EFFECTS OF OILS RICH IN OMEGA-3 FAs AND OMEGA-6 FAs SUPPLEMENTATION ON RUMINAL FERMENTATION AND CHANGE IN FATTY ACIDS IN THE RUMEN OF CATTLE

Chayapol Meeprom

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

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ผลของการเสริมน้ำมันที่เป็นแหล่งของกรดไขมันโอเมก้า 3 และกรดไขมันโอเมก้า 6 ต่อกระบวนการหมักย่อยและการเปลี่ยนแปลงกรดไขมันในกระเพาะหมักของโค

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วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาปรัชญาดุษฎีบัณฑิต
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ปีการศึกษา 2560

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

Thesis Examining Committee

(Assoc. Prof. Dr. Suthipong Uriyapongson)
Chairperson

(Assoc. Prof. Dr. Wisitiporn Suksombat)
Member (Thesis Advisor)

(Assoc. Prof. Dr. Amonrat Molee)
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(Prof. Dr. Santi Maensiri)
Vice Rector for Academic Affairs and Internationalization

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

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ในการศึกษานี้ประกอบไปด้วย 5 การทดลองได้แก่

การทดลองที่ 1 การศึกษาผลของการเสริมน้ำมันที่เป็นแหล่งของกรดไขมันโอเมก้า 3 ในปริมาณ 3 เปอร์เซ็นต์ของวัตถุแห้งทั้งหมด โดยแบ่งกลุ่มทดลองเป็น 4 กลุ่มได้แก่ 1) กลุ่มไม่ทำการเสริมน้ำมัน (ควบคุม) 2) กลุ่มเสริมน้ำมันลินสีด (LSO) 3) กลุ่มเสริมน้ำมันลินสีดร่วมกับน้ำมันปลา (LSO+FO)ที่อัตราส่วน 1 ต่อ 1 โดยน้ำหนัก 4) กลุ่มเสริมไขมันไหลผ่านจากน้ำมันลินสีด (Ca-LSO) ผลการทดลองพบว่าการเสริม LSO+FO สามารถเพิ่มระดับ *t11*-C18:1 และ C22:6n-3 ได้อย่างมีนัยสำคัญทางสถิติ ซึ่งในขณะเดียวกันระดับของ C18:0 ได้มีระดับที่ลดลง อย่างไรก็ตามพบว่าในชั่วโมงที่ 4 และ 6 หลังจากการให้อาหารปริมาณสัดส่วนของ acetic acid ภายในกระเพาะหมักมีระดับที่ลดลง

การทดลองที่ 2 การศึกษาผลของการเสริมน้ำมันที่เป็นแหล่งของกรดไขมันโอเมก้า 6 ในปริมาณ 3 เปอร์เซ็นต์ของวัตถุแห้งทั้งหมด โดยแบ่งกลุ่มทดลองเป็น 4 กลุ่มได้แก่ 1) กลุ่มไม่ทำการเสริมน้ำมัน (ควบคุม) 2) กลุ่มเสริมน้ำมันถั่วเหลือง (SBO) 3)กลุ่มเสริมน้ำมันปลา (FO) 4) กลุ่มเสริมน้ำมันถั่วเหลืองร่วมกับน้ำมันปลาที่อัตราส่วน 1 ต่อ 1 โดยน้ำหนักผลการทดลองพบว่า การเสริม FO และ SBO+FO มีผลให้ปริมาณของ C18:0 ภายในกระเพาะหมักลดลงอย่างมีนัยสำคัญทางสถิติ ส่วนปริมาณของ *t11*-C18:2 และ *c9, t11*-C18:2 เพิ่มขึ้นอีกทั้งการเสริม SBO และ SBO+FO ทำให้สัดส่วนของ acetic acid ณ ชั่วโมงที่ 2 หลังจากการให้อาหารลดลงร่วมทั้งความเป็นกรดด่างภายในกระเพาะหมัก

การทดลองที่ 3 การศึกษาถึงการเสริมสัดส่วนของ LSO ต่อ FO ในสัดส่วนต่างๆในปริมาณ 3 เปอร์เซ็นต์ของวัตถุแห้งทั้งหมด โดยแบ่งการทดลองเป็น 3 กลุ่มได้แก่ 1) สัดส่วนของ LSO ต่อ FO ที่อัตราส่วน 2 ต่อ 1 โดยน้ำหนัก 2) สัดส่วนของ LSO ต่อ FO ที่อัตราส่วน 1 ต่อ 1 โดยน้ำหนัก 3) สัดส่วนของ LSO ต่อ FO ที่อัตราส่วน 1 ต่อ 2 โดยน้ำหนัก ผลการทดลองพบว่าการเสริม LSO+FO ที่สัดส่วนของ LSO ต่อ FO ที่อัตราส่วน 1 ต่อ 1 โดยน้ำหนักสามารถเพิ่มปริมาณของ C20:5n-3 และ C22:6n-3 ภายในกระเพาะหมักได้อย่างมีนัยสำคัญทางสถิติ ($P<0.05$) รวมทั้งสามารถเพิ่มปริมาณ

ของ *t11*-C18:1 ได้ อย่างไรก็ตาม ได้พบผลเชิงลบทางด้านการย่อยสลายของเยื่อใยที่ไม่ละลายในกรด ($P<0.05$)

การทดลองที่ 4 การศึกษาถึงการเสริมสัดส่วนของ SBO ต่อ FO ในสัดส่วนต่างๆ ในปริมาณ 3 เปอร์เซ็นต์ของวัตถุแห้งทั้งหมด โดยแบ่งการทดลองเป็น 3 กลุ่มได้แก่ 1) สัดส่วนของ SBO ต่อ FO ที่อัตราส่วน 2 ต่อ 1 โดยน้ำหนัก 2) สัดส่วนของ SBO ต่อ FO ที่อัตราส่วน 1 ต่อ 1 โดยน้ำหนัก 3) สัดส่วนของ SBO ต่อ FO ที่อัตราส่วน 1 ต่อ 2 โดยน้ำหนัก ผลการทดลองพบว่า การเสริม SBO+FO อัตราส่วน 1 ต่อ 1 โดยน้ำหนักสามารถเพิ่มความเข้มข้นของ *t11*-C18:1 ภายในกระเพาะหมักได้อย่างมีนัยสำคัญทางสถิติ ($P<0.05$) ณ ชั่วโมงที่ 2 และ 6 หลังจากทำการให้อาหาร รวมทั้งปริมาณของ C20:5n-3 และ C22:6n-3 ได้มีปริมาณเพิ่มขึ้นเช่นกัน ($P<0.05$) อย่างไรก็ตามการเสริม SBO+FO อัตราส่วน 1 ต่อ 2 โดยน้ำหนักพบว่า ณ ชั่วโมงที่ 4 หลังจากการให้อาหารสัดส่วนของ acetic acid มีสัดส่วนที่ลดลง อย่างไรก็ตามไม่พบว่าการเสริมน้ำมันทุกสัดส่วนไม่มีผลต่อการย่อยสลายวัตถุแห้ง โปรตีน เยื่อใยที่ไม่ละลายในสารเป็นกลาง และเยื่อใยที่ไม่ละลายในสารเป็นกรดภายในกระเพาะหมัก

การทดลองที่ 5 การศึกษาที่ระดับของ SBO+LSO+FO ในอัตราส่วน 1 ต่อ 1 ต่อ 1 โดยน้ำหนัก โดยการทดลองจะแบ่งออกเป็น 3 กลุ่ม โดยกลุ่มที่ 1 จะทำการเสริมที่ระดับ 2 เปอร์เซ็นต์ของวัตถุแห้งทั้งหมด กลุ่มที่ 2 ทำการการเสริมที่ระดับ 3 เปอร์เซ็นต์ของวัตถุแห้งทั้งหมดและกลุ่มที่ 3 ทำการเสริมที่ระดับ 4 เปอร์เซ็นต์ของวัตถุแห้งทั้งหมด จากการทดลองพบว่าการเสริมน้ำมันผสมที่ระดับ 4 เปอร์เซ็นต์ของวัตถุแห้งทั้งหมดมีผลให้ระดับของ *t11*-C18:1 C20:5-3 และ C22:6n-3 ภายในกระเพาะหมักเพิ่มขึ้นอย่างมีนัยสำคัญทางสถิติ ($P<0.05$) ณ ทุกช่วงเวลาหลังจากทำการให้อาหาร รวมทั้งปริมาณสัดส่วนของ propionic acid และ ปริมาณของแอมโมเนียไนโตรเจนภายในกระเพาะหมักได้เพิ่มขึ้นเช่นเดียวกัน

จากการทดลองสามารถสรุปได้ว่า การเสริม SBO+LSO+FO ที่สัดส่วน 1 ต่อ 1 ต่อ 1 โดยน้ำหนักสามารถเพิ่มสารตั้งต้นในการสังเคราะห์กรดไขมันที่เป็นประโยชน์และปริมาณกรดไขมันที่เป็นประโยชน์ต่อสุขภาพได้

สาขาวิชาเทคโนโลยีการผลิตสัตว์ ลายมือชื่อนักศึกษา_____

ปีการศึกษา 2560 ลายมือชื่ออาจารย์ที่ปรึกษา_____

CHAYAPOL MEEPROM : EFFECTS OF OILS RICH IN OMEGA-3 FAs AND OMEGA-6 FAs SUPPLEMENTATION ON RUMINAL FERMENTATION AND CHANGE IN FATTY ACIDS IN THE RUMEN OF CATTLE. THESIS ADVISOR : ASSOC. PROF. WISITIPORN SUKSOMBAT, Ph.D., 219 PP.

BIO-HYDROGENATION/RUMINAL FERMENTATION/LINSEED OIL/SOYBEAN OIL/FISH OIL/FISTULATED CATTLE

The present study comprising 5 experiments as follows:

Experiment 1 was conducted to evaluate the effects of feeding 3% of total feed DM from oil rich in omega-3 FAs including no oil (control), linseed oil (LSO), 1:1 w/w linseed oil and fish oil (LSO+FO) and calcium salt from linseed oil (Ca-LSO). The results found that feeding LSO+FO significantly increased *t11*-C18:1 and C22:6n-3 whereas C18:0 was decreased. The ruminal acetic acid content was reduced at 4 and 6 h after feeding ($P<0.05$).

Experiment 2 was carried out to determine the effects of applying 3% of total feed DM from oil rich in omega-6 FAs including no oil (control), soy, bean oil (SBO), fish oil (FO), 1:1 w/w SBO+FO. The results revealed that FO and SBO+FO applications significantly reduced the ruminal concentration of C18:0 but increased *t11*-C18:1 and *c9, t11*- C18:2 contents. Supplementation of SBO and SBO+FO reduced the molar proportion of acetic acid at 2 h after feeding and significantly decreased ruminal pH.

Experiment 3 was conducted to investigate the effects of adding 3% of total

feed DM at different ratios from LSO and FO including 2:1 w/w LSO+FO, 1:1 LSO+FO and 1:2 w/w LSO+FO. The addition of 1:2 w/w LSO+FO significantly increased ruminal C20:5n-3 and C22:6n-3 concentrations ($P<0.05$). Additionally, 1:1 w/w LSO+FO significantly increased the concentration of *t11*-C18:1, however, there was a detrimental effect on reduction in ADFD ($P<0.05$).

Experiment 4 was carried out to assess the effects of supplementing 3% of total feed DM at different ratios of SBO and FO including 2:1 w/w SBO+FO, 1:1 SBO+FO and 1:2 w/w SBO+FO. The results revealed that 2:1 w/w SBO+FO significantly increased ruminal *t11*-C18:1 at 2 and 6 h post feeding and increased the ruminal C20:5n-3 and C22:6n-3 concentrations. However, 1:2 w/w SBO+FO significantly decreased the molar proportion of acetic acid at 4 h post feeding. The degradation of DM, CP, NDF and ADF was unaffected by oil addition.

Experiment 5 was conducted to investigate the effects of feeding different levels of 1:1:1 w/w SBO, LSO and FO including 2%, 3% and 4% combination oils. Feeding 4% combination oil significantly decreased the ruminal concentration of C18:0 but increased ruminal *t11*-C18:1, C20:5n-3 and C22:6n-3 contents at all h after feeding. Additionally, it also increased the molar proportion of propionic acid and ammonia nitrogen concentration.

It can be clearly concluded in the present study that beneficial FAs or their precursors can be reasonably obtained by the addition of 1:1:1 SBO+LSO+FO at 4% of total feed DM.

School of Animal Production Technology Student's Signature _____

Academic Year 2017 Advisor's Signature _____

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TABLE OF CONTENTS

Page

TABLE OF CONTENTS (Continued)

Page

TABLE OF CONTENTS (Continued)

Page

TABLE OF CONTENTS (Continued)

Page

TABLE OF CONTENTS (Continued)

Page

TABLE OF CONTENTS (Continued)

Page

TABLE OF CONTENTS (Continued)

Page

TABLE OF CONTENTS (Continued)

Page

TABLE OF CONTENTS (Continued)

Page

LIST OF TABLES

LIST OF TABLES (Continued)

Table **Page**

LIST OF TABLES (Continued)

Table **Page**

LIST OF TABLES (Continued)

Table **Page**

LIST OF TABLES (Continued)

Table **Page**

LIST OF TABLES (Continued)

Table **Page**

LIST OF FIGURES

LIST OF ABBREVIATIONS

ADF	=	Acid detergent fiber
CLA	=	Conjugated Linoleic acids
C10:0	=	Capric acid
C12:0	=	Lauric acid
C14:0	=	Myristic acid
C16:0	=	Palmitic acid
C18:0	=	Stearic acid
t11-C18:1	=	Vaccenic acid
C18:1n9c	=	Oleic acid
C18:2n6t	=	Linolelaidic acid
C18:2n6c	=	Linoleic acid
C18:3n3	=	α -Linoleic acid
C20:0	=	Arachidic acid
C20:1	=	Gondoic acid
C22:0	=	Behenic acid
C20:4n-6	=	Arachidonic acid
C20:5n-3	=	Eicosapentaenic acid
C22:6n-3	=	Docosahexaenoic acid
NDF	=	Neutral detergent fiber

CHAPTER I

INTRODUCTION

1.1 Rational of the study

The ruminal bio-hydrogenation was the process of microbial in the rumen to protect themselves from fat especially unsaturated fatty acid (Scollan et al., 2001). Unsaturated fatty acids affecting to the microbe's cell membrane while the microbes try to protect themselves from unsaturated fatty acids by adding H-atom to UFAs and change the structure of UFA to SFA (Jenkins, 1993). These cause the products from ruminant animals containing high amount of saturated fatty acids (Manoly et al., 2008). Consumption of foods rich in SFA can cause heart disease in human (Micha and Mozaffarian, 2010).

Supplementation of oils rich in polyunsaturated fatty acids (PUFA) has been of interest to enhance the beneficial fatty acids in ruminant products, specifically n-3 PUFA and n-6 PUFA, which has been associated with significant physiological and health benefits in human populations. The increasing PUFA in ruminant products is more challenging when compare with non-ruminant animals, since most of the PUFA are hydrogenated by the rumen microorganisms. Addition of oil rich in PUFA sources in diet of ruminants has been shown to increase the concentration of PUFA in the ruminant products (Palmquist, 2009). Furthermore, incomplete bio-hydrogenation of linoleic acid (C18:2n-6) and α-linolenic acid (C18:3n-3) results in developing conjugated linoleic acids (CLA) isomers (Lee and Jenkins, 2011) by increasing of

vaccenic acid (*t11*-C18:1) in the rumen as increasing a precursor of the biosynthesis of CLA (Griinari et al., 2000).

The ruminal bacteria involved in hydrogenation has been classified into two groups, A and B according to the metabolic pathway involved (Kemp and Lander, 1984) The functional of group A was to hydrogenate PUFA into *t11*-C18:1. For complete hydrogenation of PUFA only group B can hydrogenate C18:1n-9 and its isomers into C18:0. Inhibition of group B bacteria found that EPA and DHA sources addition into the diet will shift these processes to convert PUFA to saturated fatty acid (Jenkins et al., 2008).

Supplementation of oil rich in PUFA in the rumen has the potential to radically disturb ruminal pH, volatile fatty acids (VFA) and ruminal fermentation (Machmüller et al., 1998; Maia et al., 2010). However, the types and sources of PUFA fed to ruminants might have different impacts on rumen fermentation and microbial populations (Ivan et al., 2013; Liu et al., 2012).

Therefore, the objective of this study is to determine the effect of oil rich in Omega-3 FAs and Omega-6 FAs supplementation on ruminal fermentation and change in fatty acid in the rumen of cattle.

1.2 Research objectives

1.2.1 To study the effect of oil rich in Omega-3 FAs and Omega-6 FAs supplementation on ruminal bio-hydrogenation in the rumen of cattle.

1.2.2 To study the effect of oil rich in Omega-3 FAs and Omega-6 FAs supplementation on ruminal fermentation in the rumen of cattle.

1.2.3 To study the effect of oil rich in Omega-3 FAs and Omega-6 FAs supplementation on nutrient degradation in the rumen of cattle.

1.3 Research hypothesis

1.3.1 Supplementation of oil rich in EPA and DHA in combination with in oil rich in Omega 3 FAs can increase EPA, DHA and Vaccenic acid in the rumen but can reduce C18:0.

1.3.2 Supplementation of oil rich in EPA and DHA in combination with in oil rich in Omega 6 FAs can increase EPA, DHA and Vaccenic acid in the rumen but can reduce C18:0

1.3.3 Supplementation of oil rich in EPA and DHA in combination with in oil rich in Omega 3 FAs results in negative effects on ruminal fermentation and degradation.

1.3.4 Supplementation of high level of combination oils results in negative effects on ruminal fermentation and degradation.

1.4 Scope of the Study

These researches intended to study the effect of oil rich in Omega-3 FAs and Omega-6 FAs supplementation on ruminal bio-hydrogenation and fermentation in fistulated cattle.

1.5 Expected Results

1.5.1 To known the effects of supplementation of oil rich in EPA and DHA in combination with in oil rich in omega-3 FAs on ruminal bio-hydrogenation.

1.5.2 To known the effects of supplementation of oil rich in EPA and DHA in combination with in oil rich in omega-6 on ruminal bio-hydrogenation.

1.5.3 To known the ratios of the combination oils resulting in higher of EPA, DHA and *t11*-C18:1 content.

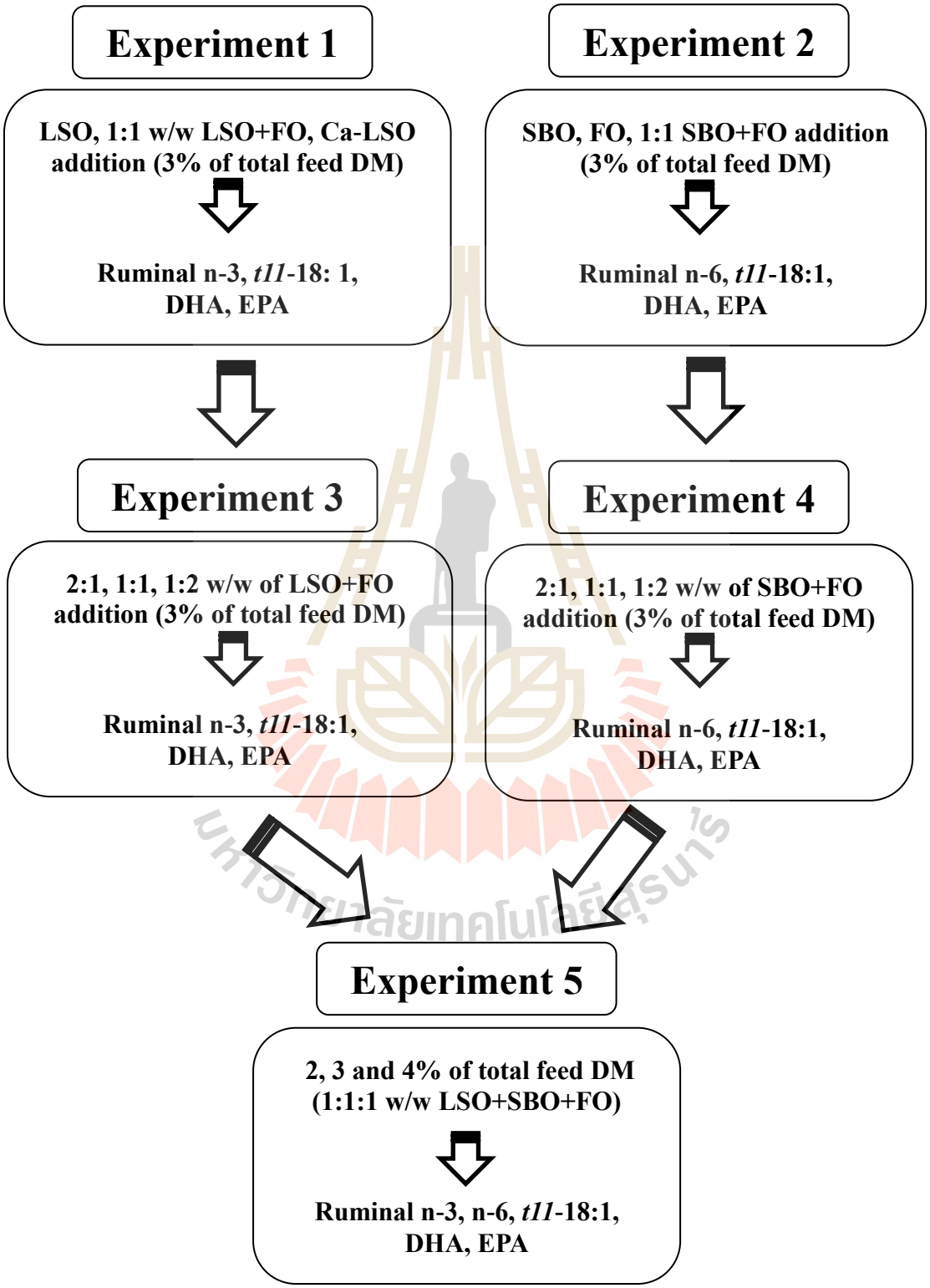
1.5.4 To known the ratios of the combination oils resulting in no negative effects on ruminal fermentation and degradation.

