

[2/]SEM = standard error of the mean.

Table 6.2 Effect of essential oils on total VFA concentration and individual VFA proportion of fistulated non-lactating dairy cows.

Item		Treatments[1/]				SEM[2/]	P-value
		CON	ALC	ZIN	CIT		
Total VFA	0 h	98.2	98.4	106.0	105.1	8.42	0.86
(m*M*)	2 h	142.2	141.8	145.0	145.0	9.99	0.99
	4 h	121.5	123.4	123.0	130.2	7.45	0.62
	6 h	121.4	121.9	123.1	123.0	8.21	0.84
Acetate	0 h	62.0	64.2	63.8	64.9	0.65	0.77
(mol/ 100 mol)[3/]	2 h	77.0	78.3	78.3	78.0	0.23	0.15
	4 h	62.9	63.4	63.6	63.5	0.41	0.33
	6 h	67.0	67.2	67.1	68.2	0.17	0.82

Table 6.2 Effect of essential oils on total VFA concentration and individual VFA proportion of fistulated non-lactating dairy cows (Continued).

Item		Treatments[1/]				SEM[2/]	P-value
		CON	ALC	ZIN	CIT		
Propionate	0 h	15.2	15.2	15.8	15.4	0.32	0.57
(mol/ 100 mol)[3/]	2 h	20.1	20.3	20.1	20.2	0.25	0.68
	4 h	18.1	18.2	18.3	18.3	0.16	0.92
	6 h	16.0	15.3	15.3	16.7	0.42	0.38
Butyrate	0 h	10.9	10.5	10.6	12.6	0.54	0.13
(mol/ 100 mol)[3/]	2 h	11.3	11.0	11.8	11.9	0.38	0.54
	4 h	10.0	11.3	10.9	10.5	0.62	0.37
	6 h	11.2	10.2	10.1	10.9	0.58	0.67
Acetate : Propionate	0 h	4.1	4.0	4.2	3.9	0.49	0.32
	2 h	2.3	2.3	2.4	2.3	0.25	0.41
	4 h	2.9	3.0	2.9	2.8	0.13	0.74
	6 h	3.5	3.7	3.8	3.4	0.28	0.46

[1/]CON = Control; ALC = Allicin; ZIN = Zingiberene; CIT = Citral.

[2/]SEM = standard error of the mean.

[3/]The proportion of individual volatile fatty acids did not include isobutyrate, isovalerate, valerate, and caproate.

Table 6.3 Effect of essential oils on rumen pH, ammonia N concentration, blood metabolites, and protozoa population of fistulated non-lactating dairy cows.

Item		Treatments[1/]				SEM[2/]	P-value
		CON	ALC	ZIN	CIT		
pH	0 h	6.59	6.38	6.28	6.23	0.13	0.30
	2 h	6.17	6.03	5.96	5.85	0.21	0.76
	4 h	6.02	5.97	5.85	5.85	0.15	0.81
	6 h	5.99	6.03	5.92	5.91	0.14	0.93

Table 6.3 Effect of essential oils on rumen pH, ammonia N concentration, blood metabolites, and protozoa population of fistulated non-lactating dairy cows (Continued).

Item		Treatments[1/]				SEM[2/]	P-value
		CON	ALC	ZIN	CIT		
NH_3-N	0 h	14.5	14.1	14.6	14.5	0.81	0.96
(mg/ 100 ml)	2 h	23.6	20.6	20.9	20.5	1.21	0.32
	4 h	17.3	15.0	14.9	15.3	0.97	0.37
	6 h	14.1	13.5	13.4	13.1	0.32	0.23
Glucose	0 h	73.0	73.3	72.5	72.3	0.97	0.88
(mg/dl)	2 h	67.5	68.5	66.3	69.0	1.26	0.48
	4 h	72.8	71.8	71.3	72.5	1.28	0.83
	6 h	74.5	74.8	72.8	73.0	1.27	0.62
BUN	0 h	17.2	17.3	17.0	17.6	0.29	0.96
(mg/ dl)	2 h	20.7	20.3	20.1	20.1	0.47	0.72
	4 h	22.0[a]	20.3[b]	20.7[b]	20.3[b]	0.47	0.04
	6 h	20.3	20.0	20.2	19.9	0.83	0.98
Protozoa	0 h	7.3	7.0	7.3	7.5	0.65	0.72
(cell/ml; $\times 10^6$)	2 h	7.0	7.0	7.1	7.3	0.88	0.86
	4 h	6.8	6.5	6.7	7.0	0.53	0.42
	6 h	7.3	7.5	7.4	7.5	0.74	0.61

[a-b]Within a row means without a common superscript letter differ.

[1/]CON = Control; ALC = Allicin; ZIN = Zingiberene; CIT = Citral.

[2/]SEM = standard error of the mean.

6.9 Discussion

Allicin

Garlic oil has a complex mix of many compounds. Four active components (allicin, diallyl sulfide, diallyl disulfide, and allyl mercaptan) are a major role for

antimicrobial activity that was tested for their effect on rumen fermentation using batch culture technique (Busquet et al., 2005a). Garlic oil has antimicrobial activity against a wide spectrum of gram-positive and gram-negative bacteria and its potential effect on modifying rumen microbial fermentation has been studied recently (Chaves et al., 2008b; Kongmun et al., 2010). In the present study, allicin increased DM and NDF disappearances without any effect on ADF and CP suggested a dose depending manner on feed digestion. Effect of garlic oil and active component on rumen fermentation and feed digestion were inconsistent and varied with active component of garlic oil (Busquet et al., 2005a), fermentation pH (Cardozo et al., 2005), or substrate incubated (Nanon et al., 2014a). Nanon et al. (2014a) suggested that combination of garlic and ginger oil as equal ratio improved DMD and partly attributed to the NDFD compared with control (no additive) *in vitro* and *in situ*. However, effect of combination of garlic and ginger oil on *in vitro* and *in situ* varied with substrates. Equally blend of garlic and ginger oil was more effective on fibrous feeds (grass hay and total mixed ration) than the grain feeds (wheat DDGS and barley grain) which was explained by increasing microbial attachment of grass hay at 6 h of *in situ* incubation, since colonization of microbial on feed particles is an important step to make feed digestion in the rumen. Although, DMD and NDFD of feed were improved by adding allicin, total VFA concentration and individual VFA proportions were unaffected by allicin addition in the present study. In contrast, supplementing with 300 or 312 mg/l garlic oil had no effect on DM, NDF, and ADF disappearances, meanwhile total VFA concentration and acetate proportion decreased but propionate and butyrate proportions increased (Busquet et al., 2005a; Busquet et al., 2005c). Garlic oil also had no effect on feed digestion, rumen fermentation, and protozoa

population in animal trial as reported by previous studies (Chaves et al., 2008a; Yang et al., 2007).

Allicin did not affect rumen pH, protozoa population, and ammonia N concentration but blood urea N was decreased. Although, allicin increased DMD and NDFD, it had no effect on blood glucose. The unexpected result was that ammonia N concentration was not affected by allicin, this might be related with reducing blood urea N concentration. This result suggested that allicin might reduce ammonia N in the rumen before absorbing through rumen wall with antimicrobial property on hyper-ammonia producing bacteria (HAPB). In contrast, supplementation with 300 or 312 mg/l garlic oil reduced ammonia N concentration and increased small peptides plus amino acids *in vitro* experiments (Busquet et al., 2005a; Busquet et al., 2005c; respectively). The present result is similar to Klevenhusen et al. (2011), who reported that garlic oil did not affect methane production and protozoa population but decreased ammonia N concentration in non-lactating sheep, even garlic oil and the organosulfur are known to exhibit a number of antimicrobial activities.