1.5.5 To known the level of the combination oils resulting higher of EPA, DHA and *t11*-C18:1 content.

1.5.6 To known the level of the combination oils resulting in no negative effects on ruminal fermentation and degradation.

1.5.7 Can use and apply the information from this study to production trial.

1.6 Conceptual Framework of the Study



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CHAPTER II

LITERETURE REVIEW

2.1 The role of omega 3 fatty acids

The fat content of EPA (20:5 n-3) and DHA (22:6 n-3) are of interest because of their potential benefits to human health. The effects of these omega-3 fatty acids on reducing risk of cardiovascular disease, type II diabetes, hypertension, cancer, and certain disruptive neurological functions and their potential mechanisms of action have been extensively reviewed (Uauy-Dagach and Valenzuala, 2000). In human nutrition, there is an effort to increase consumption of these functional food components due to the low intake of omega-3 fatty acids and the relationship of the intake of omega-3:omega-6 fatty acids; Western diets typically have an omega-6 to omega-3 ratio of 20-30: 1 whereas the ideal ratio is thought to be 4:1 or less (Razminowicz et al., 008). As a consequence, opportunities to enhance omega-3 fatty acids in many foods, including dairy products, are being explored.

EPA and DHA are absent or a minimal level in traditional cattle diets, and consequently they are typically present in very low amounts in ruminant products (0.1 % of total fatty acids). To improve the concentration of omega 3 FAs in animal products, feeds containing omega-3 FAs such as unsaturated lipid supplement, oil seed and oils can be used (Simopoulos, 2004). However, fish oils, fish by-products and marine algae are often available as cattle feedstuffs and these are rich sources of EPA and DHA. Hence, there is an increasing use of fish oils and fish meal in cattle diets.

2.2 Fat supplementation in ruminant and change in fatty acids in the rumen

The aim of fat supplementation in ruminant is to increase the concentration of energy in feed and to improve some fatty acids to produce healthy products. However, when high level of fat is added, there is a negative effect on microorganism in rumen. Normally, in cattle receiving fresh grass, the fat from fresh grass is galactolipid or galactose binding with 2 fatty acids by ester (Van Soest, 1994). Fat supplements especially polyunsaturated fatty acid (PUFA) which aim to improve some fatty acids in ruminant products will has the negative effect on ruminal fermentation by reducing cellulolytic bacteria activity and may consequently cause rumen acidosis (Moore et al., 1986). Thus, rumen microorganism will try to protect themselves from the fatty acids by changing the structure of fatty acids. These processes have important roles to improve PUFA in ruminant products. There are two main processes changing fatty acids in the rumen and preventing flow of PUFA to animal's tissues. (Figure 2.1).

Lipid metabolism in the rumen changes the fatty acid profile. The first step in the metabolism pathway of dietary fats is ruminal lipolysis. Lipids extracted from the feed can be largely hydrolysed by enzymes of rumen bacteria: *Anaerovibrio lipolytica* and *Butyrivibrio fbrisolvens* (Harfoot and Hazlewood, 1997). The next step in the metabolism pathway of lipids in the rumen is hydrogenation of unsaturated 18-carbon fatty acids into C18:0. The main substrates are C18:2n-6 and C18:3n-3 and the rate of hydrogenation increases with the degree of unsaturation (Harfoot and Hazlewood, 1997). The ruminal bacteria involved in hydrogenation have been classified into two groups, A and B, according to the metabolic pathway involved (Kemp and Lander, 1984). For complete hydrogenation of PUFA, both groups of bacteria are usually

necessary. Group A comprises a plurality of bacteria able to hydrogenate PUFA into *t11*-C18:1; this groups includes *Butyrivibrio fbrisolvens, Micrococcus* sp. and *Ruminococcus albus*. Group B, including *Fucocillus*, participates mainly in hydrogenation of C18:1n-9 and its isomers into C18:0. Two key bio-hydrogenation intermediates are *t11*-C18:1, formed from C18:2n-6 and C18:3n-3, and *c9*,*t11*-C18:2 (CLA), formed by bio-hydrogenation of C18:2n-6. The bio-hydrogenation processes in the rumen, however, are complex and apart from the main pathway involving *t11*-C18:1 and *c9*,*t11*-C18:2 as intermediates, there must be many more routes.

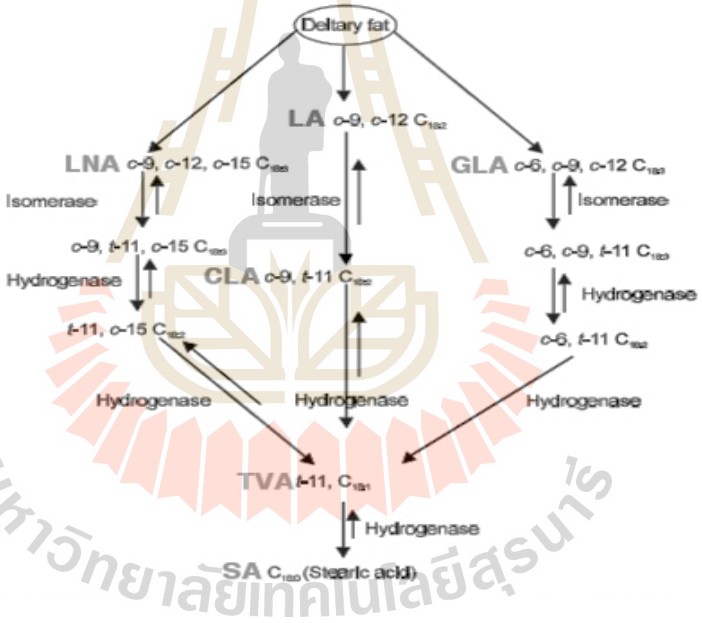

Figure 2.1 Change in fatty acids in rumen and tissue. Jenkins (1993); Drackley (2000); Mele et al. (2008)

Many researchers try to supplement another form of oil. Oil seed is one way to fix these problems because the seed covered can protect the oil from microorganism. Oil seeds including linseed and sunflower seed slowly release in the lower gut (Doreau et al., 1999). In addition, some experiments supplemented oil in the form of Ca-salt of

fatty acid to protect fatty acid from degradation in the rumen and bypassed to digest and absorb in the small intestine. Supplementation of PUFA affects microorganism especially *Butyrivibrio fibrisolven* by loss concentration in the cell and cell will hydrolysis, therefore, the double bond number is the one factor to stimulate bio-hydrogenation process. Free fatty acids with 18 carbon atoms are largely bio-hydrogenated, but the fate of fatty acids with longer chains has not been adequately studied. Ashes et al. (1992) and Offer et al. (2001) observed that a considerable proportion of EPA and DHA were not hydrogenated because the serum and plasma levels of these acids significantly increased when they were added to sheep and cow diets and similar result within the rumen. The concentrations of DHA and EPA increase meanwhile stearic acid decreases when supplements fish oil as showed in Table 2.1.

Kitessa et al. (2001a) supplemented tuna oil and protected tuna oil and found a significant decrease in C18:0 concentration when compared with control. Similarly, Doreau and Chilliard (1997) also observed a decrease in C18:0 concentration while C18:1n-9 and C18:2n-6 concentrations were decreased caused by the hydration of C18:1n-9 present in fish oil. This may be due either to changes in the microbial population or a result of changes in biochemical pathways in an attempt by the rumen microorganisms to reduce the cytotoxic effects of highly unsaturated fatty acids (Kitessa et al., 2001b). Degree of their bio-hydrogenation decreases (Gulati et al., 1999) while the proportion of C18 *trans* increases (Kitessa et al., 2001a). When added to the diets of cows, EPA and DHA disturb rumen metabolism and C18 *trans* and 10-hydroxystearic acid is formed (Kitessa et al., 2001a). Formed of C18 *trans* from C18:1n-9 and C18:2n-6 was higher among flow into small intestine as shown in Table 2.2.

Table 2.1 Effects of fish oil supplementation on change in fatty acids in the rumen

References	Treatment	Fatty acid profiles in Rumen*					
		C18:0	C18:1	C18:2	C18:3	EPA	DHA
Loor et al. (2005)	FO	NR	0.64[b]	0.75[c]	0.85	0.82	0.89
	LSO	NR	0.84[a]	0.85[b]	0.95	-	-
	SFO	NR	0.80[a]	0.92[a]	0.83	-	-
Kitessa et al. (2001a)	Control	39.4[a]	11.3[a]	4.16[a]	1.27[a]	-	0.69[c]
	Tuna oil	4.84[b]	8.66[b]	2.32[b]	0.84[b]	1.08	9.94[b]
	PTO	6.72[b]	11.9[a]	4.30[a]	1.25[a]	1.37	5.67[a]
Doreau and Chilliard (1997)	Control	54.48[a]	13.39[b]	5.54	0.48	0.03	0.10[b]
	FO+rumen	7.88[b]	36.02[a]	2.56	0.25	0.33	0.51[a]
	FO+duodenum	46.19[a]	12.9[b]	4.38	0.44	0.77	0.42[a]

[a,b] and [c] showed in column were significant different (P<0.05)

FO = Fish oil LSO = Linseed oil SFO = Sunflower oil PTO = Protected tuna oil

NR = Not reported

Table 2.2 Effect of fish oil supplementation on fatty acids duodenal flow in steer

References	Treatment	(mg) fatty acid duodenal flow in steers receiving fish oil						
		C18:0	C18:1	C18:2	C18:3	TVA	EPA	DHA
Kim et al. (2008)	control	152.7[a]	20.4[a]	7.40[a]	3.32[a]	42.5[b]	0.27[c]	0.14[a]
	FO 2.3%	115.1[b]	21.2[a]	7.64[a]	3.71[a]	73.2[a]	0.48[b]	0.39[b]
	FO 6.9%	58.9[c]	14.4[b]	3.40[b]	2.08[a]	83.4[a]	0.83[a]	1.01[a]
Loor et al. (2005)	FO	95.9[b]	26.9	27.6[b]	8.9[b]	14.4[a]	6.5[a]	3.4[a]
	LSO	398.5[a]	34.0	37.6[ab]	24.0[a]	9.4[b]	1.0[b]	0.6[b]
	SFO	346.0[a]	36.2	51.8[a]	11.0[b]	10.6[b]	2.8[b]	2.2[ab]

[a,b] and [c] showed in column were significant different (P<0.05)

FO = Fish oil LSO = Linseed oil SFO = Sunflower oil

Supplementation of fish oil increased the duodenal flow of ruminal *t11*-C18:1 (Kim et al., 2008; Loor et al., 2005) meanwhile C18:0 concentration decreased (Table 2.2). The degree of unsaturated in fish oil was toxic to microbial and may change the functional of microbes to convert *t11*-C18:1 into C18:0, however, some investigation found that *C. proteoclasticum* did not convert *t11*-C18:1 into C18:0. For the higher C20:5n-3 and C22:6n-3 flow into duodenum was effected by the lower lipolysis of C20:5n-3 and C22:6n-3 in the rumen (Chow et al., 2004), however, the ruminal bio-hydrogenation of C20:5n-3 and C22:6n-3 was still not clear (AbuGhazaleh et al., 2002). Whitlock et al. (2002) suggested that the limit of reductase in ruminal bacteria had lower hydrogenated of fatty acids containing C-atom more than 20 atoms and hypothesized that the use of oil rich in omega-3 FAs will reduce the complete hydrogenation in the rumen and increase C20:5n-3 and C22:6n-3 flow into lower gut as presented in Table 2.3.

Table 2.3 Effects of different linseed forms in combination with sources of DHA on fatty acids flow into omasum in dairy cow.

Reference	Treatment	Fatty acid flow into omasum g/day					
		C18:0	***t11*-C18:1**	**C18:1**	**C18:2**	**C18:3**	**DHA**
Sterk et al. (2012)	CS	5.368^{a}	35.6^{ab}	42.5	17.7^{ab}	21.8^{b}	ND
	EL	6.342^{a}	26.0^{b}	41.0	20.2^{a}	33.8^{a}	ND
	FL	6.331^{a}	32.6^{ab}	52.0	16.3^{ab}	15.5^{b}	ND
	DL	0.148^{b}	92.2^{a}	57.4	10.7^{b}	4.6^{c}	1.0

^{a,b} and ^{c} Showed in column were significant different ($P<0.05$).

CL = Crushed linseed; EL = Extruded whole linseed

FL = Formaldehyde – treated linseed; DL = DHA + Linseed oil

From Table 2.3, the purpose of formaldehyde-treated to protect the nutrient from ruminal microbe and the result showed that the C18:3n-3 concentration was lower than crush linseed and extruded whole linseed caused by the degradation of fatty acids in formaldehyde-treated linseed. The inhibition of ruminal bio-hydrogenation can be observed by the concentration of C18:0 that supplemented crush linseed, extruded whole linseed and formaldehyde-treated linseed had no effect on C18:0, however, addition of DHA + linseed observed lower flow of C18:0 into omasum.

2.3 Digestibility of EPA and DHA in ruminants.

Unlike in monogastric animals, almost 90% of dietary fats in ruminants reach the duodenum as non-esterified fatty acids. Fatty acids that enter the small intestine in the form of triglycerides, glycolipids and phospholipids can be released by pancreatic lipases, glycolipases and phospholipases as free fatty acids, and then absorbed. Low duodenal pH does not provide optimum conditions for the activity of these enzymes (Arienti et al., 1974). It can therefore be assumed that triglycerides are absorbed more slowly than free fatty acids in the intestine of ruminants (Doreau and Ferlay, 1994). In small intestinal epithelial cells, fatty acids are esterified and triglycerides and phospholipids are incorporated into chylomicrons and very low density lipoproteins (VLDL) and transported through lymph. In theory, lipids can also be absorbed from the large intestine, but the degree of absorption is very low (Doreau and Ferlay, 1994).

In ruminants, the digestibility of individual fatty acids depends on chain length. Digestibility was found to be highest for C16 and C18 acids (about 0.8), and slightly lower for C12, C14, C20 and C22 acids (0.65-0.70). It is generally believed that the digestibility of fatty acids increases with decreasing saturation (Doreau and Ferlay,

1994). C20:5n-3 and C22:6n-3 that escape rumen metabolism are absorbed from the small intestine as lipoproteins and transported in the aqueous media of lymph and blood to different organs and tissues in which they are stored and/or subjected to further processes. In traditional diets, C20:5n-3 and C22:6n-3 supplied preformed from the diet or from linolenic acid are preferentially incorporated into phospholipids in the muscle tissue of ruminants (Ashes et al., 1992; Wood et al., 1999), while the level of these acids in adipose tissue is low. Considerable amounts of C20:5n-3 and C22:6n-3 (up to 0.75 g/ 100 g fat) were found in subcutaneous and omental fat when lambs received a ration containing 3 g/kgDM of rumen-protected tuna oil (Kitessa et al., 2001c). When the supply of dietary fish oil was increased (80 or 120 g/kgDM), however, no C20:5n-3 and C22:6n-3 was found in subcutaneous adipose tissue (Ashes et al., 1992). Thus, the dietary level of long-chain PUFA affects lipid metabolism, although in ruminants the incorporation of long-chain n-3 PUFA into adipose tissue triglycerides is low. The reasons for this are not completely understood. C20:5n-3 and C22:6n-3 found in plasma lipoprotein triglycerides can be used by the mammary gland once they are released as free fatty acids. This reaction is catalyzed by lipoprotein lipase. Because absorbed C20:5n-3 and C22:6n-3 are transported around the body as phospholipids associated with the high-density lipoprotein (HDL) fraction, and this fraction is not a good substrate for lipoprotein lipase, the uptake by the mammary gland of absorbed C20:5n-3 and C22:6n-3 is extremely low. The concentration of C20:5n-3 and C22:6n-3 in milk fat remains low, therefore, even when the diet is enriched with these fatty acids. It was observed, however, that these acids support ruminal production of conjugated linoleic acid (Griinari and Bauman, 1999), which is incorporated mainly into the adipose tissue and mammary triglycerides of cattle.

C22:6n-3 elevates *trans*-C18:1 isomers, but is not directly converted into *trans*-C18:1 isomers in ruminal batch cultures (Klein and Jenkins, 2011). The principal sources of preformed long-chain fatty acids for milk fat production are chylomicron and plasma very low density lipoprotein triglycerides, but they fail to incorporate C20:5n-3 and C22:6n-3 into their own triglycerides to a large extent (Chilliard, 1993; Offer et al., 2001). Perhaps this explains, why the degree of incorporation of these acids into milk triglycerides is low compared with other fatty acids, although it significantly increases when fish oil is added to the diet. When the dietary concentration of C20:5n-3 and C22:6n-3 is increased, their concentration in milk also increases, although the transfer rate of these acids from diet into milk is very low. Chilliard et al. (2000) observed that the transfer rate of C20:5n-3 and C22:6n-3 into milk increased after duodenal infusion of fish oil. This shows that the main reason for such a low transfer rate is the considerable ruminal bio-hydrogenation of these acids. Even when fatty acids bypass the rumen, the proportional transfer rate is still low, which suggests that most probably other mechanisms inhibit the transfer of C20:5n-3 and C22:6n-3 into milk, including the previously described form in which C20:5n-3 and C22:6n-3 enter the mammary gland (phospholipids associated with the high-density lipoprotein fraction).

It is possible, that the C20:5n-3 and C22:6n-3 present in milk arise from *de novo* synthesis of these acids from linolenic acid, and increased supply of this acid to the mammary gland may be an alternative way of increasing the concentration of C20:5n-3 and C22:6n-3 in milk. The conversion of C18:3n-3 to C20:5n-3 is more efficient than its conversion to C22:6n-3 (Wood et al., 1999), which suggests a lower ability to achieve final conversion from EPA to DHA.

2.4 Improving EPA and DHA in ruminant products

Generally supplementation of fish oil to ruminant diets can increase the concentration of C20:5n-3 and C22:6n-3 in ruminant products (Cant et al., 1997; Chilliard and Doreau, 1997; Franklin et al., 1999; Gulati et al., 1999; Kim et al., 2008), however, different levels of addition and the mechanism by which C20:5n-3 and C22:6n-3 are synthesized cause variation in the results observed.

Supplementation of fish oil in dairy cows is toxic to the ruminal microbes and decreases milk fat (Franklin et al., 1999). *Butyrivibrio fibrisolvens* is the main bacteria playing role in ruminal bio-hydrogenation. Maia et al. (2010) found that addition oil rich in unsaturated fatty acids caused accumulation of fat inner the cell of *Butyrivibrio fibrisolvens*. The higher accumulation of lipid inner the cell membrane had negative effect on cell concentration resulting in cell hydrolysis, especially C20:5n-3 and C22:6n-3. To avoid this problem, protected form of oil can be used. However, C20:5n-3 and C22:6n-3in ruminant products can be increased by using source of C18:3n-3 oils as showed in Figure 2.2**.**

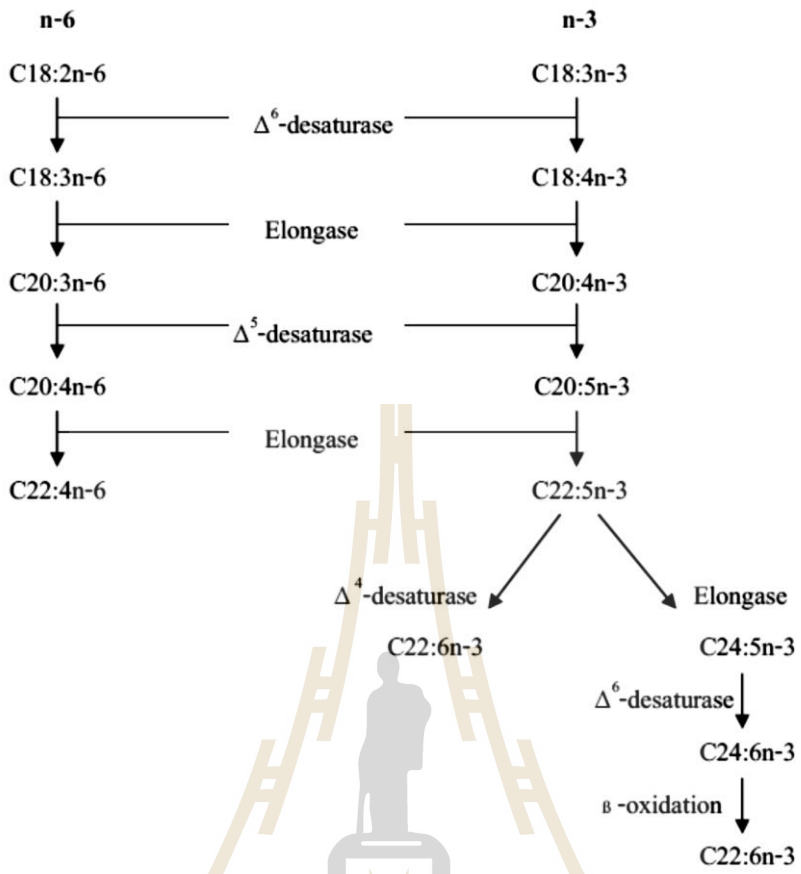

Figure 2.2 Synthesis of long chain fatty acids from unsaturated 18 carbon atom in tissue (Maia et al., 2010)

Fresh grass and linseed are rich in C18:3n-3, however, the synthesized of C20:5n-3 and C22:6n-3 is low efficiency from the limitation of desaturation and elongation enzyme. Nevertheless, higher intake of fresh grass increases acetic acid and ruminal pH, which can change fatty acids in the rumen. The higher rates of hydrogenation of C20:5n-3 and C22:6n-3are found in the rumen. Therefore, the uses of different oil forms or oil sources to improve beneficial fatty acids in animal products can be achieved as showed in Table 2.4.