Zingiberene

Zingiberene is a monocyclic sesquiterpene that is the predominant constituent of ginger oil. Ginger oil showed inhibitory effects for 10 different micro-organisms (Hammer et al., 1999), but very limited study was conducted to investigate its effect on rumen fermentation (Busquet et al., 2005b; Nanon et al., 2014a). Supplementing with 2.2 mg/l ginger oil (standardize at 180 g/kg of shagaols) did not affect ammonia N concentration, large peptides, small peptides plus amino acids N, total VFA and molar proportion of individual VFA using continuous culture (Busquet et al., 2006). However, zingiberene showed similar effect to allicin which increased DM and NDF disappearances without any effect on total VFA concentration and individual VFA

proportion as well as blood glucose. Meanwhile, zingiberene had no effect on ammonia N but reduced blood urea N. The effect of zingiberene on feed digestion was similar to Nanon et al. (2014a) who observed that equally blend of garlic and ginger oil at the dose 200 mg/kg DM substrate improved feed digestion such as DM and NDF *in vitro* and *in situ* trial via microbial attachment. The effect was more effective in high fiber feed substrate (grass hay and total mixed ration) than grain feed substrate (Wheat DDGS and barley grain).

Citral

Citral is a key component of lemongrass oil. Lemongrass has been known for antibacterial, antioxidant (Cheel et al., 2005), and antihyper-ammonia producing bacteria (McIntosh et al., 2003). However, the information of lemongrass oil affecting rumen fermentation and feed digestion is limited. The result of this study was that DMD and NDFD were improved by citral addition without changing the digestibility of CP. This is similar to Wanapat et al. (2008), who observed increased digestibility of DM and NDF with unchanged digestibility of ADF and CP when lemongrass was added in the high forage (73% diet DM) diets in steers. In addition, 200 mg/kg DM lemongrass oil consistently improved DM and NDF disappearance without changing CP disappearance for high forage diet (grass hay and total mixed ration) *in vitro* and *in situ* trial; the improve of DM disappearance was explained by increasing microbial attachment for grass hay *in situ* trial (Nanon et al., 2014a). The present result showed that CP disappearance did not change with citral addition which is similar to Nanon et al. (2014a), who suggested that lemongrass oil may particularly be effective to improve fiber digestion of roughage, thus improve DM digestion while lemongrass oil may have minimal effect on N metabolism. The effects of citral on rumen pH, total VFA concentration, individual VFA proportion, blood glucose, and protozoa

population were the same as allicin and zingiberene. Although, citral increased feed digestion, it had no effect on total VFA concentration or individual VFA proportion which is consistent with Wanapat et al. (2008), who reported that supplementing 100 g/d of lemongrass powder improved DM digestibility and total bacteria but showed no effect on VFA concentration and individual VFA proportion in beef cattle. Wanapat et al. (2013) also showed that lemongrass meal did not affect total VFA concentration in cattle.

Although ammonia N concentration was unchanged, blood urea N was decreased. This suggested that deamination may inhibit by citral as found for allicin and zingiberene. Supplementation 200 mg/kg DM lemongrass oil in high forage substrate (>50% DM diet) reduced ammonia N concentration resulting in increased concentration of large peptides N (LPep N) and small peptides plus amino acids N (SPep + AA N). This result suggested that the deamination was inhibited by lemongrass oil (Nanon et al., 2014b). The relationship between decreased ammonia N and increased SPep + AA N with lemongrass oil agreed with previous *in vivo* reports that lemongrass meal or lemongrass powder reduced proteolytic bacteria in beef cattle (Wanapat et al., 2008; Wanapat et al., 2013). This concept is supported by McEwan et al. (2002), who reported that supplementation of essential oils resulted in a reduction in the number and diversity of hyper-NH_3-producing bacteria, thereby decreased rate of ammonia N production from amino acids.

The results of present study suggested that allicin, zingiberene, and citral may have the same mode of action. The mode of action of essential oils in the rumen was suggested by Wallace et al. (2002) in two possible modes: The first is to affect the pattern of bacterial colonization of substrates; and the second is to inhibit hyper-ammonia producing bacteria involved in AA deamination.

6.10 Conclusions

Supplementation of allicin, zingiberene, and citral in fistulated non-lactating dairy cows had no effect on feed intake, ruminal pH, protozoa, ammonia N concentration, blood glucose, total VFA concentration and individual VFA proportion. However, all treatments improved DM and partly attributed to NDF disappearances which suggested that these active components of essential oils have potential to improve feed digestion. Blood urea N was reduced by treatments that mean it is associated with inhibiting deamination.