Table 2.4 Effects of linseed or fish oil or combination oil on fatty acid profile in ruminant products.

References	Treatments	species	Fatty acids profiles in ruminant products (%)							
			C18:0	C18:1n-9	C18:2n-6	C18:3n-3	*t11*-C18:1	*c9t11*	EPA	DHA
He et al. (2012)	Control	Beef cattle	8.34	37.5	1.25	0.38[b]	0.80[b]	0.55[b]	0.03	0.03[b]
	Linseed oil		8.90	37.7	1.31	0.85[a]	2.37[a]	1.36[a]	0.03	0.05[a]
Sterk et al. (2012)	CL	Dairy cow	14.25[a]	21.68[a]	1.30[b]	0.87[b]	1.31[b]	0.56[b]	NR	0.08
	EL		14.94[a]	23.33[a]	1.29[b]	0.83[b]	0.63[b]	0.35[b]	NR	0.07
	FL		13.49[a]	18.60[a]	2.12[a]	3.19[a]	1.06[b]	0.43[b]	NR	0.10
	DL		6.57[b]	10.32[b]	1.14[b]	0.46[b]	3.20[a]	1.45[a]	NR	0.07
Noci et al. (2007)	Control	Beef cattle	15.88	31.02[a]	3.17[a]	0.87[b]	8.56[a]	1.78[a]	0.26	0.06
	Linseed oil		16.10	30.56[b]	2.59[b]	1.35[a]	6.32[b]	1.26[b]	0.28	0.07

[a,b] and [c] showed in column were significant different ($P<0.05$)

CL = Crushed linseed; EL = Extruded whole linseed; FL = Formaldehyde-treated linseed; DL = DHA + Linseed oil

Table 2.4 Effects of linseed or fish oil or combination oil on fatty acid profile in ruminant products. (cont.)

References	Treatments	species	Fatty acids profiles in ruminant products (%)							
			C18:0	C18:1n-9	C18:2n-6	C18:3n-3	*t11*-C18:1	*c9t11*	EPA	DHA
Kook et al. (2002)	Control	Bull	13.51	45.66	5.32	0.27^{c}	NR	NR	0.23^{c}	0.46^{c}
	Fish oil		13.47	45.85	5.33	0.28^{c}	NR	NR	0.57^{b}	1.15^{b}
	Control	Steer	11.35	45.88	3.71	0.53^{b}	NR	NR	0.56^{b}	0.13^{d}
	Fish oil		11.06	46.26	3.79	1.21^{a}	NR	NR	1.22^{a}	2.45^{a}
Kitessa et al. (2001c)	Control	Dairy goat	12.5^{a}	24.9^{b}	2.86^{b}	0.59	2.33^{c}	NR	ND	ND
	Protected tuna oil		4.30^{c}	18.8^{c}	3.44^{a}	0.54	8.47^{a}	NR	0.47	1.01
	Unprotected tuna oil		7.26^{b}	33.0^{a}	3.47^{a}	0.32	5.93^{b}	NR	0.31	1.12
Donovan et al. (2000)	Control	Dairy cow	9.38^{a}	16.47^{a}	3.14^{a}	0.18	1.21^{c}	0.60	0.05^{d}	0.02^{b}
	Fish oil 1%		6.98^{b}	14.52^{b}	2.40^{b}	0.36$^{P0.06}$	3.07^{b}	1.58	0.22^{c}	0.06^{b}
	Fish oil 2%		4.43^{c}	11.37^{c}	2.03^{c}	0.24	6.08^{a}	2.23	0.32^{b}	0.26^{a}
	Fish oil 3%		4.03^{d}	10.89^{d}	2.35^{b}	0.22	4.69b	1.90	0.40^{a}	0.20^{a}

^{a,b} and ^{c} showed in column were significant different (P<0.05)

Table 2.4 Effects of linseed oil or fish oil or combination oil on fatty acid profile in ruminant products. (cont.)

Reference	Treatment	species	Fatty acids profiles in ruminant products (%)							
			C18:0	C18:1n-9	C18:2n-6	C18:3n-3	*t11*-C18:1	*c9t11*	EPA	DHA
IFOMA (1996)	Control	Beef cattle	14.96	34.26	2.15	0.62	1.79[b]	NR	0.31[c]	0.06
	LSO		13.76	34.84	2.08	1.02	3.48[a]	NR	0.38[b]	0.06
	FO		12.65	29.36	2.06	0.61	4.29[a]	NR	0.54[a]	0.10
	LSO+FO		12.33	30.83	2.13	0.76	4.35[a]	NR	0.37[b]	0.12

[a,b] and [c] Showed in column were significant different ($P<0.05$)

LSO = Linseed oil; FO = fish oil

The results from Kitessa et al. (2001) and Donovan et al. (2000) showed in Table 2.4, supplementation of fish oil reduced milk C18:0 concentrations of goat and cow by inhabitation converter of C18:1 and *t11*-C18:1 into C18:0 caused by C20:5n-3 and C22:6n-3 in fish oil. However, the amount of C20:5n-3 in products was lower than C22:6n-3 because the transfer rate of C20:5n-3 was lower than C22:6n-3. The transfer rate of C20:5n-3 was 7.9% and of C22:6n-3 was 20.3% (Donovan et al., 2000). Addition of linseed oil or linseed alone showed no significant difference in C20:5n-3 concentration when compare to the control group. The limitation of very long chain fatty acids is to inhibit the deposition of C20:5n-3 and C22:6n-3in ruminant products and the greater degree of unsaturated of C18:3n-3 resulted in greater ruminal bio-hydrogenation. Increasing of *c9, t11*-CLA reflected an increase in ruminal *t11*-C18:1, which was subsequently synthesized into *c9, t11*-CLA in tissue or mammary gland by delta 9 desaturase enzyme (Griinari et al., 2000). Normally the greater increase in CLA might be affected by C18:2n-6 supplementation, however, Donovan et al. (2000) and AbuGhazaleh et al. (2002) found that the CLA concentration was increased when added fish oil into the diets. The hydrolysis in the rumen of fish oil was lower than 50% whereas of plant oil was 90% (Byers and Schelling, 1988). It is not clearly that fatty acids containing more than 20 carbon atoms can be modified by partially oxidizing process or hydrogenated into 18 carbon atom, however, when supplied with C18:3n-3, the amounts of C20:5n-3 and C22:6n-3 were increased (Whitlock et al., 2002). Similarly, AbuGhazaleh et al. (2002) found 120% increasing of *t11*-C18:1 in milk. *t11*-C18:1 is the fatty acid produced from hydrogenated of C18:2n-6 and C18:3n-3 (Harfoot and Hazlewood, 1997). AbuGhazaleh et al. (2002) hypothesized that supplementation of fish oil could associate with the production of ruminal *t11*-C18:1 and CLA in ruminant products.

2.5 The role of Conjugated Linoleic Acid (CLA)

Biomedical studies with animal models have documented the anticarcinogenic and anti-atherogenic effects of *c9, t11* CLA (Ip et al.,1994). Since *c9,t11* CLA is, by a considerable margin, the most predominant CLA isomer in milk fat, enhancing the CLA content of milk is realistically only related to increases in this isomer. The *c9, t11* CLA is the major CLA isomer in ruminant fat representing about 75 to 90% of the total CLA synthesized from *t11*-C18:1 in the tissue (Demeyer and Doreau, 1999), and the common name of "rumenic acid" has been proposed for this isomer because of its unique relationship to ruminants (Demeyer and Doreau, 1999). The anti-obesity effects of CLA are due to the *t10, c12* isomer; while this isomer can vary in milk fat; it never represents more than 1 or 2% of total CLA. Any CLA isomer has specific functions such as *t10, c12*-CLA can inhibit development of adiposity and *c9, t11*- CLA can inhibit the number of pre-adipocytes in adipocytes and can reduce triglyceride concentration as showed in Figure 2.3.

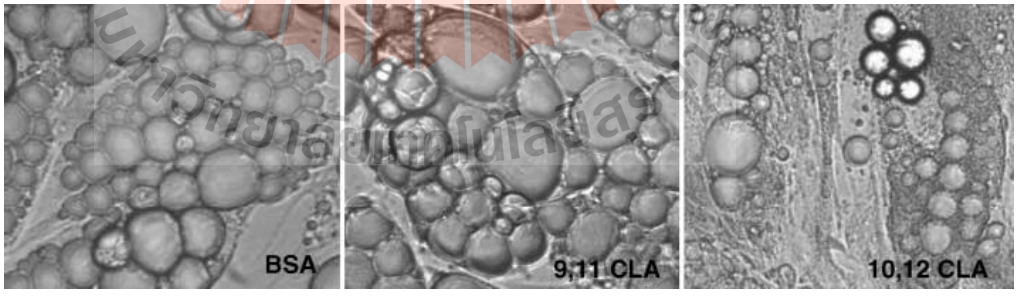

Figure 2.3 Specifically CLA isomer on change in lipid droplet morphology (Chung et al., 2005)

The investigation by Ip et al. (1994) found that supplemented 0.1 % CLA (0.015 g/d) could reduce mammary tumor in rat and diffusion of Lobulo-alveolar in mammary gland. Nevertheless, the anti-carcinogenic effect of *c9, t11*- CLA is approximately up to 85%, however, the appearance of *t10, c12* –CLA has negative effects on fat accumulation in milk and muscle by depress the expression of Stearoyl-CoA Desaturase (SCD) gene expression as showed in Figure 2.4.

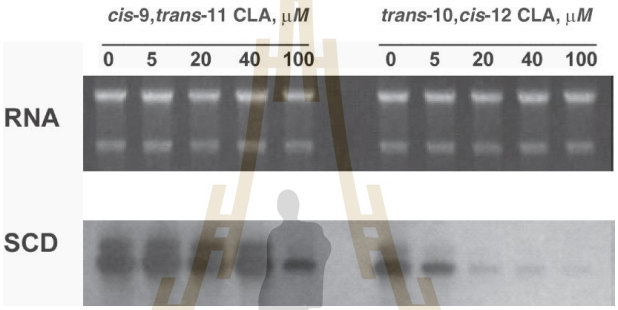

Figure 2.4 Stearoyl-CoA desaturase gene expression by CLA isomer *c9,t11* and *t10, c12* (Chung et al., 2006).

2.6 Classification of ruminal bacteria by functional change in the fatty acids

The ruminal bacteria involved in hydrogenation have been classified into two groups, A and B, according to the metabolic pathway involved (Kemp and Lander, 1984). For complete hydrogenation of PUFA, both groups of bacteria are usually necessary. Group A comprises a plurality of bacteria able to hydrogenate PUFA into *t11*-C18:1; this group includes *Butyrivibrio fibrisolvens, Micrococcus* sp. and *Ruminococcus albus*. Group B, including *Fucocillus*, participates mainly in hydrogenation of C18:1n-9, and its isomers into C18:0. Two key bio-hydrogenation

intermediates are *t11*-C18:1, formed from C18:2n-6 and C18:3n-3, and *c9,t11*- CLA, formed by bio-hydrogenation of C18:2n-6. The bio-hydrogenation processes in the rumen, however, are complex and apart from the main pathway involving *c9,t11*- CLA and *c9,t11*- CLA as intermediates, there must be many more routes. (Figure 2.5)

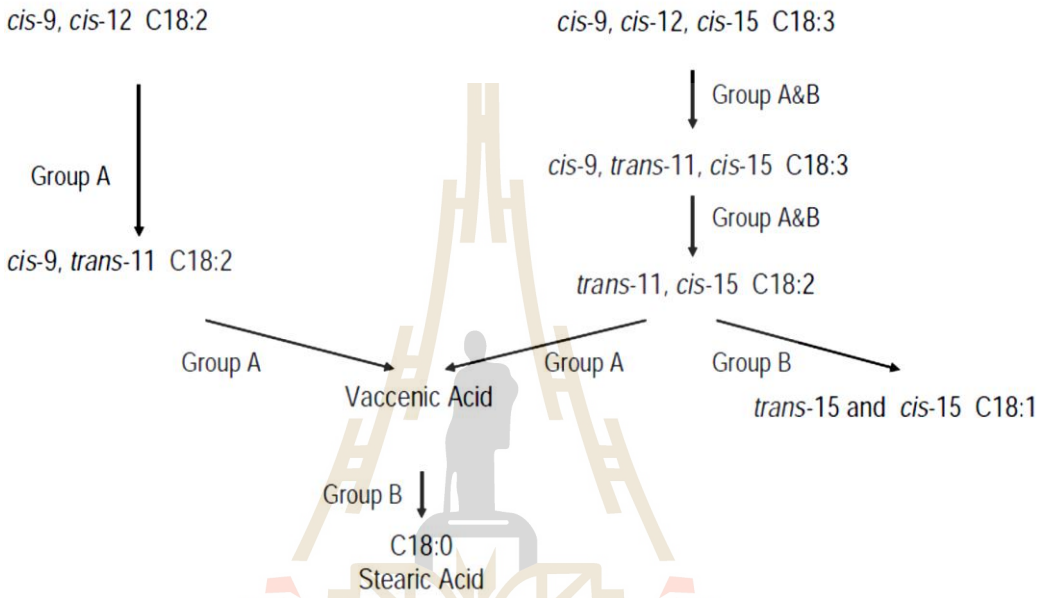

Figure 2.5 The functional change in fatty acids by 2 bacteria groups**.** (Harfoot and Hazelwood, 1988).

2.7 Change in fatty acids in the rumen

Feeding management and rumen ecology have influenced on ruminal hydrogenation. Generally, the last step of bio–hydrogenation is to produce *t11*-C18:1. The abnormal stage in rumen particularly greater acidity in the rumen, smallest *t10* - C18:1 production could be produced when the cow received high amount of concentrate (Jenkins et al., 2008). The lower ruminal pH enhanced the growing of *Bifidobacterium*, *Propionibacterium*, *Lactococcus*, *Streptococcus* and *Lactobacillus*.

The production of *t10*-C18:1 in the rumen when absorbed in lower gut can synthesized *t10,c12*-CLA or *t10* shift in hydrogenation pathways (Hinrichsen et al., 2006). Production of *t10*-C18:1 in the rumen can indicate the involute change in fatty acids in hydrogenation process.

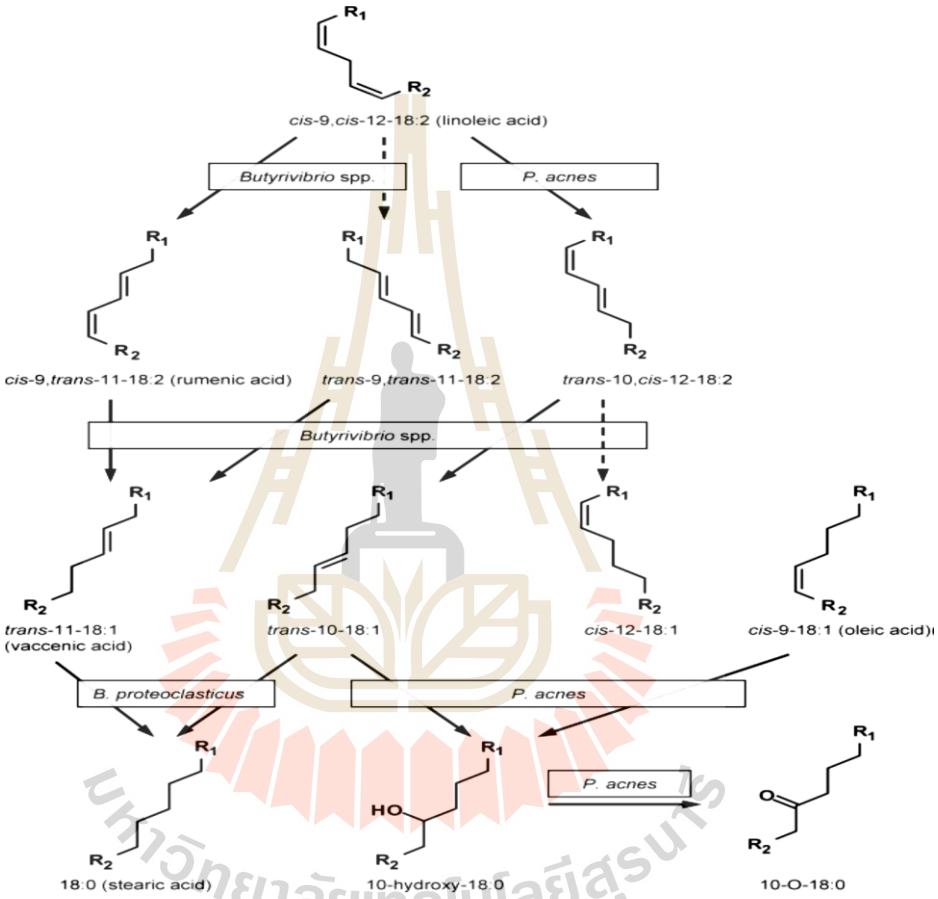

Figure 2.6 Fatty acids changing by *Butyrivibrio.spp*, *B.proteoclasticus* and *P.acnes* in the rumen (Mckain et al., 2010).

From Figure 2.6, *B. fibrisolvens* and *B. proteoclasticum* have the ability to complete hydrogenation of C18:2n-6 into *c9,t11* - C18:2, *t9,t11* - C18:2 and *t11*-C18:1 as the final step (Mckain et al., 2010). However, *B. fibrisolvens* and *C. proteoclasticum* cannot convert *t11*-C18:1 into C18:0, only *P.acnes* can produce C18:0 as the final step

of bio-hydrogenation (Scollan et al., 2001; Bauman et al., 2000).

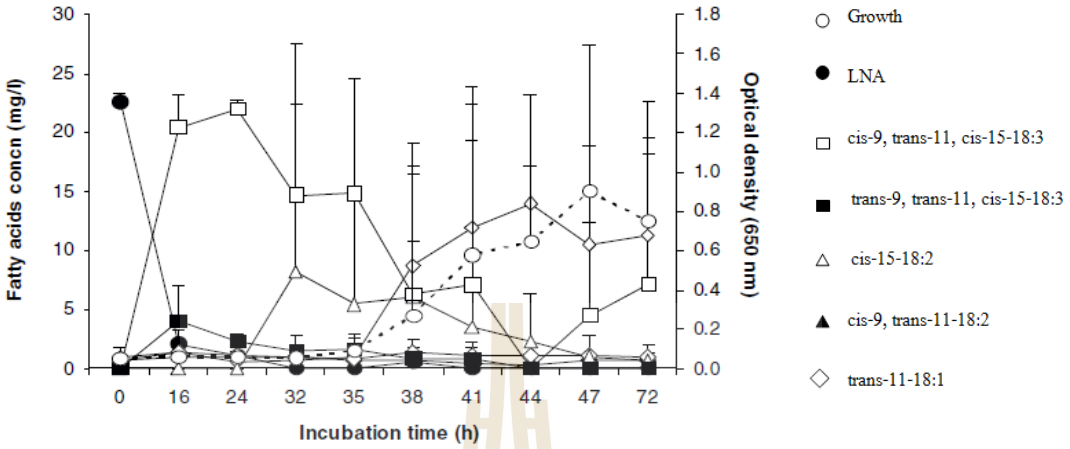

Figure 2.7 The relationship between fatty acids concentration on *B. fibrisolvens* received C18:3n-3 (Maia et al., 2010).

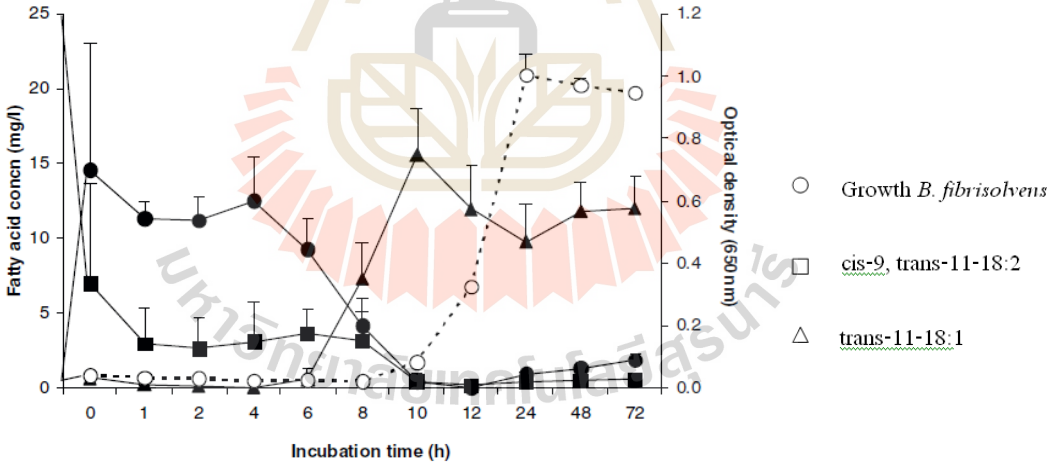

Figure 2.8 The relationship between fatty acids concentration on *B. fibrisolvens* received C18:2n-6 (Maia et al., 2010).