6.11 References

Busquet, M., Calsamiglia, S., Ferret, A., Carro, M. D., and Kamel, C. (2005a). Effect of garlic oil and four of its compounds on rumen microbial fermentation. **J. Dairy Sci.** 88: 4393-4404.

Busquet, M., Calsamiglia, S., Ferret, A., Cardozo, P. W., and Kamel, C. (2005c). Effects of cinnamaldehyde and garlic oil on rumen microbial fermentation in a dual flow continuous culture. **J. Dairy Sci.** 88: 2508-2516.

Busquet, M., Calsamiglia, S., Ferret, A., and Kamel, C. (2005b). Screening for effects of plants on dairy cattle rumen microbial fermentation in a continuous culture system. **Anim. Feed Sci. Technol.** 123: 597-613.

Busquet, M., Calsamiglia, S., Ferret, A., and Kamel, C. (2006). Plant extracts affect *in vitro* rumen microbial fermentation. **J. Dairy Sci.** 89: 761-771.

Cardozo, P. W., Calsamiglia, S., Ferret, A., and Kamel, C. (2005). Screening for the effects of natural plant extracts at different pH on *in vitro* rumenmicrobial

fermentation of a high-concentrate diet for beef cattle. **J. Anim. Sci.** 83: 2572-2579.

Chaves, A. V., Stanford, K., Dugan, M. E. R., Gibson, L. L., McAllister, T. A., Van Herk, A., and Benchaar, A. (2008a). Effects of cinnamaldehyde, garlic and juniper berry essential oils on rumen fermentation, blood metabolites, growth performance, and carcass characteristics of growing lambs. **Livest. Sci.** 117: 215-224.

Chaves, A.V., He, M. L., Yang, W. Z., Hristov, A. N., McAllister, T. A., and Benchaar, C. (2008b). Effects of essential oils on proteolytic, deaminative and methanogenic activities of mixed ruminal bacteria. **Can. J. Anim. Sci.** 88: 117-122.

Cheel, J., Theoduloz, C., Rodriìguez, J., and Schmeda-Hirschmann, G. (2005). Free radical scavengers and antioxidants from lemongrass (Cymbopogon citratus (DC.) Stapf.). **J. Agric. Food Chem.** 53: 2511-2517.

Hammer, K. A., Carson, C. F., and Riley, T. V. (1999). Antimicrobial activity of essential oils and other plant extracts. **J. Appl. Microbiol.** 86: 985-990.

Hosoda, K., Kuramoto, K., Eruden, B., Nishida, T., and Shioya, S. (2006). The effects of three herbs as feed supplements on blood metabolites, hormones, antioxidant activity, IgG concentration, and ruminal fermentation in Holstein steers. **Asian-Aust. J. Anim. Sci.** 19: 35-41.

Klevenhusen, K., Zeitz, J. O., Duval, S., Kreuzer, M., and Soliva, C. R. (2011). Garlic oil and principal component diallyl disulfide fail to mitigate methane, but improve digestibility in sheep. **Anim. Feed Sci. Technol.** 166: 356-363.

Kongmun, P., Wanapat, M., Pakdee, P., and Navanukraw, C. (2010). Effect of coconut oil and garlic powder on in vitro fermentation using gas production technique. **Livest. Sci.** 127: 38-44.

McEwan, N. R., Graham, R. C., Wallace, R. J., Losa, R., Williams, P., and Newbold, C. J. (2002). Effect of essential oils on ammonia production by rumen microbes. **Reprod. Nutr. Dev.** 42(Suppl. 1): S65.

McIntosh, F. M., Williams, P., Losa, R., Wallace, R. J., Beever, D. A., and Newbold, C. J. (2003). Effect of essential oil on ruminal metabolism and their protein metabolism. **Appl. Environ. Microbiol.** 69: 5011-5014.

Nanon, A., Suksombat, W., and Yang, W. Z. (2014a). Effects of essential oils supplementation on *in vitro* and in situfeed digestion in beef cattle. **Anim. Feed Sci. Technol.** 196: 50-59.

Nanon, A., Suksombat, W., and Yang, W. Z. (2014b). Assessment of lemongrass oil supplementation in a dairy diet on *in vitro* ruminal fermentation characteristics using the rumen simulation technique. **Can. J. Anim. Sci.** 94: 731-736.

Ramos-Morales, E., Martínez-Fernández, G., Abecia, L., Martin-García, A. I., Molina-Alcaide, E., and Yáñez-Ruiz, D. R. (2013). Garlic derived compounds modify ruminal fatty acid biohydrogenation and induce shifts in the *Butyrivibrio* community in continuous-culture fermenters. **Anim. Feed Sci. Technol.** 184: 38-48.

Valero, M., and Salmeroìn, M. C. (2003). Antibacterial activity of 11 essential oils against Bacillus cereus in tyndallized carrot broth. **Int. J. Food Microbiol.** 85: 73-81.

Van Soest, P. J., Robertson, J. B., and Lewis, B. A. (1991). Methods for dietary fiber, neutral fiber and nonstarch polysaccharides in relation to animal nutrition. **J. Dairy Sci.** 74: 3588-3595.

Wallace, R. J., McEwan, N. R., McIntosh, F. M., Teferedegne, B., and Newbold, C. J. (2002). Natural products as manipulators of rumen fermentation. **Asian-Aust. J. Anim. Sci.** 15: 1458-1468.

Wanapat, M., Cherdthong, A., Pakdee, P., and Wanapat, S. (2008). Manipulation of rumen ecology by dietary lemongrass (Cymbopogon citratus Stapf.) powder supplementation. **J. Anim. Sci.** 86: 3497-3503.

Wanapat, W., Kang, S., Khejornsart, P., and Wanapat, S. (2013). Effects of plant herb combination supplementation on rumen fermentation and nutrient digestibility in beef cattle. **Asian-Aust. J. Anim. Sci.** 26: 1127-1136.

Yang, W. Z., Benchaar, C., Ametaj, B. N., Chaves, A. V., He, M. L., and McAlister, T. A. (2007). Effects of garlic and juniper berry essential oils on ruminal fermentation and on the site and extent of digestion in lactating cows. **J. Dairy Sci.** 90: 5671-5681.

CHAPTER VII

OVERALL CONCLUSION AND IMPLICATION

7.1 Conclusion

The purposes of the present study were to determine the effect of essential oils (EOs) on feed digestion and rumen fermentation in laboratory and animal.

The first experiment was conducted to determine what types and doses of EOs can improve ruminal microbial fermentation. The results demonstrated that adding EOs increased DM disappearance but decreased ammonia N concentration. High dose (1600 mg/kg DM substrate) of each EOs had negative effects on feed digestion and rumen fermentation. However, the moderate dose (200 mg/kg DM substrate) was cost-effective dose for each EOs by improving DM digestibility (DMD). The reduced ammonia N concentration was consistent in both batch cultures. The EOs had negligible effect on methane production. These results suggested that EOs used in the present study could be potentially developed as rumen modifier to improve feed digestion and rumen fermentation. However, cinnamon oil and clove oil were intensively studied in the last decade and the results of all EOs showed the same trend. Therefore, cinnamon and clove were not used in the next experiment. The dose of 200 mg/kg DM substrate of each essential oil was used in the experiment two to experiment four for main dose based on feed digestion and ammonia N concentration results.