Differentiation of C18:2n-6 is toxic to the growing of *B. fibrisolvens* at 10 h (Table 2.7). The C18:2n-6 will be isomerized into *c9, t11*- C18:2 and reduced into *t11*-

C18:1. Maia et al. (2010) suggested that C18:3n-3 is more toxic than C18:2n-6 especially gram-positive bacteria as showed in Figure 2.8. The highest growing of *B.fibrisolvens* on C18:2n-6 was at 10 h (Figure 2.7), however, the highest growing of *B.fibrisolvens* on C18:3n3 was at 37 h. This is related to the toxic of two fatty acids. *B. fibrisolvens* and *B. proteoclasticus* are the main bacteria involved in bio-hydrogenation process especially *t10, c12* - C18:2 into *t10* - C18:1, and *t9,t11* - C18:2; *c9,t11* - C18:2 into *t11*-C18:1 by reductase enzyme. After producing *t11*-C18:1, *B. Proteoclasticus* and *P.acnes* will add H-atom in carbon chain and change unsaturated to saturated FAs as showed in Table 2.5.

Table 2.5 Hydrogenation of fatty acids by *B. fibrisolvens, B.proteoclasticus and P.acnes* (McKain et al., 2010).

Bacteria	**Substrates**	**Products**
B. fibrisolvens	*c9,t11*-C18:2	*t11*-C18:1
	t10,c12-C18:2	*t10*-C18:1
	t10,c12-C18:2	*t12*-C18:1
	t10,c12-C18:2	*c12*-C18:1
	t9,t11-C18:2	*t10*-C18:1
B. proteoclasticus	*t10*-C18:1	C18:0
	t11-C18:1	C18:0
	c9-C18:1	C18:0
P. acnes	*t10*-C18:1	10-O-C18:0
	t10-C18:1	10-OH-C18:0
	c9-C18:1	10-O-C18:0
	c9-C18:1	10-OH-C18:0

Table 2.6 Ruminal bio-hydrogenation from 18 carbon atom fatty acids.

Reference	Fatty Acid substrate	Fatty acid concentration (mg/1)					
		***c9,t11* - C18:2**		***t9,t11*- C18:2**		***t10,c12* - C18:2**	
		Initial	**Final**	**Initial**	**Final**	**Initial**	**Final**
McKain et al. (2010)	C18:0	3.39	2.76	3.05	2.43	3.41	2.88
	t10 - C18:1	-	-	-	-	0.21	11.52
	t11- C18:1	0.22	30.64	0	3.22	-	-
	c9 - C18:1	1.53	1.55	1.18	1.21	-	-
	c12 - C18:1	-	-	-	-	-	3.57
Boeckaert et al. (2008)	C18:0	4.15	3.66	3.01	2.95	4.24	2.70
	t10 - C18:1	-	-	-	-	0.52	13.84
	t11 - C18:1	0.37	31.25	0.05	2.54	-	-
	c9 - C18:1	-	-	-	-	-	-
	c9,t11 - C18:2	26.60	0.39	1.70	2.26	0.21	0.18
	t10,c12 – C18:2	-	-	-	-	18.09	1.39
Kepler et al. (1966)	C18:0	3.32	2.91	3.46	2.58	3.33	3.05
	t10 - C18:1	1.54	1.25	-	-	-	-
	t11 - C18:1	0.42	40.05	-	3.63	-	-
	c9,t11 - C18:2	20.06	0.40	1.75	0.84	0.35	0.22

Bio-hydrogenation of *c9, t11*–C18:2; *t9, t11*-C18:2; *t10, c12* - C18:2 as the substrate by *B. fibrisolvens* found that *B. fibrisolvens* can convert 0.22 mg/l *c9,t11*–C18:2 into 30.64 mg/l *t11*-C18:1 (McKain et al., 2010). *B. fibrisolvens*, group A

bacteria, are inefficient to convert those FAs into C18:0, only group B bacteria particular *Butyrivibrio proteoclasticus* and *Propionibactiriam acnes* can change *t10*-C18:1 and *t11*- C18:1 into C18:0.

2.8 Increasing of ruminal vaccenic acid by EPA and DHA sources

The main fatty acids containing 18 carbon atom, C18:2n-6 and C18:3n-3, when hydrogenate by group A bacteria such as *Butyrivibrio fibrisolvens*, *Micrococcus sp*. and *Ruminococcus albus*, the final step is *t11*-C18:1, as the substrate to synthesize CLA in the tissue. It can be hypothesized that inhibitory of group B bacteria by using oil rich in EPA and DHA in combination with oil rich in omega 6 fatty acids can increase vaccenic acid content in the rumen, as showed in Table 2.7.

As in Table 2.7, Jalč et al. (2009) supplemented different ratios of microbial oil and fish oil found an increase in *t11*-C18:1 content in the rumen whereas 1:1 mixed oil decreased C18:0 content by inhibiting the final step of bio-hydrogenation, but increased the concentration of ruminal *t11*-C18:1 (Gulati et al., 2000). These results are in agreement with AbuGhazaleh et al. (2002) who observed an increase in *t11*-C18:1 coinciding with a reduction in C18:0 when fish oil, soy bean oil in combination with fish oil were supplemented compared to soy bean oil alone. Additionally, AbuGhazaleh et al. (2004) also reported an increase in *t11*-C18:1 and a reduction in C18:0 when DHA and EPA were spplied. In contrast, Vlaeminck et. al. (2008) showed no significant difference in ruminal *t11*-C18:1 and C18:0 concentrations when algae mixed with DHA was added. The lack of effect on change in ruminal *t11*-C18:1 was probably due to natural source of DHA from algae.

Table 2.7 Effects of oil rich in EPA and DHA supplementation on ruminal hydrogenation.

References	Treatment	Fatty acid profiles in rumen*					
		C18:0	**TVA**	**C18:1**	**C18:2**	**C18:3**	***c9,t11***
Jalč et al. (2009)	Control	0.82[a]	0.15[b]	0.22	0.21	-	0.13[b]
	1:1 MO + FO	0.62[b]	0.69[a]	0.86	0.22	-	0.24[a]
	3:1 MO + FO	0.83[a]	0.78[a]	0.88	0.18	-	0.26[a]
	5:1 MO + FO	0.94[a]	0.82[a]	0.67	0.17	-	0.32[a]
Vlaeminck et al. (2008)	Algae	-	6.403	0.296[b]	0.144[b]	0.234[b]	0.023
	Algae+DHA	-	4.348	0.451[a]	0.220[a]	0.432[a]	0.023
AbuGhazaleh et al. (2002)	Control	33.52[b]	2.61[b]	9.20[a]	15.07[a]	1.64	0.09[b]
	FO	23.01[c]	4.56[a]	7.92[b]	9.12[b]	1.56	0.26[a]
	SBO	36.34[a]	4.61[a]	7.94[b]	10.71[b]	1.65	0.18[a]
	SBO + FO	31.55[b]	4.39[a]	7.28[b]	9.31[b]	1.45	0.21[a]

[a,b] and [c] showed in column were significant different ($P<0.05$)

MO = microbial oil; FO = fish oil; SBO = soy bean oil

* Jalč et al. (2009) and Vlaeminck et al. (2008) reported in mg/incubation unit

Table 2.7 Effects of oil rich in EPA and DHA supplementation on ruminal hydrogenation (cont.).

References	Treatment	Fatty acid profiles in Rumen*				
		C18:0	TVA	C18:1	C18:2	C18:3
(AbuGhazaleh and Jenkins, 2004)	Control	20.6[a]	6.8[c]	5.6[c]	4.8[c]	0.2
	5 mg DHA	10.4[b]	11.0[a]	7.8[b]	5.9[c]	0.2
	10 mg DHA	10.3[b]	9.4[ab]	7.9[b]	7.6[b]	0.2
	15 mg DHA	10.2[b]	9.2[ab]	9.2[a]	7.3[b]	0.3
	20 mg DHA	10.3[b]	7.9[b]	8.1[b]	8.4[a]	0.3
	Control	18.7[a]	7.4[c]	6.4[b]	7.0[c]	0.4
	5 mg EPA	12.6[b]	11.3[a]	8.0[a]	7.7[c]	0.4
	10 mg EPA	11.1[b]	10.2[a]	8.8[a]	8.4[b]	0.4
	15 mg EPA	11.4[b]	9.7[b]	9.4[a]	10.0[a]	0.4

[a,b] and [c] showed in column were significant different ($P<0.05$)

Presently, pathways of EPA and DHA hydrogenation still unclear regarding an increase in *t11*-C18:1. Some reports have concluded that either EPA and DHA may inhibit group B bacteria or the functional activity in bacteria may be abnormal.

Studies on flow of fatty acids into duodenum indicated that fatty acids did not subject to complete hydrogenation since flow of *t11*-C18:1 and *c9, t11*-CLA into duodenum was increased by fish oil in combination with oil rich in C18:2n-6 when compared to control as showed in Figure 2.9.

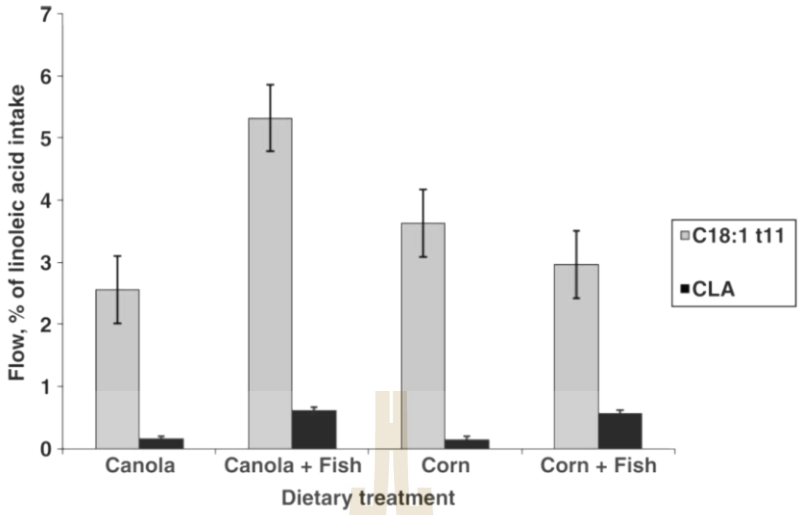

Figure 2.9 Effects of dietary fat supplementation on duodenal flow of t11-C18:1 and c9, t11-C18:2 (Duckett and Gillis , 2010).

From Figure 2.9, supplemented source of C18:2n-6 resulted in greater *t11*-C18:1 flow into duodenal when compared to no fish oil supplementation because use of fish oil improves the bio-hydrogenation process that enhances the incomplete ruminal hydrogenation. In addition, use of canola oil with fish oil also resulted in higher *t11*-C18:1 than corn oil with fish oil. However, when canola oil or corn oil alone was supplemented, *t11*-C18:1 flow in to duodenal was higher in corn oil than than canola oil. Canola oil is the source of C18:1n-9 while corn oil is the source of C18:2n-6 which has opportunity to convert to *t11*-C18:1 by isomerization and hydrogenation. For C18:1n-9 only, isomerization process will also produce *t11*-C18:1. C18:2n-6 has higher toxic than C18:1n-9 and has more influence on hydrogenation. Nevertheless, using fish oil can inhibit ruminal hydrogenation and shows the greater *t11*-C18:1 flow into duodenum.

2.9 Improving of CLA in ruminant products by EPA and DHA source

Improving the CLA content in ruminant products in the previous report found that use of fish oil could increase *t11*-C18:1 content in the rumen and flow into duodenum. The synthesis of CLA in dairy cow's milk starts from the absorption of *t11*-C18:1 in small intestine and transports to the tissue in mammary gland and then CLA is synthesized. Dairy and beef cattle have the same enzyme to added double bond at position 9th of carbon length known as delta 9 desaturase (Griinari and Bauman, 1999).

It is possible to increase CLA content in ruminant products by supplementation of fish oil (AbuGhazaleh et al., 2002; Kitessa et al., 2001c; Donovan et al., 2000). Supplementation of fish oil with source of C18:2n-6 reduced C18:0 content by inhibiting final step of ruminal bio-hydrogenation from oil rich in EPA and DHA (Kitessa et al., 2001a) but increased *t11*-C18:1. Supplementation of fish oil in combination with oil rich in C18:2n-6 increased *t11*-C18:1 and CLA content in ruminant product (Whitlock et al., 2002) as showed in Table 2.8.

Supplementation of soybean oil, fish oil and combination oil increased CLA and *t11*-C18:1 content in milk (Table 2.8) but decreased C18:0 content in milk. The decreasing C18:0 in ruminant product produces healthy food for consumer. Although beef and dairy cattle are different in fat deposition but the main of differentiation is *de novo* synthesis. The fatty acids from diets are similar. The CLA content in ruminant product depends on *t11*-C18:1 content in the rumen and expression of delta 9 desaturase enzyme.

Table 2.8 Effect of fish oil in combination with in oil rich in C18:2n-6 on fatty acid profile in milk of dairy cows.

Reference	Treatment	Fatty acids profiles (%)					
		C18:0	**C18:1**	**C18:2**	**C18:3**	**TVA**	***c9t11***
AbuGhazaleh et al., 2002	Control	10.44[b]	16.12[b]	2.61[c]	0.54[b]	1.02[b]	0.40[b]
	FO	8.11[b]	15.08[b]	2.20[d]	0.85[a]	2.34[a]	0.88[a]
	ESB	12.14[a]	18.85[a]	4.52[a]	0.87[a]	2.41[a]	0.87[a]
	FO+ESB	9.94[b]	18.15[a]	3.49[b]	0.87[a]	2.06[a]	0.80[a]

[a,b,c] and [d] Showed in column were significant different (P<0.05)

ND = Not detected NR= Not Reported

FO=Fish oil ESB= Extruded Soybean oil

2.10 References

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CHAPTER III

RUMINAL BIO-HYDROGENATION AND FERMENTATION IN RESPONSE TO OIL RICH IN OMEGA-3 FATTY ACIDS ADDITION TO FISTULATED CATTLE'S DIETS

3.1 Abstract

The aim of this experiment was to study the effects of oil rich in omega-3 FAs supplementation on ruminal bio-hydrogenation and fermentation in fistulated cattle. Four fistulated cattle were assigned into 4 dietary treatments in a 4 ×4 Latin square design. All cattle were fed approximately 4 kg/d of 14% CP concentrae and 2.4 kg/d of rice straw. Treatments were: 1) control; 2) supplemented with linseed oil at 3% of feed dry matter; 3) supplemented with linseed oil and fish oil 1:1 w/w at 3% of feed dry matter; 4) supplemented with calcium salt of linseed oil at 3% of feed dry matter. Each period in the Latin square design lasted 21 d, with the first 7 d for adaptation. The results found that supplementation of linseed oil with fish oil reduced acetic acid content in the rumen fluid at 4 and 6 h after feeding ($P<0.05$) However, no difference was found on pH, DMD, CPD, NDFD, ADFD, propionate and butyrate proportion.

3.2 Introduction

The products from ruminant contain high amount of saturated fatty acids (SFA)

because the process of bio-hydrogenation occurs in the rumen due to microorganisms that converted unsaturated fatty acids (UFA) into SFAs (Scollan et al., 2001).Occurrence of bio hydrogenation is from that the animal receives fat or oil containing UFA affecting to the microbe's cell membrane. The microbes try to protect themselves from UFAs by adding H-atom to UFAs and change the structure of UFA to SFA. (Jenkins, 1993)

SFA in ruminant products are generally considered to have negative effects on human health. The effect of SFA on the relative proportions of high and low density lipoprotein cholesterol results in coronary heart disease (CHD) (Hu et al., 2001; WHO, 2003). Now healthy foods are interesting among consumers and there are various methods to solve these problems particularly decrease in the ratio of omega 6 to omega3, increase the proportion of C20:5n-3 and C22:6n-3 in the products. Nutritionists are important roles to improve theses fatty acids with no detrimental effect on animal performance.

To improve omega-3 FA content, supplementation of oil rich in omega 3 in feed can accumulate omega-3 FAs in beef or milk. Because the cattle cannot synthesize omega-3 FAs by themselves, they must receive form feed. Linseed oil contains the essential C18:3n-3, which the body converts into C20:5n-3 and C22:6n-3, the n-3 fatty acids found in fish oil. 20:5n-3 and 22:6n-3 usually from fish oil, has been shown to reduce inflammation and help to prevent certain chronic diseases, such as heart disease and arthritis. However, when the cattle receive omega-3 FAs the microbe in the rumen will change the profile of fat, so called bio-hydrogenation process. The process can shift by using seed oil or Rumen protected fat or combination of oils to inhibit this process and pass to lower gut and synthesize omega-3 fatty acid family in the tissues. The fermentation in the rumen is important when the cattle are

supplemented with oils because it will affect animal performance. Thus, this study was conducted to evaluate the effects of oil rich in omega-3 FAs supplementation on ruminal fermentation in fistulated cattle.

3.3 Materials and Methods

3.3.1 Animals and Feeding

All experiment procedures were conducted following the Ethical Principles and Guidelines for the Use of Animal issued by National Research Council of Thailand. Four fistulated cattle were assigned in 4 treatments in a 4×4 Latin square design. All cattle were fed approximately 4 kg/d of 14% CP concentrate and 2.4 kg/d of rice straw. Treatments were: 1) Control; 2) supplemented with linseed oil (LSO) at 3% of feed dry matter (DM); 3) supplemented with linseed oil (LSO) and fish oil (FO) 1:1 w/w at 3% of feed DM; 4) supplemented with calcium salt of linseed oil (Ca-LSO) at 3% of feed DM. Ingredients of concentrate and chemical composition of concentrate and rice straw used in the experiment are presented in Table 3.1 while the fatty acid composition of feed and oils used in the present study are presented in Table 3.2. All cattle also had free access to clean water and were individually housed in a free-stall unit and individually fed according to treatments. The experiment lasted for 84 days (4 periods) with 21 d in each period, the first 7 d of each period for adaptation to diets followed by 14 d for ruminal sample collection and *in sacco* disappearance trial.

3.3.2 Preparing Calcium Soap of Linseed Oil

Calcium soap of LSO was prepared by precipitation method (Garg, 1998) with minor modifications. The exact procedure used to prepare calcium soap is as follows.

1. Hundred grams of acid oil was mixed to 1 L of water and stirred vigorously for 5 minutes.

2. Two hundred ml of 11%NaOH was added.

3. The contents were heated and stirred until the fatty acids were dissolved completely.

4. While hot, the resulting blend was slowly added with 200 ml of 20% $CaCl_2$ solution.

5. The calcium soap formed was separated and washed with tap water.

6. Excess water was removed by squeezing the calcium soap through muslin cloth.

7. Finally, the calcium soap was air dried in a dark room and stored at subzero temperature until used for feeding.

3.3.3 Sample Collection

To evaluate fatty acids profile in rumen content and ruminal fermentation, on the last day of each experimental period (d 21), Samples of ruminal contents were collected on d 21 of each period at 0, 2, 4 and 6 h after the morning feeding. Ruminal contents (approximately 450 g of whole ruminal contents) were removed by hand from four different locations in the rumen and mixed. Additional ruminal contents were taken and squeezed through four layers of cheesecloth and 100 ml of ruminal fluid was added to each sample. One portion of rumen fluid was immediately analyzed for pH (pH meter model UB-5, Denver Instrument, Germany). Ruminal samples were then placed into plastic bags and stored on ice until processing in the laboratory. Every sample was mixed one more time by hand, subsampled (approximately 200 g), and frozen (-20°C).

3.3.4 Laboratory Analyses

3.3.4.1 Feed chemical composition analysis

Feeds offered were weighed daily to calculate dry matter intakes (DMI). Samples were taken on 2 consecutive days weekly and dried at 60°C for 48 hours and at the end of the experiment, feed samples were pooled to make representative samples for proximate and detergent analyses. Samples were ground through 1 mm screen and analyzed for chemical composition. Dry matter (DM) was determined by hot air oven at 60°C for 48 h while CP was analyzed by Kjeldahl method (AOAC, 1995). Ether extract was determined by using petroleum ether in a Soxtec System (AOAC, 1995). Fiber fraction, neutral detergent fiber and acid detergent fiber were determined using the method described by Van Soest et al. (1991), adapted for Fiber Analyzer. Ash content was determined by ashing in a muffle furnace at 600°C for 3 h. The chemical analysis was expressed on the basis of the final DM.

3.3.4.2 Fatty acids in feed analysis

Fatty acids composition of concentrates, rice straw, linseed oil fish oil and Ca-linseed oil were extracted using a modified of the method used by Folch et al. (1957) and Metcalfe et al. (1966). Fifteen gram of each sample was homogenized for 2 min with 90 ml of chloroform-methanol (2:1) (Nissel AM-8 Homogenizer, Nihonseikikaisha, LTD., Japan). Each sample was then further homogenized for 2 min with 30 ml of chloroform. Then, each sample was separated in separating funnel and 30 ml of deionized water and 5 ml of 0.58% NaCl was added. The under layer of fatty acid methyl esters (FAME) was removed and placed in screw-cap test tube and stored at -20ºC until methylation. Fatty acid methyl esters (FAME) were prepared by the procedure described by Ostrowska et al. (2000). The procedure involved placing approximately 30 mg of the extracted oil into a 15 ml reaction tube

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

3.3.4.3 Fatty acids in rumen analysis

Rumen fluid of each period was extracted for fatty acid using a modified method used by Romeu-Nadal et al. (2004). From a well-mixed aliquot of Rumen fluid, 20 ml was placed in 50 ml centrifuge tubes. Then added 27 ml of dichloromethane - methanol solution (2:1, v/v) to each tube. The mixture was shaken mechanically for 15 min and centrifuged at 2500 rpm for 8 min at 4°C. Approximately 8 ml of distilled water was pipette into each tube and, after shaking for a further 15 min, the sample was, again centrifuged at 2500 rpm for 8 min at 4°C. As much of the upper aqueous fraction as possible was carefully removed with a pipette. The organic layer was washed with 8 ml of a saturated solution of the sodium chloride, and finally

mixed mechanically for 15 min and centrifuged for 8 min at 2500 rpm at 4°C. Again, the upper aqueous fraction was carefully removed with a pipette. The organic fraction was carefully transferred to a separating funnel and filtered through 1PS paper (Whatman, Maidstone, UK) containing anhydrous sodium sulfate, and 3-5 ml of dichloromethane was passed through the filter. The fat solution was taken in pre-weighed conical flask. Finally the extract was concentrated by removing dichloromethane in a rotatory evaporator and dried under a gentle stream of nitrogen. The weight difference of the conical flask before/after was assumed to be fat. The fat was stored at -20°C and redissolved in dichloromethane (3%, w/v) immediately analyzing by gas chromatography (GC) (7890A GC System, Agilent Technology, USA), equipped with a 100 m x 0.25 mm x 0.2 μm film fused silica capillary column (SP1233, Supelco Inc, Bellefonte, PA, USA). Injector and detector temperatures were 250°C. The column temperature was kept at 70°C for 4 min, then increased at 13 °C/min to 175°C and held at 175°C for 27 min, then increased at 4°C/min to 215°C and held at 215°C for 17 min, then increased at 4°C/min to 240°C and held at 240°C for 10 min.

3.3.4.4 Volatile fatty acids and Ammonia nitrogen analysis

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 2N H_2SO_4, 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. The supernatant was analyzed for ammonia N by Kjeldahl and concentrations of VFA were determined by GC (Hewlett Packard GC system HP6890 A; Hewlett Packard, Avondale, PA) equipped with a 30 m x 0.32 mm x 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.

3.3.4.5 Degradability Determination of DM, CP, NDF and ADF

Concentrate and rice straw were ground through a 2 mm screen for *in sacco* ruminal disappearance determination. Approximately 5 g of 2 mm ground samples e were placed into 8 x 11 cm nylon bags with 47 μm pore size. Samples were suspended in the rumen of each fistulated cattle for 0 (pre feeding), 2, 4, 6, 12, 24, 48 (concentrate) and 72 h (rice straw), and were then removed and washed in water and then dried at 65°C for 48 h. After weighing each bag individually, the residues were subjected to DM determination. The contents of the bags were then assayed for CP NDF and ADF content (CPD, NDFD and ADFD). The NDF and ADF analyses were conducted sequentially using an ANKOM200 Fiber analyzer unit based on the procedure described by Van Soest et al. (1991). Sodium sulfite (10 g/l NDF solution) and heat-stable bacterial amylase (2 ml/l NDF solution) were used in the analysis of NDF and ADF. The degradability value was obtained by subjecting nutrient losses at arbitrary of time using NEWAY EXCEL (Chen, 1996).

3.3.5 Statistical Analysis

All data were analyzed as a 4 x 4 Latin squares design using ANOVA procedure of SAS (SAS, 1996). Significant differences among treatment were assessed by Duncan's new multiple range test. A significant level of $P<0.05$ was used (Steel and Torrie, 1980).

3.3.6 Experimental Site

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

3.3.7 Duration

The duration of the present experiment was from January to April 2015.

3.4 Result

3.4.1 Chemical composition of experimental diet

The ingredients, chemical and FA compositions of the individual feed used in the current study are presented in Table 3.1 and 3.2. Fat content in Ca-LSO was low (70.4%) which was due to the preparation process (Table 3.1). LSO had the highest proportion of C18:3n-3 (53.67 g/100 g fat) while FO had the highest proportion of C22:6n-3 (37.25 g/100 g fat). In the concentrate, C18:1n-9 (29.42 g/100 g fat) and C12:0 (22.76 g/100 g fat) were the main fatty acids (FA), whereas C18:3n-3, C16:0 and C18:1n-9 were the main FA in Ca-LSO (35.94, 31.32 and 20.81 g/100 g fat respectively). The main FA in rice straw was C16:0 (45.67 g/100 g fat) (Table 3.2)

Table 3.1 Chemical composition of the experimental diets**.**

Items	Concentrate[1]	LSO	FO	Ca-LSO	Rice straw
Dry matter	**93.4**			**96.3**	**92.2**
	----------% of DM----------				
Ash	8.7			29.6	15.3
Crude protein	12.6				2.4
Ether extract	4.0	100	100	70.4	1.3
Crude fiber	17.1				34.9
Neutral detergent fiber	39.3				72.4
Acid detergent fiber	16.5				50.3
Acid detergent lignin	3.4				10.5

LSO = linseed oil; FO = fish oil; Ca-LSO = calcium salt of linseed oil

[1]kg/100 kg concentrate: 30 dried cassava chip, 4 ground corn, 10 rice bran, 25 palm meal, 15 coconut meal, 6 dried distillers grains with solubles, 0.5 sodium bicarbonate, 6 molasses, 1 dicalciumphosphate (16%P), 1.5 urea, 0.5 salt and 0.5 premix. Premix: provided per kg of concentrate including vitamin A, 5,000 IU; vitamin D3, 2,200 IU; vitamin E, 15 IU; Ca, 8.5 g; P, 6 g; K, 9.5 g; Mg, 2.4 g; Na, 2.1 g; Cl, 3.4 g; S, 3.2 g; Co, 0.16 mg; Cu, 100 mg; I, 1.3 mg; Mn, 64 mg; Zn, 64 mg; Fe, 64 mg; Se, 0.45 mg.

Table 3.2 Fatty acid compositions (g/100 g of total fatty acids) of concentrate, rice straw and oils used in the experiment.

Fatty acids	Concentrate	Rice straw	Linseed oil	Fish oil	Ca-LSO
C12:0	22.76	6.44	2.91	2.18	ND
C14:0	7.84	8.15	0.35	4.37	ND
C16:0	16.76	45.67	22.76	27.84	31.32
C18:0	2.49	0.12	0.21	6.18	1.45
C18:1 n9	29.42	24.92	14.90	12.43	20.81
C18:2 n6	17.07	11.75	2.72	1.68	4.19
C18:3 n3	0.29	ND	53.67	0.91	35.94
C22:6 n3	ND	ND	ND	37.25	ND
Others	3.38	2.85	2.48	5.16	6.29

Ca-LSO = calcium salt of linseed oil; ND = Not detected.;

Others = C8:0 + C15:0 + C20:1 + C21:0 + C23:0

3.4.2 Intake of main components and major fatty acids

The present experiment was designed to limit concentrate and rice straw consumptions and to control the ratio of concentrate to rice straw at 60:40 (DM basis). Control cattle ate significantly less ($p<0.05$) DM and fat than other cattle due to no fat supplement while Ca-LSO cattle consumed significantly higher DM than others reflecting the balance of fat intake among treatments. Since Ca-LSO contained 70.4% fat, to obtain approximate 180 g fat from Ca-LSO, 260 g Ca-LSO was added to the diet (Table 3.3). Total fat intake was similar among oil supplemented cattle and they ate significantly higher fat than the control cattle. The intake of individual FA differed due

to the composition of the various supplemental oils (Table 3.3). When individual FA intake was calculated (Table 3.3), cattle on LSO and LSO+FO diets consumed more C12:0 than those cattle on control and Ca-LSO diets. LSO cattle ate more C18:3n-3 than others. Higher C18:3n-3 intake of LSO cattle was caused by higher C18:3n-3 content in LSO. Cattle on LSO+FO diet ate more C14:0, C18:0 and C22:6n-3 than other cattle whereas cattle on Ca-LSO consumed more C16:0, C18:1n-9 and C18:2n-6 than other cattle. Higher C22:6n-3 consumption of LSO+FO was a result of higher content of C22:6n-3 in FO.

Table 3.3 DM, CP, fat and fatty acid intakes of experimental cattle.

Items	Control	LSO	LSO + FO	Ca-LSO	SEM	P-value
DM intake (kg/d)						
Concentrate	3.74	3.74	3.74	3.74	-	-
Rice straw	2.21	2.21	2.21	2.21	-	-
Oil	-	0.18	0.18	-	-	-
Ca-LSO	-	-	-	0.26	-	-
Total	5.95^{c}	6.13^{b}	6.13^{b}	6.21^{a}	0.003	0.001
CP intake (g/d)						
Concentrate	471	471	471	471	-	-
Rice straw	54	54	54	54	-	-
Total	525	525	525	525	-	-
Fat intake (g/d)						
Concentrate	150	150	150	150	-	-
Rice straw	29	29	29	29	-	-
Oil	-	180	180	-	-	-
Ca-LSO	-	-	-	183	-	-
Total	179^{b}	359^{a}	359^{a}	362^{a}	0.001	0.001

^{abc} Within a row means without a common superscript letter differ.

Table 3.3 DM, CP, fat and fatty acid intakes of experimental cattle (cont.).

Items	Control	LSO	LSO + FO	Ca-LSO	SEM	P-value
FA intake (g/d)						
C12:0	24.67^{b}	29.29^{a}	29.11^{a}	25.52^{b}	0.170	0.007
C14:0	9.71^{c}	10.48^{b}	13.08^{a}	10.03^{c}	0.063	<0.001
C16:0	26.54^{d}	56.81^{c}	60.09^{b}	68.60^{a}	0.168	<0.001
C18:0	2.58^{d}	2.94^{c}	6.81^{a}	4.58^{b}	0.019	<0.001
C18:1n9	35.29^{d}	55.75^{b}	54.15^{c}	63.88^{a}	0.240	<0.001
C18:2n6	19.92^{c}	24.11^{b}	23.43^{b}	26.11^{a}	0.134	<0.002
C18:3n3	0.30^{d}	69.87^{a}	35.68^{c}	47.69^{b}	0.035	<0.001
C22:6n3	-	-	24.14	-	-	-
Others	4.05^{d}	7.40^{c}	9.13^{b}	12.47^{a}	0.029	<0.001
Total	127^{b}	257^{a}	257^{a}	259^{a}	18.67	<0.001

SEM = standard error of the mean; Others = C8:0 + C15:0 + C20:1 + C21:0 + C23:0

^{abc} Within a row means without a common superscript letter differ

3.4.3 Fatty acid profile in rumen content

At 2, 4 and 6 h after feeding, ruminal content from control, LSO, and Ca-LSO cattle contained higher concentration of ruminal C18:0 than that from LSO+FO cattle (Table 3.4). Increases in C18:0 reflecting ruminal biohydrogenation of C18:1n-9, C18:2n-6 and C18:3n-3 in control, LSO and Ca-LSO diets. At all h after feeding, ruminal content from LSO+FO cattle had higher C14:0, *t11*-C18:1 and C22:6n-3 than those from other cattle (Table 3.4). Supplementation of LSO in

combination with FO greatly increased *t11*-C18:1 at 6 h after feeding in the current study.

Table 3.4 Effect of linseed oil, fish oil and Ca-Linseed oil supplementation on fatty acid profile in fistulated cattle (g/100g fatty acids)**.**

Fatty acids	Control	LSO	LSO + FO	Ca-LSO	SEM	P-value
Pre - feeding						
C12:0	6.94	7.86	7.58	6.82	0.534	0.745
C14:0	4.88	5.74	5.94	3.95	0.292	0.175
C16:0	24.89	23.33	24.02	24.18	0.629	0.200
C18:0	57.83	57.75	57.42	59.94	0.654	0.298
C18:1n-9	3.85	3.62	3.66	3.80	0.066	0.586
C18:2n-6	1.61	1.69	1.37	1.30	0.146	0.590

LSO = linseed oil; FO = fish oil; Ca-LSO = calcium salt of linseed oil;

SEM = standard error of the mean

Table 3.4 Effect of linseed oil, fish oil and Ca-Linseed oil supplementation on fatty acid profile in fistulated cattle (g/100g fatty acids) (Cont.).

Fatty acids	Control	LSO	LSO + FO	Ca-LSO	SEM	P-value
2 h after feeding						
C12:0	7.57	10.35	12.63	9.65	0.628	0.120
C14:0	3.62^{c}	5.96^{b}	10.33^{a}	5.75^{b}	0.282	0.002
C16:0	31.08^{a}	20.04^{b}	38.89^{a}	21.52^{b}	1.303	0.011
C18:0	52.14^{a}	48.66^{a}	9.83^{b}	47.21^{a}	2.231	0.004
C18:1n-9	1.84	4.53	2.35	2.38	0.611	0.394
C18:2n-6	2.17	2.37	1.57	2.86	0.258	0.357
C18:3n-3	0.43^{c}	2.01^{a}	1.24^{b}	1.51^{ab}	0.088	0.008
t11-C18:1	0.55^{d}	5.72^{c}	14.71^{a}	8.88^{b}	0.460	0.010
c9,t11-C18:2	0.58	0.10	0.48	0.17	0.081	0.176
c9,c11-C18:2	0.00	0.08	0.31	0.07	0.040	0.114
t10,c12-C18:2	0.00	0.15	0.00	0.00	0.044	0.847
C22:6n-3	0.00^{b}	0.00^{b}	7.64^{a}	0.00^{b}	0.376	0.002

LSO = linseed oil; FO = fish oil; Ca-LSO = calcium salt of linseed oil

SEM = standard error of the mean

^{abcd} Within a row means without a common superscript letter differ.

Table 3.4 Effect of linseed oil, fish oil and Ca-Linseed oil supplementation on fatty acid profile in fistulated cattle (g/100g fatty acids) (cont.)**.**

Fatty acids	Control	LSO	LSO + FO	Ca-LSO	SEM	P-value
4 h after feeding						
C12:0	3.58^{c}	7.02^{bc}	11.33^{b}	16.25^{a}	0.717	0.007
C14:0	5.74^{bc}	4.75^{c}	10.19^{a}	6.60^{b}	0.203	0.001
C16:0	41.55^{a}	19.17^{b}	41.13^{a}	19.04^{b}	0.732	0.001
C18:0	44.23^{b}	53.32^{b}	11.47^{c}	44.08^{a}	0.861	0.001
C18:1n-9	0.67^{b}	6.07^{a}	6.06^{a}	7.78^{a}	0.302	0.002
C18:2n-6	3.24^{a}	2.64^{ab}	1.47^{b}	2.88^{a}	0.176	0.053
C18:3n-3	0.12^{b}	1.76^{a}	2.22^{a}	1.75^{a}	0.085	0.002
t11-C18:1	0.84^{c}	4.78^{b}	11.29^{a}	1.59^{c}	0.240	0.001
c9,t11-C18:2	0.00	0.07	0.09	0.00	0.031	0.517
c9,c11-C18:2	0.00	0.07	0.12	0.00	0.035	0.512
t10,c12-C18:2	0.00^{b}	0.39^{a}	0.00^{b}	0.00^{b}	0.012	0.001
t9,t11-C18:2	0.00	0.00	0.14	0.00	0.040	0.478
C22:6n-3	0.00^{b}	0.00^{b}	4.51^{a}	0.00^{b}	0.367	0.016

LSO = linseed oil; FO = fish oil; Ca-LSO = calcium salt of linseed oil; SEM = standard error of the mean

^{abcd} Within a row means without a common superscript letter differ.

Table 3.4 Effect of linseed oil, fish oil and Ca-Linseed oil supplementation on fatty acid profile in fistulated cattle (g/100g fatty acids) (cont.)**.**

Fatty acids	Control	LSO	LSO + FO	Ca-LSO	SEM	P-value
6 h after feeding						
C12:0	4.36^{b}	9.01^{a}	7.23^{ab}	7.98^{a}	0.445	0. 050
C14:0	4.29	4.93	6.95	4.35	0.407	0.149
C16:0	16.65^{d}	18.68^{c}	28.14^{a}	22.02^{b}	0.340	0.001
C18:0	15.16^{c}	54.85^{a}	8.53^{d}	45.65^{b}	0.613	0.001
C18:1n-9	2.13^{b}	4.85^{ab}	6.81^{ab}	11.07^{a}	0.943	0.070
C18:2n-6	2.23^{ab}	0.00^{b}	0.93^{b}	4.30^{a}	0.463	0.067
C18:3n-3	0.00^{b}	0.42^{ab}	0.28^{ab}	1.03^{a}	0.133	0.122
t11-C18:1	55.17^{a}	7.26^{c}	39.06^{b}	3.51^{c}	0.724	0.001
c9,t11-C18:2	0.00	0.00	0.12	0.07	0.021	0.148
c9,c11-C18:2	0.00	0.00	0.12	0.00	0.021	0.152
t10,c12-C18:2	0.00	0.00	0.11	0.09	0.039	0.558
C22:6n-3	0.00^{b}	0.00^{b}	1.69^{a}	0.00^{b}	0.100	0.018

LSO = linseed oil; FO = fish oil; Ca-LSO = calcium salt of linseed oil;
SEM = standard error of the mean
^{ab} Within a row means without a common superscript letter differ.

3.4.4 Ruminal Fermentation

At 2 h after feeding, pH, NH_3-N, acetate, propionate, butyrate and acetate: propionate ratio were unaffected by dietary treatments (Table 3.5). Ruminal pH, acetate and butyrate were similar among treatments at 4 h post-feeding, however, ruminal NH_3-N of LSO+FO cattle was significantly higher ($p<0.01$) than other cattle. Propionic acid proportion of cattle on LSO+FO diet was also higher ($p<0.05$) than cattle on LSO and Ca-LSO but was similar to cattle on control diet. As a result, acetate: propionate ratio of cattle LSO+FO was lower than other cattle.

At 6 h post-feeding, dietary treatments did not affect ruminal pH, NH_3-N, acetate and butyrate concentrations; however, LSO+FO significantly increased ($p<0.05$) molar proportion of propionate and significantly reduced ($p<0.05$) acetate: propionate ratio (Table 3.5).

Table 3.5 Effect of linseed oil, fish oil and Ca-Linseed oil supplementation on pH, ammonia nitrogen (mg/100 ml) and volatile fatty acids (mol/100 mol) in fistulated cattle**.**

Item	Control	LSO	LSO + FO	Ca-LSO	SEM	P-value
Pre-feeding						
pH	6.35	6.36	6.35	6.34	0.032	0.833
NH_3N	8.12	7.48	8.92	7.63	0.673	0.321
Acetic acid	72.43	73.48	71.35	72.68	1.512	0.723
Propionic acid	17.53	16.63	17.51	17.17	0.863	0.704
Butyric acid	10.04	9.89	11.14	10.15	0.732	0.783
A:P ratio	4.13	4.41	4.07	4.23	0.482	0.642
2 h after feeding						
pH	6.48	6.43	6.40	6.41	0.023	0.251
NH_3N	12.61	11.49	11.13	13.11	0.634	0.512
Acetic acid	70.24	71.60	68.99	71.99	0.488	0.212
Propionic acid	17.97	18.52	19.54	17.84	0.414	0.412
Butyric acid	11.79	9.87	11.46	10.17	0.135	0.072
A:P ratio	3.91	3.92	3.59	4.09	0.098	0.306

LSO = linseed oil; FO = fish oil; Ca-LSO = calcium salt of linseed oil; A:P ratio = acetate: propionate ratio; SEM = standard error of the mean

[ab]Within a row means without a common superscript letter differ

Table 3.5 Effect of linseed oil, fish oil and Ca-Linseed oil supplementation on pH, ammonia nitrogen (mg/100 ml) and volatile fatty acids (mol/100mol) in fistulated cattle (cont.)**.**

Item	Control	LSO	LSO + FO	Ca-LSO	SEM	P-value
4 h after feeding						
pH	6.03	6.03	6.06	6.04	0.032	0.293
NH_3N	4.67^{b}	4.52^{b}	8.01^{a}	4.80^{b}	0.091	0.001
Acetic acid	72.83	72.33	67.86	72.56	0.372	0.055
Propionic acid	16.28^{a}	17.77^{b}	19.53^{a}	16.96^{b}	0.101	0.018
Butyric acid	10.89	9.89	12.61	10.49	0.359	0.159
A:P ratio	4.47^{a}	4.14^{b}	3.48^{c}	4.34^{a}	0.013	0.002
6 h after feeding						
pH	6.31	6.33	6.32	6.30	0.064	0.363
NH_3N	4.98	3.61	4.69	5.02	0.402	0.473
Acetic acid	72.94	73.52	68.93	73.61	0.363	0.052
Propionic acid	17.01^{b}	16.71^{b}	18.72^{a}	16.25^{b}	0.102	0.017
Butyric acid	10.05	9.78	12.35	10.14	0.364	0.170
A:P ratio	4.28^{a}	4.48^{a}	3.69^{b}	4.62^{a}	0.030	0.012

LSO = linseed oil; FO = fish oil; Ca-LSO = calcium salt of linseed oil; A:P ratio = acetate: propionate ratio; SEM = standard error of the mean

^{ab} Within a row means without a common superscript letter differ

3.4.5 Degradability of DM, CP, NDF and ADF

Feeding LSO, LSO+FO and Ca-LSO had no effects on dry matter degradability (DMD) of concentrate and rice straw (P>0.05) at 0.02, 0.05 and 0.08

fraction/h outflow rate when compare to control diet (Table 3.6). The readily soluble fraction (*a*) of concentrate of control, LSO, LSO+FO and Ca-LSO cattle were 28.57, 28.40, 27.65 and 30.40 % respectively which were not significantly different. However, *a* fraction of concentrate was higher than that of rice straw (14- 15 % readily soluble fraction (*a*). The potential degradable fraction (*b*) of concentrate and rice straw was unaffected (P>0.05) by dietary treatments (Table 3.6).