The second experiment was focused on feed digestion based on the first experiment and divided into two parts; the first part was still screening test for the optimal dose of lemongrass oil (LEM) and equally blend of garlic and ginger oil (CEO) using close gap between doses with various substrates (wheat DDGS, barley grain, grass hay, and total mixed ration); the second part selected the best dose from the first part and then conducted the experiment in fistulated non-lactating cattle. Lemongrass oil was used as single essential oil because little literature has been reported. Lemongrass oil has been shown benefit results for animal host. Garlic oil has been intensively studied in recent years but only one literature reported the effect of ginger oil on rumen fermentation and feed digestion. Addition of different composition of EOs or specific combinations of EOs secondary metabolites may enhance efficiency of rumen microbial fermentation. Increasing dose of EOs from 0, 100, 200, to 300 mg/kg DM substrate increased DMD in batch culture trial. Therefore, supplementation of LEM or CEO at the dose of 200 mg/kg DM was used in the second part. The results demonstrated that LEM or CEO were consistent with *in situ* rumen DMD. In addition, LEM and CEO were more effective to improve DMD of high fiber feeds such as grass hay and total mixed ration (50% of forage). In the second part, microbial attachment was measured to explain the improvement of feed digestion. Microbial attachment increased for grass hay at 6 h when LEM or CEO was added. However, EOs had no effect on methane production. These results suggested that the EO used in the present study could be potentially developed as rumen modifier to improve rumen fermentation and feed digestion, especially roughage feeds in ruminant animals.

The third experiment was conducted in Rumen Simulation Technique (RUSITEC). Lemongrass oil was used at 100 and 200 mg/kg DM together with monensin. Treatments had no effect on nutrient digestibility, fermentation characteristics, gas production, and methane production. Lemongrass oil did not change DMD which was unexpected result based on previous studies. However, lemongrass oil increased LPep N and SPep N concentrations and as expected, decreased ammonia N concentration. Supplementation of monensin did not affect N fraction concentration. These results suggested that addition of LEM altered profiles of peptide, AA, and NH_3-N in the fermentation media, and increased microbial protein synthesis without changing fermentation VFA and nutrient digestibility. The results also suggested that LEM inhibited the deamination process by likely altering microbial populations, in particular proteolytic bacteria.

The fourth experiment was to determine the effect of main active component such as allicin, zingiberene, and citral instead of garlic, ginger, and lemongrass oil, respectively on feed digestion, rumen fermentation and blood metabolites in fistulated non-lactating dairy cows. Two ml of each treatment was top-dressing on diet divided into 2 meals per day. Feed intake was not influenced by treatments. As expected, treatments increased DMD and NDFD without any effect on digestibility of ADF and CP. Improvement of DMD and partly attributed to NDFD suggested that these active component of garlic, ginger, and lemongrass have potential to improve feed digestion. Supplementing with allicin, zingiberene, and citral had no effect on ruminal pH, fermentation VFA concentration, blood glucose, and protozoa population as well as ammonia N concentration. However, blood urea N was decreased by treatments which might associate with inhibited deamination in the rumen.

7.2 Implications

The present study suggests that :

Essential oils show consistent results with improving DMD and NDFD but reduce ammonia N concentration which will be benefit for animal to receive more energy and protein, both are important for maintenance and production. The animal should be supplemented with 200 mg/kg EOs. DM and NDF digestibilities were increased by adding EOs suggesting that animal will receive more energy. This is very important for early lactating cows because cows at this state subject to negative energy balance resulted from using energy for maintenance and production but decreased feed intake. This will be risk for ketosis. Meanwhile, increasing NDFD associated with increasing milk fat results in higher milk price in Thailand.

Roughage in Thailand is high in fiber but low in protein thus concentrate feed (high in protein) has been used in high ratio to increase protein percentage of total ration resulting in high feed cost. In the rumen, protein is hydrolyzed to oligo-peptides by proteolytic bacteria afterwards prevotella degrades oligo-peptides to dipeptides. Then various species of bacteria produce dipeptidases and metaloproteases for degrading dipeptides to amino acids afterwards deamination present. Amino acids is then converted to ammonia by hyper ammonia-producing bacteria including *Clostridium sticklandii* and *Peptostreptococcus anaerobius*. Supplementation of EOs increased LPep N and SPep + AA N but decreased ammonia N concentration resulting in increased ruminal by-pass protein from inhibition of proteolytic activity in deamination process, resulted in lower price of feed cost. Future research should conduct by using early lactating dairy cow and supplement with active component of EOs (i.e., allicin, zingiberene, and citral) and should focus on milk production and milk composition.

APPENDIX

Reagents preparation (Goering and Van Soest, 1970)

1. Buffer solution

- Ammonium bicarbonate (NH_4HCO_3) 4 g
- Sodium bicarbonate ($NaHCO_3$) 35 g
- Dissolved in water and brought up to 1 L in volumetric flask.