For crude protein degradability (CPD) of concentrate, fat supplements had no effects on readily soluble fraction (*a*), potential degradable fraction (*b*) and effective degradability at out flow rate 0.02, 0.05 and 0.08 fraction/h when compare to control diet (Table 3.7). Neutral detergent fiber degradability (NDFD) and acid detergent fiber degradability of rice straw (ADFD) showed the same result (Table 3.8), with NDFD of rice straw at out flowrate 0.05 fraction/h of 0.28, 0.27, 0.29 and 0.28 for control, LSO, LSO+FO and Ca-LSO respectively and ADFD of rice straw at out flowrate 0.05 fraction/h of 0.21, 0.21, 0.20 and 0.22 respectively (Table 3.8).

Table 3.6 Effect of linseed oil, fish oil and Ca-Linseed oil supplementation on dry matter degradability (DMD) of concentrate and rice straw in fistulated cattle.

Item	Control	LSO	LSO+FO	Ca-LSO	SEM	P-Value
Dry matter degradability of concentrate						
a	28.57	28.40	27.65	30.40	0.967	0.723
b	46.77	45.57	46.85	46.60	1.367	0.976
a + *b*	75.33	74.30	74.50	77.00	1.443	0.863
c, per h	0.086	0.102	0.094	0.072	0.011	0.721
dg, 0.02/h	0.66	0.65	0.66	0.67	0.010	0.978
dg, 0.05/h	0.58	0.58	0.58	0.58	0.009	0.834
dg, 0.08/h	0.53	0.53	0.53	0.52	0.009	0.970
Dry matter degradability of rice straw						
a	14.73	14.20	15.27	14.63	1.287	0.416
b	58.23	58.42	58.40	57.57	1.653	0.453
a + *b*	72.96	72.62	73.67	72.20	1.588	0.475
c, per h	0.021	0.020	0.021	0.023	0.003	0.159
dg, 0.02/h	0.38	0.38	0.37	0.37	0.010	0.745
dg, 0.05/h	0.26	0.24	0.26	0.24	0.007	0.624
dg, 0.08/h	0.22	0.20	0.21	0.20	0.008	0.826

LSO = linseed oil; FO = fish oil; Ca-LSO = calcium salt of linseed oil

SEM = standard error of the mean;

a = the intercept of the degradation curve at time zero;

b = the potential degradability of the component;

c = the rate constant for the degradation of 'b'.

Table 3.7 Effect of linseed oil, fish oil and Ca-Linseed oil supplementation on crude protein degradability (CPD) of concentrate in fistulated cattle.

Item	Control	LSO	LSO+FO	Ca-LSO	SEM	P-Value
Crude protein degradability of concentrate						
a	17.73	17.20	16.27	15.63	1.287	0.416
b	58.23	58.42	58.40	60.57	1.654	0.766
a + *b*	75.86	75.62	74.67	76.20	1.588	0.742
c, per h	0.021	0.020	0.024	0.020	0.004	0.159
dg, 0.02/h	0.38	0.38	0.37	0.37	0.010	0.745
dg, 0.05/h	0.26	0.24	0.25	0.24	0.007	0.624
dg, 0.08/h	0.22	0.20	0.21	0.20	0.008	0.827

LSO = linseed oil; FO = fish oil; Ca-LSO = calcium salt of linseed oil

SEM = standard error of the mean;

a = the intercept of the degradation curve at time zero;

b = the potential degradability of the component;

c = the rate constant for the degradation of 'b'.

Table 3.8 Effect of linseed oil, fish oil and Ca-Linseed oil supplementation on neutral detergent fiber degradability (NDFD) and acid detergent fiber (ADFD) of rice straw in fistulated cattle.

Item	Control	LSO	LSO+FO	Ca-LSO	SEM	P-Value
Neutral detergent fiber degradability of rice straw						
a	13.51	14.05	13.80	13.23	0.358	0.723
b	64.20	64.80	64.55	64.65	0.980	0.989
a + *b*	77.71	78.85	78.30	77.88	0.628	0.811
c, per h	0.015	0.013	0.015	0.018	0.002	0.109
dg, 0.02/h	0.41	0.40	0.41	0.41	0.011	0.918
dg, 0.05/h	0.28	0.27	0.29	0.28	0.008	0.343
dg, 0.08/h	0.24	0.23	0.25	0.23	0.007	0.333
Acid detergent fiber degradability of rice straw						
a	5.35	5.15	5.30	5.55	0.185	0.262
b	54.35	54.35	53.55	52.75	0.873	0.778
a + *b*	59.70	59.50	58.85	58.30	1.025	0.961
c, per h	0.013	0.016	0.014	0.015	0.002	0.351
dg, 0.02/h	0.34	0.34	0.34	0.35	0.003	0.535
dg, 0.05/h	0.21	0.21	0.20	0.22	0.007	0.808
dg, 0.08/h	0.17	0.16	0.16	0.16	0.008	0.986

LSO = linseed oil; FO = fish oil; Ca-LSO = calcium salt of linseed oil SEM = standard error of the mean; *a* = the intercept of the degradation curve at time zero; *b* = the potential degradability of the component; *c* = the rate constant for the degradation of 'b'.

3.5 Discussion

3.5.1 Fatty Acid Profile in Ruminal Content

When LSO was added in combination with FO in the present study, ruminal C18:0 content was significantly reduced while C16:0 and *t*rans-11 C18:1 contents were significantly increased which were similar to Doreau and Chilliard (1997), Kitessa et al. (2001a, b) and Loor et al. (2005). It is widely known that ruminal microorganism eliminates unsaturated 18 carbon fatty acids such as C18:2n-6 and C18:3n-3 through bio-hydrogenation. Fish oil addition resulted in decreased C18:0 meanwhile increased *trans*-C18:1 in the rumen (Jenkins et al., 2008). In the bio-hydrogenation process, two groups of bacteria are in operate. One group can hydrogenate 18 carbons unsaturated fatty acids into C18:0, however, FO addition will shift these processes by inhibition of bacterial conversion unsaturated fatty acids to saturated fatty acid. (Jenkins et al., 2008). Loor et al. (2005) also observed an increase in C16:0 when FO was added to the diet compared with sunflower oil (SFO) and LSO. Similarly, Kitessa et al. (2001a) supplemented protected tuna oil and tuna oil found an increase in C16:0 concentration in the rumen, abomasum and cholesterol plasma lipid, however, no significant difference in free fatty acid, phospholipids and triacylglycerol in plasma lipid was observed. Fish oil contains C20:5n-3 and C22:6n-3, adding C22:6n-3 to the rumen alters a variety of fatty acids. Feeding C22 polyenoic fatty acids sharply increased the proportion of *t11*-C18:1 in the rumen. AbuGhazaleh et al. (2002) previously reported that *t11*-C18:1 accumulated in all cultures over time with higher accumulations associated with higher levels of C22:6n-3 supplementation. In addition, AbuGhazaleh and Jenkins (2004) also reported a positive correlation between C22:6n-3 supplementation and *trans-11* C18:1. However, they were not able to identify the

source of *t11*-C18:1. *t11*-C18:1 was the major source to synthesize *c9,t11*-C18:2 (CLA) in animal tissue. C22:6n-3 supplementation increased *t11*-C18:1 isomer and inhibited the bio-hydrogenation of oleic and linoleic acids with 1, 2, 3, or 4% C22:6n-3 supplementation (AbuGhazaleh and Jenkins, 2004). They also reported decreased C18:0 in all C22:6n-3 cultures by 24 h. Doreau and Chilliard (1997) previously reported total C18:1 fatty acids increased from 13 to 36% and C18:0 decreased from 54 to 7.9% in duodenal contents when fish oil was added to the rumen. Moreover, Donovan et. al. (2000) supplemented fish oil at 0, 1, 2, and 3% in lactating dairy cows and reported increases in total C18:1 isomer, *trans-11* C18:1, and *c9,t11*-C18:2 as C22:6n-3 levels rose to 2% then stabilized through 3% C22:6n-3. They also reported an inverse response of C18:0. These results indicate increased levels of *trans*-18:1, particularly *t11*-C18:1, are consistent across many studies with C22:6n-3 supplementation while effects on C18:0, C18:2n-6, and C18:3n-3 are more inconsistent. Inclusion of C22:6n-3 in the diet of ruminant is considered a desirable strategy to increase the content of these nutritionally important fatty acids in meat and milk. However, these polyenoic fatty acids may affect efficiency of the rumen microbial system (Kitessa et al., 2001b). In cattle, LSO in the diet increased *t11*-C18:1, *c9,t11*- C18:2, and C18:3n-3 at the duodenum (Doreau et al., 2009b), whereas FO resulted in greater flows of *t11*-C18:1, C20:5n- 3, and C22:6n-3 (Shingfield et al., 2003; Kim et al., 2008; Lee et al., 2008). Both C18:2n-6 and C18:3n-3 decreased the bio-hydrogenation of C22:6n-3 and increased *t11*-C18:1 accumulation *in vitro* (Chow et al., 2004; Wąsowska et al., 2006; Boeckaert et al., 2007), suggesting that a mixture of LSO and FO would result in greater ruminal escape of C18:3n-3 and C22:6n-3 and may increase the availability of C18:1n-9, C18:2n-6 and CLA bio-hydrogenation intermediates for absorption. Shingfield et al. (2011) also reported that inclusion of

LSO in the diet increased C16:0, C18:0, *trans*-C18:1, CLA, and C18:3n-3 at the duodenum, whereas FO increased the flow of C14:0, C16:0, total C16:1, *trans*-C18:1, but decreased C18:0 at the duodenum.

3.5.2 Ruminal Fermentation

Ruminal pH was unaffected by dietary treatments (Table 3.5). Similar results have been reported (Fievez et al., 2003; Beauchemin et al., .(2007 Doreau et al. (2009a) also demonstrated that linseed oil did not affect the rumen fermentation pattern .In addition, Harvatine and Allen (2006) suggested that the use of saturated and unsaturated lipids had a minor or insignificant effect on ruminal fermentation parameters. However, Messana et al. (2013) reported that in animals receiving the highest dietary lipid content (60 g/kg), rumen pH decreased quadratically ($p<0.001$) with an increase in the lipid content. Shingfield et al. (2003) found a significant decrease in pH when fish oil was supplemented because of reduction in DMI related to decreased pH. However, in all treatments of the present study, the ruminal pH remained between 6.03 and 6.54; thus, the pH did not have a significant effect on ruminal fermentation. The lowest pH was observed at 4 h after feeding (6.03-6.06) while at other h after feeding, the pH was higher than 6.30 in all treatments.

Ammonia nitrogen has been reported to vary due to many factors such as the level of feeding, degradability of protein in the rumen and feeding frequency. Neveu et al. (2014). Ammonia nitrogen uses for the efficiency of amino acid synthesis and microbial growth, and was not affected by oil supplementation. The present study found that LSO+FO significantly increased ruminal ammonia nitrogen content at later h after feeding (4 h after feeding) (Table 3.5). Similar previous result was also reported that fish oil supplementation increased NH_3-N (Keady and Mayne, 1999). In addition, another study found inconsistent results, with significant increases decreases in NH_3-N

when C18:3n-3 sources was supplemented in sheep (Zhang et al., 2008).

VFA was the product produced from fermentation of bacteria in the rumen. Bergman (1990) founded that VFA was used for cattle energy up to 80%. The present study found an increase in propionate at 4 and 6 h post-feeding resulting in a decrease in acetate: propionate ratio. Shingfield et al. (2011) supplemented LSO and FO alone or as an equal mixture and reported that supplements of FO shifted rumen fermentation toward propionate at the expense of acetate with no change in molar proportions of butyrate. Earlier studies reported that FO has no major effect on fermentation characteristics in growing cattle (Lee et al., 2008; Kim et al., 2008), but enhanced the ratio of glucogenic: lipogenic precursors in the rumen of steers (Shingfield et al., 2010). It is probable that changes in rumen fermentation patterns are related to the effect of FO on nutrient digestion in the rumen and alterations in the relative abundance of specific microbial populations. In the present study, inclusion of LSO in the diet had no effect on rumen fermentation patterns, consistent with previous reports in cattle (Doreau et al., 2009a). In other experiments, LSO (Ueda et al., 2003) or linseed (Gonthier et al., 2004) supplying 3 to 4% of additional lipid in the diet have been shown to increase molar proportions of propionate at the expense of acetate. Given that FO altered ruminal VFA, whereas LSO had no effect, it appears that the changes in rumen fermentation to LSO+FO are due to fish oil.

It can be concluded in the present study that linseed oil in combination with fish oil significantly reduced stearic acid but significantly increased palmitic and *trans* vaccenic acid in the rumen. Linseed oil in combination with fish oil significantly increased ammonia nitrogen at later h after feeding and significantly increased the molar proportion of propionic acid but significantly reduced the acetic: propionic acid ratio.

3.5.3 Degradability of DM, CP, NDF and ADF

DMD, CPD, NDFD and ADFD were not affected by oil supplementation in the current study. This is in agreement with Toral et al. (2009) who reported that addition of 3 and 10g/d in the diets of sheep had no effects on DMD CPD and NDFD when compare to control. Similar to Keady and Mayne (1999) who observed no effect of fish oil supplementation, even when a shift in the rumen fermentation pattern was observed. The consequences of oil supplementation reported elsewhere, however, include reductions, no effects or even increases in fiber degradation (Wachira et al., 2000; Sinclair et al., 2005). Vargas et al. (2011), in *in vitro* study, noted that addition of 50 g/total feed linseed oil had no effects on OM, NDF and CP disappearances. This is in agreement with Jalc et al. (2007) who did not find differences in DM or NDF degradation when the diet was supplemented with C18:1n-9, C18:2n-6 or C18:3n-3. On the contrary, Patra and Yu (2013) and Yang et al. (2009) reported that supplementation of oils rich in C18:2-n6, C18:3n-3, C20:5n-3 and C22:6n-3 could be toxic to microbial membrane in the rumen mainly in fibrolytic bacteria and had negative influence on degradability in the rumen, especially NDFD.

3.6 Conclusion

Supplementation of linseed oil in combination with fish oil at 3% of total feed DM reduced acetic acid molar proportion in the rumen fluid at 4 and 6h after feeding ($P<0.05$). However, the concentration of *t11*-C18:1 and C22:6n-3 was increased while of C18:0 decreased when compare to other treatments. All of oil supplemented diet had no effects on pH, DMD, CPD, NDFD, ADFD, propionate and butyrate proportion when compare to non-supplemented control.

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CHAPTER IV

RUMINAL BIO-HYDROGENATION AND FERMENTATION IN RESPONSE TO OIL RICH IN OMEGA-6 FATTY ACID COMBINATION WITH FISH OIL ADDITION TO FISTULATED CATTLE'S DIETS

4.1 Abstract

The objective of current experiment was to study the effects of oil rich in omega-6 FAs supplementation on ruminal bio-hydrogenation and fermentation in fistulated cattle. Four fistulated cattle were assigned in 4 treatments in a 4×4 Latin squares design. All cattle were fed approximately 4 kg/d of 14% CP concentrate and 2.4 kg/d of rice straw. Treatments were: 1) control; 2) supplemented with soybean oil at 3% of feed dry matter; 3) supplemented with fish oil at 3% of feed dry matter; 4) supplemented with soybean oil in combination with fish oil 1:1 w/w at 3% of feed dry matter;. Each period in the Latin square design lasted 21 d, with the first 7 d for adaptation. The results found that supplementation of fish oil and combination oil reduced the concentration of C18:0 in the rumen content when compare to control and soybean oil but increased C20:5n-3 and C22:6n-3 content ($P<0.05$). However, concentrations of *t11*-C18:1 and *c9,t11*-C18:2 in the rumen content were significantly increased ($P<0.05$) by soybean oil and mixed oil addition. Supplementation of soybean oil and soybean oil in combination with fish oil reduced molar proportion of acetic

acid in the rumen fluid at 2 h after feeding but increased molar proportion of propionic acid. The ruminal pH was significantly decreased ($P<0.05$) by soybean oil and soybean oil in combination with fish oil addition. Supplementation of fish oil had greater ammonia nitrogen at 2, 4 and 6 h post-feeding. However addition of oils had no effects on DMD, CPD, NDFD, and ADFD in *in situ* study ($P>0.05$).

4.2 Introduction

The consumption of beef and dairy products has increased by increasing world population and consumers focus and realize on nutrients of food consumed each day (WHO, 2003) Fatty acids represent 30-35% of total energy intake in many industrial countries and the most important dietary sources of fatty acids are vegetable oils, dairy products, meat products, grain and fatty fish or fish oils.

Biomedical studies with animal models have documented the anticarcinogenic and anti-atherogenic effects of *c9,t11*-C18:2 (Ip et al.,1994). Since *c9,t11*-C18:2 is, by a considerable margin, the most predominant CLA isomer in milk fat, enhancing the CLA content of milk is realistically only related to increases in this isomer. *c9,t11*-C18:2 was the major CLA isomer in ruminant fat representing about 75 to 90% of the total CLA synthesized from vaccenic acid in the tissue (Demeyer and Doreau, 1999) and the common name of "rumenic acid" has been proposed for this isomer because of its unique relationship to ruminants (Demeyer and Doreau, 1999). The anti-obesity effects of CLA are due to the *t10,c12* isomer; while this isomer can vary in milk fat; it never represents more than 1 or 2% of total CLA and thus, food products derived from ruminants are unlikely to provide sufficient amounts of this isomer to have biological effects on body fat. Fat and fatty acids in beef and dairy products depend on the feed

ingredient, nutrient composition, digestive systems and processes that occur via the animal.

The benefits of adding fat to the diets of cattle may be limited by its negative effect on fermentation in the rumen, mainly through reduction of the cellulolytic activity of rumen microflora. *Butyrivibrio fibrisolvens* is one of the major microorganisms involved in microbial fatty acid alterations in the rumen (Maia et al., 2010). Maia et al. (2010) found that when unsaturated fatty acids are incorporated into the microbial cell membrane of *Butyrivibrio fibrisolvens*, they increase the permeability of the cell membrane, negatively impacting the integrity of the cell. Maia et al. (2010) identified this loss of cell integrity as a mechanism of PUFA toxicity and identified that the highly unsaturated fish oil fatty acids, C20:5n-3 and C22:6n-3, are highly toxic to ruminal microorganisms. In order to minimize the toxic effects of PUFA and to utilize fats for energy, ruminal microorganisms are effective at metabolizing fats into their components and eliminating the toxic double bonds. Additionally the fermentation in the rumen is important when the cattle are supplemented with oils because it will affect animal performance. Thus, this study was conducted to evaluate the effects of oil rich in omega-6 FAs supplementation with fish oil on ruminal bio-hydrogenation and fermentation in fistulated cattle.

4.3 Materials and Methods

4.3.1 Animals and Feeding

All experiment procedures were conducted following the Ethical Principles and Guidelines for the Use of Animal issued by National Research Council of Thailand. Four fistulated cattle were assigned in 4 treatments in a 4×4 Latin square

design. All cattle were fed approximately 4 kg/d of 14% CP concentrate and 2.4 kg/d of rice straw. Treatments were: 1) control 2) supplemented with soybean oil (SBO) at 3% of feed dry matter (DM); 3) supplemented with fish oil (FO) at 3% of feed DM; 4) supplemented with soybean oil (SBO) in combination with fish oil (FO) 1:1 w/w at 3%of feed DM. All cattle also had free access to clean water and were individually housed in a free-stall unit and individually fed according to treatments. The experiment lasted for 84 days (4 periods) with 21 d in each period, the first 7 d of each period for adaptation to diets followed by 14 d for ruminal sample collection and *in sacco* disappearance trial.

4.3.2 Sample Collection

To evaluate fatty acids profile in rumen content and ruminal fermentation, the procedures of sample collection, preservation of samples and pH measurement were the same as in Chapter 3 (Section 3.3.3).

4.3.3 Laboratory Analyses

4.3.3.1 Feed chemical composition analysis

Sample collection of feeds and feed chemical composition analyses were the same as in Chapter 3 (Section 3.3.4.1).

4.3.3.2 Analysis of fatty acids in feeds

The procedures of fatty acid composition analysis were the same as in Chapter 3 (Section 3.3.4.2).

4.3.4.3 Analysis of fatty acids in rumen content

Preparation and analysis of rumen fluid samples were the same as in Chapter 3 (Section 3.3.4.3).

4.3.3.4 Volatile fatty acid and ammonia nitrogen analysis

Ruminal volatile fatty acids (VFA) and ammonia N were determined in rumen fluid samples as in Chapter 3 (Section 3.3.4.4).

4.3.3.5 Determination DM, CP, NDF and ADF degradabilities

Preparation of feed samples and determination of DM, CP, NDF and ADF degradabilities were the same as in Chapter 3 (Section 3.3.4.5).

4.3.4 Statistical Analysis

All data were analyzed as a 4x4 Latin squares design using ANOVA procedure of SAS (SAS, 1996). Significant differences among treatment were assessed by Duncan's new multiple range test. A significant level of $P<0.05$ was used (Steel and Torrie, 1980).

4.3.5 Experimental Site

The experiment was conducted a University's Farm and The Center of Scientific and Technological Equipment, Suranaree University of Technology.

4.3.6 Duration

The duration of the present experiment was from September to December 2015.

4.4 Result

4.4.1 Chemical composition of experimental diet

Chemical compositions of the concentrate, rice straw, soybean oil (SBO) and fish oil (FO) used in the experiment are show in Table 4.1.

The fatty acid compositions of the concentrate, rice straw, SBO and FO used in the experiment are shown in Table 4.2. C18:2n6 was the major fatty acid in the SBO

approximately 44.74% of total fatty acid. FO had the highest proportion of C22:6n-3 and C20:5n3 (30.42% and 7.93 of total fatty acid respectively). In the concentrate, C18:1n-9 (29.54% of total fatty acid) and C12:0 (22.74% of total fatty acid) were the main fatty acids (FA). The main FA in rice straw was C16:0 (45.77% of total fatty acid).

Table 4.1 Chemical composition of the experimental diets.

Items	Concentrate	Soybean oil	Fish oil	Rice straw
Dry matter	90.2	100	100	92.2
	..... % of DM.....			
Ash	9.2			16.1
Crude protein	13.6			2.4
Ether extract	3.6	100	100	1.6
Crude fiber	17.4			39.2
Neutral detergent fiber	40.1			74.3
Acid detergent fiber	18.5			51.3
Acid detergent lignin	3.9			11.1

[1]kg/100 kg concentrate: 30 dried cassava chip, 4 ground corn, 10 rice bran, 25 palm meal, 15 coconut meal, 6 dried distillers grains with solubles, 0.5 sodium bicarbonate, 6 molasses, 1 dicalciumphosphate (16%P), 1.5 urea, 0.5 salt and 0.5 premix. Premix: provided per kg of concentrate including vitamin A, 5,000 IU; vitamin D3, 2,200 IU; vitamin E, 15 IU; Ca, 8.5 g; P, 6 g; K, 9.5 g; Mg, 2.4 g; Na, 2.1 g; Cl, 3.4 g; S, 3.2 g; Co, 0.16 mg; Cu, 100 mg; I, 1.3 mg; Mn, 64 mg; Zn, 64 mg; Fe, 64 mg; Se, 0.45 mg.

Table 4.2 Fatty acid compositions (g/100 g of total fatty acids) of concentrate, rice straw and oils used in the experiment.

Fatty acids	Concentrate	Rice straw	Soybean oil	Fish oil
C12:0	22.74	6.35	0.43	2.17
C14:0	7.78	8.21	1.09	4.38
C16:0	16.58	45.77	13.74	28.02
C18:0	2.52	0.08	5.26	6.09
C18:1n-9	29.54	24.77	33.87	14.44
C18:2n-6	17.17	11.32	44.74	1.69
C18:3n-3	0.21	ND	0.35	0.93
C20:5n-3	ND	ND	ND	7.93
C22:6n-3	ND	ND	ND	30.42
Others	3.46	3.50	0.52	3.93

SBO = soybean oil; FO = fish oil

ND = Not detected; Others = C8:0 + C15:0 + C20:1 + C21:0 + C23:0

4.4.2 Intake of main components and major fatty acids

The current study was designed to limit concentrate and rice straw consumptions and to control the ratio of concentrate to rice straw at 60:40 (DM basis). Oils supplemented cattle had significantly higher DM and fat intakes when compare to the control cattle. Adding oils at 3% of total feed DM was equivalent to 180 g/day as shown in Table 4.3.

FA intakes were similar among oil supplemented cattle but were significantly higher than the control cattle. However, intake of individual FA differed

due to the composition of the various supplemental oils (Table 4.3). When individual FA intake was calculated (Table 4.3), cattle on SBO diet received more C18:1n9 and C18:2n-6 than those cattle on control, FO and SBO FO diets. Greater C18:2n-6 intake of SBO cattle was caused by higher C18:2n-6 content in SBO. Cattle on FO diet received more C14:0, C16:0, C18:0, C20:5n-3 and C22:6n-3 than other cattle. This is because FO contained high proportion of C20:5 and C22:6n-3. The control cattle consumed less FA because of lower fat intake (Table 4.3).

Table 4.3 DM, CP, fat and fatty acid intakes of experimental cattle.

Items	Control	SBO	FO	SBO+FO	SEM	P-value
DM intake (kg/d)						
Concentrate	3.73	3.73	3.73	3.73	-	-
Rice straw	2.21	2.21	2.21	2.21	-	-
Oil	-	0.18	0.18	0.18	-	-
Total	5.93[b]	6.13[a]	6.13[a]	6.13[a]	0.004	0.001
CP intake (g/d)						
Concentrate	507	507	507	507	-	-
Rice straw	52	52	52	52	-	-
Total	559	559	559	559	-	-
Fat intake (g/d)						
Concentrate	134	134	134	134	-	-
Rice straw	38	38	38	38	-	-
Oil		180	180	180	-	-
Total	173[b]	353[a]	353[a]	353[a]	0.003	0.001

SBO = soybean oil; FO = fish oil

[ab] Within a row means without a common superscript letter differ

Table 4.3 DM, CP, fat and fatty acid intakes of experimental cattle (cont.).

Items	Control	SBO	FO	SBO+FO	SEM	P-value
FA intake (g/d)						
C12:0	22.74^{d}	25.43^{c}	27.69^{a}	26.52^{b}	0.160	0.001
C14:0	10.21^{d}	11.69^{c}	16.13^{a}	13.91^{b}	0.005	0.001
C16:0	29.88^{d}	48.45^{c}	67.73^{a}	58.10^{b}	0.001	0.001
C18:0	2.57^{d}	9.67^{c}	10.79^{a}	10.22^{b}	0.002	0.001
C18:1n-9	36.90^{d}	82.65^{a}	56.42^{c}	69.53^{b}	0.020	0.001
C18:2n-6	20.56^{d}	80.98^{a}	22.86^{c}	51.92^{b}	0.011	0.001
C18:3n-3	0.21^{d}	0.68^{c}	1.47^{a}	1.07^{b}	0.001	0.001
C20:5n-3	0.00^{c}	0.00^{c}	10.70^{a}	5.40^{b}	0.001	0.001
C22:6n-3	0.00^{c}	0.00^{c}	41.07^{a}	20.54^{b}	0.001	0.001
Others	4.49^{d}	5.20^{c}	9.80^{a}	7.50^{b}	0.002	0.001
Total	130^{b}	265^{a}	265^{a}	265^{a}	0.070	0.001

SBO =soybean oil; FO =fish oil

SEM = standard error of the mean; Others = C8:0 + C15:0 + C20:1 + C21:0 + C23:0

^{abcd} Within a row means without a common superscript letter differ

4.4.3 Fatty acid profile in rumen content

Supplementation of SBO, FO and SBO+FO resulted in higher ruminal concentration of *t11*-C18:1 at 2, 4 and 6 h after feeding (Table 4.4). Ruminal content from SBO and control cattle contained significantly higher ruminal concentration of C18:0 than those from FO and SBO+FO cattle (Table 4.4). At all h after feeding, ruminal content from FO and SBO+FO cattle had higher C16:0, C20:5n-3 and C22:6n-

3 than those from control and SBO cattle (Table 4.4). However, the concentration of *c9,t11*-C18:2 in ruminal content from FO cattle was similar to from that of control cattle but significantly less than those from SBO and SBO+FO cattle. SBO and SBO in combination with FO greatly decreased C18:0 at all h after feeding in the current study.

Table 4.4 Effect of soybean oil, fish oil and soybean oil combination with fish oil supplementation on fatty acid profile in fistulated cattle (g/100g fatty acids).

Fatty acids	Control	SBO	FO	SBO+FO	SEM	P-value
Pre - feeding						
C12:0	12.91	12.58	13.07	12.47	0.155	0.413
C14:0	9.18	8.80	8.83	8.50	0.262	0.884
C16:0	33.89	34.44	34.69	34.66	0.142	0.754
C18:0	38.76	37.51	37.15	38.73	0.363	0.367
C18:1n-9	2.49	2.88	2.65	2.28	0.389	0.833
C18:2n-6	1.31	1.71	1.60	1.63	0.243	0.984
t11-C18:1	1.45	2.19	2.00	1.73	0.102	0.365

SBO = soybean oil; FO = fish oil; SEM = standard error of the mean

Table 4.4 Effect of soybean oil, fish oil and soybean oil combination with fish oil supplementation on fatty acid profile in fistulated cattle (g/100g fatty acids) (cont.).

Fatty acids	Control	SBO	FO	SBO+FO	SEM	P-value
2 h after feeding						
C12:0	7.42	6.62	7.13	7.85	0.275	0.382
C14:0	5.84	5.06	6.04	5.90	0.503	0.322
C16:0	34.21^{a}	18.37^{b}	33.09^{a}	26.73^{ab}	1.411	0.045
C18:0	48.04^{a}	28.70^{b}	6.47^{c}	8.28^{c}	1.194	0.001
C18:1n-9	1.45^{b}	7.24^{a}	8.94^{a}	8.46^{a}	0.707	0.044
C18:2n-6	2.19^{b}	5.52^{a}	1.69^{b}	2.11^{b}	0.341	0.042
C18:3n-3	0.48	0.56	0.55	0.58	0.025	0.222
t11-C18:1	0.37^{c}	18.84^{b}	22.88^{ab}	26.72^{a}	0.670	0.001
c9,t11-C18:2	0.00^{b}	9.13^{a}	0.00^{b}	9.25^{a}	0.125	0.001
C20:5n-3	0.00^{c}	0.00^{c}	1.31^{b}	0.56^{a}	0.071	0.005
C22:6n-3	0.00^{b}	0.00^{b}	11.90^{a}	3.55^{b}	0.617	0.004

SBO = soybean oil; FO = fish oil; SEM = standard error of the mean

^{abc} Within a row means without a common superscript letter differ.

Table 4.4 Effect of soybean oil, fish oil and soybean oil combination with fish oil supplementation on fatty acid profile in fistulated cattle (g/100g fatty acids) (cont.).

Fatty acids	Control	SBO	FO	SBO+FO	SEM	P-value
4 h after feeding						
C12:0	3.53^{b}	6.04^{a}	5.75^{ab}	7.30^{a}	0.338	0.046
C14:0	5.64	5.40	5.77	5.71	0.474	0.986
C16:0	41.33^{a}	20.53^{c}	34.15^{b}	28.47^{b}	0.957	0.003
C18:0	34.13^{a}	29.99^{b}	8.03^{c}	7.68^{c}	0.780	0.001
C18:1n-9	1.21^{c}	6.41^{ab}	5.15^{b}	9.46^{a}	0.477	0.007
C18:2n-6	3.20^{ab}	4.58^{a}	1.07^{b}	1.31^{b}	0.362	0.044
C18:3n-3	0.11^{b}	0.57^{a}	0.66^{a}	0.56^{a}	0.044	0.025
t11-C18:1	10.85^{c}	21.59^{b}	25.72^{ab}	29.41^{a}	0.821	0.001
c9,t11-C18:2	0.00^{b}	4.88^{a}	0.00^{b}	5.97^{a}	0.291	0.002
C20:5n-3	0.00^{c}	0.00^{c}	1.16^{a}	0.75^{b}	0.029	0.001
C22:6n-3	0.00^{c}	0.00^{c}	12.53^{a}	3.36^{b}	0.201	0.001

SBO = soybean oil; FO = fish oil; SEM = standard error of the mean

^{abc} Within a row means without a common superscript letter differ.

Table 4.4 Effect of soybean oil, fish oil and soybean oil combination with fish oil supplementation on fatty acid profile in fistulated cattle (g/100g fatty acids) (cont.).

Fatty acids	Control	SBO	FO	SBO+FO	SEM	P-value
6 h after feeding						
C12:0	5.36^{b}	6.74^{ab}	8.81^{ab}	11.14^{a}	0.773	0.086
C14:0	5.40^{b}	4.45^{b}	8.05^{a}	6.98^{a}	0.309	0.017
C16:0	21.65^{b}	21.91^{b}	33.91^{a}	30.27^{a}	1.154	0.012
C18:0	48.17^{a}	37.97^{b}	7.40^{c}	8.30^{c}	1.920	0.013
C18:1n-9	2.03^{b}	4.82^{ab}	5.04^{ab}	6.99^{a}	0.454	0.051
C18:2n-6	2.23^{a}	1.80^{a}	0.96^{b}	0.97^{b}	0.116	0.025
C18:3n-3	0.06^{b}	0.61^{a}	0.65^{a}	0.78^{a}	0.032	0.002
t11-C18:1	15.10^{c}	16.96^{bc}	24.56^{ab}	29.43^{a}	1.146	0.020
c9,t11-C18:2	0.00^{c}	4.73^{a}	0.00^{c}	1.83^{b}	0.064	0.001
C20:5n-3	0.00^{c}	0.00^{c}	0.57^{a}	0.26^{b}	0.016	0.001
C22:6n-3	0.00^{c}	0.00^{c}	10.05^{a}	3.01^{b}	1.151	0.063

SBO = soybean oil; FO = fish oil; SEM = standard error of the mean

^{abc} Within a row means without a common superscript letter differ.

4.4.4 Ruminal Fermentation

Addition of SBO or FO into the diet significantly decreased ruminal pH when compare to the control and SBO+FO diets at 2 h after feeding while A:P ratio was lower than those control and SBO+FO diets (Table 4.5). At 4 and 6 h after feeding, ruminal pH was not affected by dietary treatments.

The current study found that SBO and FO had effect on ruminal VFA concentrations at and 2 h after feeding (Table 4.5). SBO and FO supplementation significantly reduced molar proportion of acetic acid at 2 h post-feeding but significantly increased molar proportion of propionic acid. At 4 and 6 h post-feeding supplementation of oils had no effect on ruminal volatile fatty acids and A:P ratio, however, FO added to the diet significantly increased ammonia nitrogen in rumen fluid at 2, 4 and 6 after feeding.

Table 4.5 Effect of soybean oil, fish oil and soybean oil combination with fish oil supplementation on pH, ammonia nitrogen (mg/100 ml) and volatile fatty acids (mol/100 mol) in fistulated cattle.

Item	Control	SBO	FO	SBO+FO	SEM	P-value
Pre - feeding						
pH	6.94	6.87	6.89	6.93	0.019	0.238
NH_3N	8.92	8.99	8.81	8.87	0.093	0.747
Acetic acid	67.63	67.77	67.97	67.64	0.771	0.273
Propionic acid	16.64	16.57	16.86	16.87	0.576	0.181
Butyric acid	15.73	15.66	15.17	15.49	0.772	0.391
A:P ratio	4.06	4.09	4.03	4.01	0.094	0.119
2 h after feeding						
pH	6.92^{a}	6.76^{b}	6.76^{b}	6.88^{a}	0.016	0.027
NH_3N	14.52^{b}	15.18^{b}	19.23^{a}	15.74^{b}	0.380	0.024
Acetic acid	64.93^{a}	57.06^{b}	55.94^{b}	62.58^{a}	0.528	0.006
Propionic acid	20.96^{b}	27.84^{a}	28.69^{a}	23.05^{b}	0.376	0.002
Butyric acid	14.10	15.10	15.38	14.37	0.262	0.294
A:P ratio	3.10^{a}	2.06^{c}	1.96^{c}	2.72^{b}	0.038	0.001

SBO = soybean oil; FO = fish oil; A:P ratio = acetate: propionate ratio

SEM = standard error of the mean

^{ab}Within a row means without a common superscript letter differ

Table 4.5 Effect of soybean oil, fish oil and soybean oil combination with fish oil supplementation on pH, ammonia nitrogen (mg/100 ml) and volatile fatty acids (mol/100 mol) in fistulated cattle (cont.).

Item	Control	SBO	FO	SBO + FO	SEM	P-value
4 h after feeding						
pH	6.66	6.58	6.78	6.51	0.054	0.345
NH_3N	5.68^{b}	5.64^{b}	8.27^{a}	6.93^{ab}	0.334	0.048
Acetic acid	62.64	64.79	63.75	64.27	0.290	0.136
Propionic acid	23.62	22.18	24.07	22.07	0.558	0.448
Butyric acid	13.74	13.07	12.18	13.66	0.561	0.674
A:P ratio	2.66	2.92	2.69	2.92	0.070	0.355
6 h after feeding						
pH	6.72	6.52	6.54	6.42	0.053	0.299
NH_3N	6.09^{b}	6.50^{b}	8.74^{a}	7.68^{ab}	0.247	0.048
Acetic acid	66.94	68.48	63.67	64.03	1.336	0.476
Propionic acid	23.63	20.13	24.35	22.13	0.681	0.198
Butyric acid	9.43	11.39	11.98	13.84	0.735	0.251
A:P ratio	3.05	3.40	2.63	3.02	0.092	0.111

SBO = soybean oil; FO = fish oil; A:P ratio = acetate: propionate ratio

SEM = standard error of the mean

^{ab}Within a row means without a common superscript letter differ

4.4.5 Degradability of DM CP NDF and ADF

Addition of oils to the diet, in this study, had no effects on the readily soluble fraction (*a*) and the potentially degradability fraction (*b*) of concentrate and rice straw (Table 4.6). When the effective degradabilities were calculated, they were not significant different at all out flow rates (P>0.05). Similarly, when considered for the crude protein degradability (CPD) of concentrate, the readily soluble fraction (*a*),

potentially degradability fraction (*b*) and effective degradabilities at out flow rate 0.02, 0.05 and 0.08 /h were unaffected by oil supplementation when compare to control diet (Table 4.7). The neutral detergent fiber degradability (NDFD) and acid detergent fiber degradability (ADFD) of rice straw were also unaffected (Table 4.8).

Table 4.6 Effect of soybean oil, fish oil and soybean oil combination with fish oil supplementation on dry matter degradability (DMD) of concentrate and rice straw in fistulated cattle.

Item	Control	SBO	FO	SBO+FO	SEM	P-Value
Dry matter degradability of concentrate						
a	23.58	23.70	22.55	23.18	0.926	0.109
b	52.82	52.50	50.75	51.11	1.472	0.305
a + *b*	76.40	76.20	73.30	74.29	1.328	0.273
c, per h	0.045	0.043	0.042	0.045	0.012	0.315
dg, 0.02/h	0.67	0.67	0.66	0.67	0.007	0.923
dg, 0.05/h	0.60	0.60	0.61	0.62	0.005	0.636
dg, 0.08/h	0.59	0.59	0.57	0.58	0.004	0.500
Dry matter degradability of rice straw						
a	19.50	19.30	19.90	19.53	0.260	0.206
b	40.24	40.33	42.37	41.40	2.538	0.809
a + *b*	59.74	59.63	62.27	60.94	2.284	0.850
c, per h	0.021	0.025	0.017	0.030	0.003	0.482
dg, 0.02/h	0.41	0.41	0.41	0.43	0.008	0.197
dg, 0.05/h	0.32	0.32	0.31	0.33	0.005	0.304
dg, 0.08/h	0.28	0.28	0.27	0.29	0.004	0.105

SBO = soybean oil; FO = fish oil; SEM = standard error of the mean

a = the intercept of the degradation curve at time zero;

b = the potential degradability of the component;

c = the rate constant for the degradation of 'b'.

Table 4.7 Effect of soybean oil, fish oil and soybean oil combination with fish oil supplementation on crude protein degradability (CPD) of concentrate in fistulated cattle.

Item	Control	SBO	FO	SBO+FO	SEM	P-Value
crude protein degradability of concentrate						
a	29.34	29.23	30.23	30.07	1.233	0.134
b	61.53	61.83	62.20	61.87	0.986	0.194
a + *b*	90.87	91.06	92.43	91.94	0.248	0.278
c, per h	0.223	0.230	0.207	0.198	0.008	0.435
dg, 0.02/h	0.86	0.86	0.87	0.87	0.003	0.636
dg, 0.05/h	0.80	0.80	0.81	0.81	0.005	0.826
dg, 0.08/h	0.74	0.75	0.76	0.77	0.005	0.700

SBO = soybean oil; FO = fish oil; SEM = standard error of the mean

a = the intercept of the degradation curve at time zero;

b = the potential degradability of the component;

c = the rate constant for the degradation of 'b'.

Table 4.8 Effect of soybean oil, fish oil and soybean oil combination with fish oil supplementation on neutral detergent fiber degradability (NDFD) and acid detergent fiber degradability (ADFD) of rice straw in fistulated cattle.

Item	Control	SBO	FO	SBO+FO	SEM	P-Value
Neutral detergent fiber degradability of rice straw						
a	12.65	12.73	12.30	13.60	0.289	0.363
b	55.75	55.47	56.43	54.45	0.522	0.437
a + *b*	68.40	68.20	68.73	68.05	0.586	0.464
c, per h	0.017	0.017	0.015	0.019	0.002	0.472
dg, 0.02/h	0.38	0.38	0.38	0.40	0.013	0.713
dg, 0.05/h	0.27	0.27	0.26	0.24	0.012	0.756
dg, 0.08/h	0.23	0.23	0.22	0.25	0.003	0.125
Acid detergent fiber degradability of rice straw						
a	12.13	12.30	11.05	10.15	1.141	0.271
b	50.25	49.27	53.75	50.95	8.605	0.357
a + *b*	62.38	61.57	64.80	61.10	9.075	0.384
c, per h	0.021	0.021	0.019	0.020	0.005	0.230
dg, 0.02/h	0.35	0.35	0.34	0.36	3.848	0.344
dg, 0.05/h	0.26	0.26	0.24	0.25	1.154	0.125
dg, 0.08/h	0.21	0.21	0.19	0.20	1.230	0.129

SBO = soybean oil; FO = fish oil; SEM = standard error of the mean

a = the intercept of the degradation curve at time zero;

b = the potential degradability of the component;

c = the rate constant for the degradation of 'b'.