2. Macromineral solution

- Sodium hydrogen phosphate, dibasic (Na_2HPO_4) 5.7 g
- Potassium phosphate, monobasic (KH_2PO_4) 6.0 g
- Magnesium sulfate, heptahydrate ($MgSO_4.7H_2O$) 0.6 g
- Dissolved in water and brought up to 1 L in volumetric flask.

NOTE : Buffer and Macromineral solution could be stored refrigerated for up to 3 months and at room temperature for up to 1 month.

3. Micromineral solution

- Calcium chloride, dihydrate ($CaCl_2.2H_2O$) 13.2 g
- Manganese chloride, tetrahydrate ($MnCl_2.4H_2O$) 10.0 g
- Cobalt chloride, hexahydrate ($CoCl_2.6H_2O$) 1.0 g
- Ferric chloride, hexahydrate ($FeCl_2.6H_2O$) 8.0 g
- Dissolved in water and brought up to 100 mL in volumetric flask.

NOTE : Micromineral solution could be stored refrigerated for up to 12 months.

4. 0.1% (wt/vol) Resazurin

- Dissolved 0.1 g of resazurin 100 mL water.
- Stored in dark (amber coloured) bottle at 4 °C (in fridge).

5. Medium preparation (on the day the *in vitro* was started)

**This recipe was for 1 L, increased volume as required

- Weighed out 2.5 g tryptone and dissolved completely in 500 mL water
- Added 0.125 mL micromineral solution
- Added 250 mL buffer solution and 250 mL macromineral solution
- Added 1.25 mL 0.1% resazurin solution
- Placed container with medium in water bath (39 °C) and bubbled CO_2 through solution for 45 minutes
- Weighed out 0.313 g L-cysteine hydrochloride and 0.313 g sodium sulphide and added directly to medium
- Bubbled CO_2 through solution for another 15 minutes or until solution turned grey to clear.
    - A purple/pink colour indicated the presence of oxygen.
    - A grey/clear colour indicated the solution was reduced.
- Kept medium in water bath and headspace saturated with CO_2 until medium + inoculums was going to be transferred to incubation vials (at this point rumen fluid could be collected).

6. Buffer preparation (Artificial saliva) (McDougall, 1948)

- Sodium hydrogen phosphate, dibasic (Na_2HPO_4)	55.36 g
- Sodium bicarbonate ($NaHCO_3$)	147 g
- Sodium chloride (NaCl)	7.05 g

- Potassium chloride (KCl)	8.55 g
- Magnesium chloride, hexahydrate ($MgCl_2 \cdot 6H_2O$)	0.915 g
- Calcium chloride, dihydrate ($CaCl_2 \cdot 2H_2O$)	0.503 g
- Ammonium sulfate ($^{15}(NH_4)_2SO_4$)	

CURRICULUM VITAE

Mr. Atitthan Nanon was born on the 30th of July 1986 in Nakhon Ratchasima. Thailand. He graduated with the Bachelor degree from school of Animal Production Technology, Institute of Agricultural Technology, Suranaree University of Technology. After graduation, he obtained the scholarship from the Royal Golden Jubilee (RGJ) Ph.D. Program, The Thailand Research Fund (TRF), to presence a Doctor degree at the same university. He conducted the research in the topic of use of essential oils in dairy cows for manipulation of rumen microbial fermentation. The result of this project has been published in several journals; effects of essential oils supplementation on *in vitro* and *in situ* feed digestion in beef cattle for Animal Feed Science and Technology journal (vol. 196: page 50-59), assessment of lemongrass oil supplementation in a dairy diet on *in vitro* ruminal fermentation characteristics using the rumen simulation technique for Canadian Journal of Animal Science (vol. 94: page 731-736), and use of essential oils for manipulation of rumen microbial fermentation for Thai Journal of Veterinary Medicine (In press). He also had opportunity to go learn and work with expert of essential oils (Dr. WenZhu Yang) at Agriculture and Agri-Food Canada, Lethbridge Research Centre, Alberta, Canada during January 2012 to March 2013.