4.5 Discussion

4.5.1 Fatty Acid Profile in Ruminal Content

The rate of hydrogenation increases with the increased degree of unsaturation (Harfoot and Hazlewood, 1997). The ruminal bacteria involved in hydrogenation have been classified into two groups, A and B, according to the metabolic pathway involved (Kemp and Lander, 1984). For complete hydrogenation of PUFA, both groups of bacteria are usually necessary. Group A comprises a plurality of bacteria able to hydrogenate PUFA into *t11*-C18:1; this group includes *Butyrivibrio fibrisolvens, Micrococcus* sp. and *Ruminococcus albus*. Group B, including *Fucocillus*, participates mainly in hydrogenation of C18:1 and its isomers into C18:0. Two key bio-hydrogenation intermediates are *t11*-C18:1 and C18:0 (Abughazaleh et al., 2002). The increasing of *t11*-C18:1 in current study was affected by the addition of soybean oil and fish oil. Supplementations of soybean oil or fish oil increased the concentration of *t11*-C18:1 when compare to the control group and its concentration was greater when soybean oil was added in combination with fish oil. Similarly, Toral et al. (2010) added sunflower oil into the diet and found significant increase in the amount of *t11*-C18:1. The greater concentration of *t11*-C18:1 along with the lower concentration of C18:0 with the fish oil addition relative to incomplete bio-hydrogenation (Loor et al., 2004; AbuGhazaleh and Jacobson, 2007; Fuentes et al., 2009). Increase in the concentration of *t11*-C18:1 with oil supplement resulted from the increase in inputs of dietary C18 unsaturated fatty acids, the precursors for *t11*-C18:1. Furthermore, supplementation of a high-concentrate diet with fish oil providing <25% C20:5n-3 and C22:6n-3 relative to C18:2n-6 from sunflower oil was effective in enhancing flow of *t11*-18:1 to maintain synthesis of *c9,t11*-C18:2 in the mammary gland. This result

confirmed *in vitro* data showing similar accumulation of *trans*-18:1 due to C22:6n-3 compared with soybean oil at 6 times the level of C22:6n-3 supplementation (AbuGhazaleh and Jenkins, 2004).

Fish oil addition will shift these processes by inhibition of bacterial conversion unsaturated fatty acids to saturated fatty acid (Jenkins et al., 2008) as observed in the present study. Supplemented fish oil and fish oil mixed with soybean oil reduced the concentration of C18:0. Similarly, Kim et al. (2008) supplemented fish oil at 2.3% and 6.9% in steer and showed that the concentration of C18:0 in duodenum was linearly decreased when compare to control group. In addition, supplementation of different ratio of microbial oil in combination with fish oil also reduced these fatty acids in ruminal content (Jalč et al., 2009). AbuGhazaleh and Jenkins (2004) observed several changes in the ruminal batch culture fatty acid profile when C22:6n-3 was supplemented. DHA supplementation decreased C18:0 and inhibited the bio-hydrogenation of C18:1n-9 and C18:2n-6 with 1, 2, 3, or 4% C22:6n-3 supplementation.

The greater ruminal C16:0 in FO cattle was affected by the fatty acid intake of fish oil in FO supplementation group. Fish oil contained higher C16:0 (45.77 g/100 of total fatty acids) when compare to soybean oil (13.74 g/100 g of total fatty acids). Similar result was previously reported by (Kitessa et al. (2001) who supplemented protected tuna oil and tuna oil and found an increase in C16:0 concentration in the rumen content. Loor et al. (2005) supplemented fish oil at 2.5% of total feed DM, sunflower oil at 5% of total feed DM and linseed oil at 5% of total feed DM in Holstein cows and reported that fish oil cow had higher concentration of C16:0 when compared to sunflower oil and linseed oil cows.

The cattle received fish oil had greater ruminal concentration of C20:5 n-3 and C22:6n-3 when compared to those cattle received control and soybean oil. The main PUFAs in fish oil were C22:6n-3 and C20:5n-3 (30.42 and 7.93 g/100g of total fatty acids respectively). Loor et al. (2005) reported that fish oil supplementation in cows increased the concentration of C20:5n-3 and C22:6n-3 in the rumen while linseed and sunflower oil addition did not found those fatty acids. Similarly, Kitessa et al. (2001a) supplemented tuna oil and rumen protected tuna oil in goat and found higher C20:5n-3 and C22:6n-3 in the rumen compared to control group. Dohme et. al. (2003) reported *in vitro* rates of DHA lipolysis and bio-hydrogenation both occurred in ruminal batch cultures, but that increasing levels of fish oil decreased the percent of both lipolysis and bio-hydrogenation at 24 h. Lipolysis rates fell from 83% to 58% and bio-hydrogenation rates decreased from >90% to <30% as fish oil increased from 12.5 mg to 125 mg per culture. AbuGhazaleh and Jenkins (2004) also found that DHA disappeared from ruminal batch cultures inoculated with Holstein rumen fluid. They reported the percentage of DHA that disappeared from cultures decreased from 60% to 7% as DHA level in cultures increased from 1% to 4% of the diet.

For ruminal *c9,t11*-C18:2 or rumenic acid, the present study found that supplementation of soybean oil and combination oil had greater concentration when compared to control and fish oil animals. Abughazaleh et al. (2002) also previously reported significant increase in *c9,t11*-C18:2 in the rumen when soybean oil, fish oil and combination oil were added to the diet. Jalč et al. (2009) supplemented different ratios of oil rich in C18:2n-6 in combination with fish oil founded that increasing proportion of C18:2n-6 linearly increased *c9,t11*-C18:2. However, these fatty acids are not the main precursor of CLA in ruminant products; the main precursor to synthesize CLA is from *t11*-C18:1. (Bauman et al., 2000).

4.5.2 Ruminal Fermentation

In the current study, supplementations of fish oil and soybean oil reduced ruminal pH. Multiple studies have found that fishmeal, algae, and fish oil all decreased dry matter intake (Wright et. al. 2003, Donovan et. al, 2000, and Whitlock et. al., 2002), although Amorocho et. al. (2009) actually observed an increase in dry matter intake when catfish oil was included in the concentrate. Shingfield et al. (2003)found significant decrease in pH when fish oil was supplemented because of reduction in DMI related to lower pH. However, in the current study, concentrate and rice straw intakes were limited resulting in lower ruminal pH. Nevel and Demeyer (1996) suggested that, in cattle received oil rich in C18:2n-6, the ruminal pH changed from 6.8 to 5.2. Amorocho et. al. (2009) reported decrease in rumen pH when cat fish oil was added whereas Boeckaert et. al. (2008) reported that supplementing DHA algae had no effect on ruminal pH compared to control. In addition, Latham et al. (1972) showed that low rumen pH resulted in lower levels of lipolytic activity and bio-hydrogenation of unsaturated FA in ruminal fluid. Most rumen microbes are sensitive to low pH conditions as acidity in the rumen impact microbial growth and enzymes activities (Martin et al., 2002; Jenkins et al., 2008).

Ruminal ammonia nitrogen is the source of nitrogen for bacterial growth in the rumen. The result in the present study showed that supplementation of fish oil reduced the concentration of ammonia nitrogen in the rumen, which is similar to Zhang et al. (2008) who observed significant decreases in NH_3-N when fish oil combine with linoleic acid sources were supplemented in sheep. In contrast, Gudla et al. (2012) showed no significant difference in ammonia nitrogen when soybean oil in combination with fish oil was added compared to non-oil supplement control.

The effects of fish oil supplementation on ruminal volatile fatty acids

found that the proportion of acetic acid decreased while propionic acid increased at 2h post feeding which was similar to Gudla et al. (2012). The lower acetate concentration with the oil supplemented diets found that the DNA abundance for cellulolytic bacteria (R. *flavefaciens*, B. *fibrisolvens* and R. *albus*) was reduced with the low forage diets (Martin et al., 2002). Amorocho et. al. (2009) reported that cat fish oil decreased the acetate to propionate ratio which was similar to Lee et al., (2008), Keady and Mayne (1999) and Kim et al. (2008). FO modified rumen fermentation, causing a decrease in the molar A: P ratio. Earlier studies reported that FO has no major effect on fermentation characteristics in growing cattle, but enhanced the ratio of glucogenic: lipogenic precursors in the rumen of steers (Shingfield et al., 2010). It is probable that changes in rumen fermentation patterns are related to the effect of FO on nutrient digestion in the rumen and alterations in the relative abundance of specific microbial populations. Toral et al. (2016) supplemented fish oil and sunflower oil and showed significant increase in molar proportion of propionic acid when compared to control group. Jalč et al. (2009) supplemented oil rich in omega 6 in combination with fish oil in different ratio also found that oil supplemented diets had greater propionic acid when compare to the control group while acetic acid decreased.

4.5.3 Degradability of DM CP NDF and ADF

In the current study, supplementation of SBO, FO and SBO + FO had no effects on DMD, CPD, NDFD and ADFD which is in agreement with the result from Toral et al. (2009) who supplemented FO with sources of C18:2n-6 in sheep. Evandro Maia Ferreira et al. (2015) supplemented fish oil at 2.5, 5 and 7.5 g/total feed DM in Lambs and found no significant difference in DMD, CPD, OMD any NDFD. They also demonstrated that the supply of up to 7.5 g/kg DM of fish oil together with 32.5 g/kg DM of soybean oil did not compromise the NDF digestibility in the diets

with high concentrate diets. Other authors also found similar results (Lee et al., 2008; Shingfield et al., 2010; Toral et al., 2009, 2010). Oliveira et al. (2007) utilized lipids in the form of soybean grains and soybean oil and verified that soybean oil negatively affected fiber digestibility. Therefore, in addition to the level of ether extract, the source can also influence digestibility and animal performance. Patra and Yu (2013; 2015) suggested that additions of long chain fatty acid can inhibit with complementary mechanisms of actions on methanogenesis and may alter the archaeal communities, and consequently may decrease methane production additively without negatively impacting upon rumen fermentation and degradability. Furthermore, the *in vitro* study from Szczechowiak et al. (2016) found that supplemented soybean oil blended with fish oil had no effects on DM, OM and NDF degradability, however, supplemented SBO+FO significantly increased CP degradability. Ferreira et al. (2015) suggested that animals receiving diets with 40 g/kg DM of soybean oil exhibited lower ruminal ammonia concentrations in comparison to the control treatment. This finding may be attributed to a lower ruminal CP digestion by animals in this treatment, which is compatible with lower CP digestibility in the total digestive tract. The ruminal ammonia concentration linearly increased with the increase of fish oil blend levels in the diet. If a greater substitution of fish oil blends for soybean oil reduced ruminal microbial growth, it can also be explained that an increased ruminal ammonia concentration was due to lower utilization of ammonia available in the rumen for microbial growth.

4.6 Conclusion

Supplemented fish oil and SBO+FO reduced the concentration of C18:0 in the rumen content when compared to control and soybean oil cattle, but increased ruminal C20:5n-3 and C22:6n-3 content ($P<0.05$). In addition, soybean oil and mixed oil increased the concentration of *t11*-C18:1 and *c9,t11*-C18:2 in the rumen content ($P<0.05$). Supplementation of soybean oil and soybean oil in combination with fish oil reduced acetic acid content in the rumen fluid at 2 h after feeding but increased propionic acid content. The ruminal pH were decreased ($P<0.05$) by soybean oil and soybean oil in combination with fish oil supplementation. Addition of fish oil showed greater ammonia nitrogen at 2, 4 and 6 h post-feeding. However, addition of oils had no effects on DMD, CPD, NDFD, and ADFD in *in situ* study ($P>0.05$).

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CHAPTER V

RUMINAL BIO-HYDROGENATION AND FERMENTATION IN RESPONSE TO DIFFERENT RATIO OF OIL RICH IN OMEGA-3 FAs AND FISH OIL IN FISTULATED CATTLE'S DIETS

5.1 Abstract

The objective of current experiment was to investigate the effects of different ratio of linseed oil and fish oil addition on ruminal bio-hydrogenation and fermentation in fistulated cattle. Three fistulated cattle were assigned into 3 treatments in a 3×3 Latin square design. All cattle were fed approximately 4 kg/d of 14% CP concentrate and 2.4 kg/d of rice straw. Treatments were: 1) supplemented linseed oil in combination with fish oil 2:1 w/w at 3% of total feed DM; 2) supplemented linseed oil in combination with fish oil 1:1 w/w at 3% of total feed DM; 3) supplemented linseed oil in combination with fish oil 1:2 w/w at 3% of total feed DM. Each period in the Latin square design lasted 21 d, with the first 7 d for adaptation. The results found that supplementation of 1:2 w/w linseed oil in combination with fish oil at 3% of total feed DM significantly increased C20:5n-3 and C22:6n-3 in the rumen ($P<0.05$). Additionally, 1:1 w/w linseed oil mixed with fish oil significantly increased the concentration of *t11*-C18:1. There were no significant differences in ruminal pH, ammonia nitrogen and volatile fatty acids. However, at 6 h after feeding, the molar

proportion of propionic acid tended to be increased by 2:1 w/w linseed oil and fish oil addition. Feeding 1:1 w/w LSO+FO resulted in detrimental effect on reduction in ADFD ($P<0.05$) but no significant differences on DMD, CPD and NDFD ($P>0.05$) was observed.

5.2 Introduction

Recently, the dietary recommendation for humans of the highly unsaturated n-3 fatty acids, specifically C20:5n-3 and C22:6n-3, has increased from 0.15 to 0.65 g/d (Kris-Etherton et al., 2000). Several authors have demonstrated that intestinal supply (Scholljegerdes et al., 2001) and muscle tissue composition (Mandell et al., 1997; Scollan et al., 2001) of fatty acids in beef cattle were affected by fatty acid composition of dietary full-fat safflower seeds. Feeding lipids high in long chain polyunsaturatedfatty acids (PUFA) can enhance the fatty acid concentrations in beef cattle (Mandell et al., 1997; Scollan et al., 2001) and milk from dairy cattle (Lawless et al., 1998; Whitlock et al., 2002).

The results from chapter 3 found an increase in the proportion of ruminal PUFA and a reduction in ruminal SFA when linseed oil was added in combination with fish oil 1:1 w/w at 3% of total feed DM when compared to that addition of linseed oil or Ca-linseed oil. The concentrations of C18 PUFA, particular C18:2n-6 and C18:3n-3 decreased as they are hydrogenated completely to C18:0 with formation of intermediates like conjugated linoleic acid (*c9,t11*-C18:2) and vaccenic acid (*t11*-C18:1) as the most important known ones (Harfoot and Hazlewood, 1997). Dohme et al. (2003) founded that fish oil had lower lipolysis and bio-hydrogenation of C20:5n-3 and C22:6n-3 compared to C18:2n-6 which was similar to the result of Gulati et al.

(1999). This effect was also observed *in vivo* resulting in an enhanced duodenal flow of C20:5n-3 and C22:6n-3 (Wachira et al., 2000). Addition of fish oil in dairy cows showed significant increase in the milk content of *c9,t11*-C18:2 and *t11*-C18:1 (Donovan et al., 2000). These FAs are main intermediates in the rumen bio-hydrogenation of C18:3n-3 and/or C18:2n-6. As only small amounts of C18:2n-6 and C18:3n-3 was present in fish oil, it was hypothesized that supplementation of fish oil inhibited the complete bio-hydrogenation of C18:2n-6 and C18:3n-3 derived from sources other than fish oil (Bauman et al., 1999; AbuGhazalehet al., 2002; Whitlock et al., 2002). Feeding fish oil with oils high in linoleic or linolenic acid have been shown to be effective way to enhance the *c9,t11*-C18:2 level in milk (Donovan et al., 2000). AbuGhazaleh et al. (2003) concluded that supplementing dairy cows' diets with a combination of fish oil and other high linoleic or linolenic sources was the most efficient dietary regimen for increasing milk *c9,t11*-C18:2 CLA. Therefore the aim of this experiment was to evaluate the effects of different ratios of linseed oil in combination with fish oil supplementation in fistulated cattle on ruminal bio-hydrogenation and fermentation.

5.3 Materials and Methods

5.3.1 Animals and Feeding

All experiment procedures were conducted following the Ethical Principles and Guidelines for the Use of Animal issued by National Research Council of Thailand. Three fistulated cattle were assigned in 3 treatments in a 3×3 Latin square design. All cattle were fed approximately 4 kg/d of 14% CP concentrate and 2.4 kg/d of rice straw. Treatments were: 1) supplemented 2:1 w/w linseed oil (LSO) in

combination with fish oil (FO) at 3%of feed DM 2) supplemented 1:1 w/w linseed oil (LSO) in combination with fish oil (FO) at 3%of feed DM; 3) supplemented 1:2 w/w linseed oil (LSO) in combination with fish oil (FO) at 3%of feed DM. All cattle also had free access to clean water and were individually housed in a free-stall unit and individually fed according to treatments. The experiment lasted for 63 days (3 periods) with 21 d in each period, the first 7 d of each period for adaptation to diets followed by 14 d for ruminal sample collection and *in sacco* disappearance trial.

5.3.2 Sample Collection

To evaluate fatty acids profile in rumen content and ruminal fermentation, the procedures of sample collection, preservation of samples and pH measurement were the same as in Chapter 3 (Section 3.3.3)

5.3.3 Laboratory Analyses

5.3.3.1 Feed chemical composition analysis

Sample collection of feeds and feed chemical composition analyses were the same as in Chapter 3 (Section 3.3.4.1)

5.3.3.2 Fatty acids in feed analysis

The procedures of fatty acid composition analysis were the same as in Chapter 3 (Section 3.3.4.2).

5.3.4.3 Fatty acids in rumen analysis

Preparation and analysis of rumen fluid samples were the same as in Chapter 3 (Section 3.3.4.3).

5.3.3.4 Volatile fatty acids and Ammonia nitrogen analysis

Ruminal volatile fatty acids (VFA) and ammonia N were determined in rumen fluid samples as in Chapter 3 (Section 3.3.4.4).

5.3.3.5 Determination Degradability of DM CP NDF and ADF

Preparation of feed samples and determination of DM, CP, NDF and ADF degradabilities were the same as in Chapter 3 (Section 3.3.4.5).

5.3.4 Statistical Analysis

All data were analyzed as a 3x3 Latin squares design using ANOVA procedure of SAS (SAS, 1996).Significant differences among treatment were assessed by Duncan's new multiple range test. A significant level of $P<0.05$ was used (Steel and Torrie, 1980).

5.3.5 Experimental Site

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

5.3.6 Duration

The duration of the present experiment was from January to April 2016.

5.4 Result

5.4.1 Chemical composition of experimental diet

Chemical compositions of the concentrate, rice straw, linseed oil (LSO) and fish oil (FO) used in the experiment are show in Table 5.1. The concentrate used in this experiment contained 89.6% dry matter, 14.1% crude protein and 3.7% crude fat. Dry matter, crude protein and crude fat of rice straw were 88.7%, 2.1% and 1.8% respectively. Oils in the current study contained 100% fat.

Table 5.1 Chemical composition of the experimental diets.

Items	Concentrate	Linseed oil	Fish oil	Rice straw
Dry matter	89.6	100	100	88.7
	..... % of DM.....			
Ash	8.2			18.1
Crude protein	14.1			2.1
Ether extract	3.7	100	100	1.8
Crude fiber	15.2			40.6
Neutral detergent fiber	40.1			76.1
Acid detergent fiber	20.4			53.2
Acid detergent lignin	4.9			17.1

[1]kg/100 kg concentrate: 30 dried cassava chip, 4 ground corn, 10 rice bran, 25 palm meal, 15 coconut meal, 6 dried distillers grains with solubles, 0.5 sodium bicarbonate, 6 molasses, 1 dicalciumphosphate (16%P), 1.5 urea, 0.5 salt and 0.5 premix. Premix: provided per kg of concentrate including vitamin A, 5,000 IU; vitamin D3, 2,200 IU; vitamin E, 15 IU; Ca, 8.5 g; P, 6 g; K, 9.5 g; Mg, 2.4 g; Na, 2.1 g; Cl, 3.4 g; S, 3.2 g; Co, 0.16 mg; Cu, 100 mg; I, 1.3 mg; Mn, 64 mg; Zn, 64 mg; Fe, 64 mg; Se, 0.45 mg.

Fatty acid compositions of the concentrate, rice straw, LSO and FO used in the experiment are shown in Table 5.2. The C18:2n-3 proportion was the major fatty acid in the LSO (53.67% of total fatty acid). FO had the highest proportion of C22:6n-3 and C20:5n-3 (30.42% and 8.03 of total fatty acid respectively). In the concentrate, C18:1n-9 (29.58% of total fatty acid) and C12:0 (22.72% of total fatty acid) were the main fatty acids (FA) while the main FA in rice straw was C16:0 (45.70% of total fatty acid).

Table 5.2 Fatty acid compositions (g/100 g of total fatty acids) of concentrate, rice straw and oils used in the experiment.

Fatty acids	Concentrate	Rice straw	Linseed oil	Fish oil
C12:0	22.72	6.31	2.90	2.15
C14:0	7.80	8.25	0.35	4.40
C16:0	16.54	45.70	22.75	28.01
C18:0	2.50	0.15	0.22	6.10
C18:1n-9	29.58	24.74	14.90	14.40
C18:2n-6	17.19	11.35	2.73	1.73
C18:3n-3	0.25	ND	53.67	0.93
C20:5n-3	ND	ND	ND	8.03
C22:6n-3	ND	ND	ND	30.42
Others	3.42	3.50	2.48	3.73

ND = Not detected; Others = C8:0 + C15:0 + C20:1 + C21:0 + C23:0

5.4.2 Intake of main components and major fatty acids

The current study was designed to limit concentrate and rice straw consumptions and to control the ratio of concentrate to rice straw at 60:40 (DM basis). (Table 5.3) All cattle consumed all concentrate, rice straw and oils offered.

The present study found that increases in the proportion of fish oil linearly increased the intake of C14:0 (16.56, 17.78 and 18.99 g/d), C16:0 (83.54, 85.12 and 86.70 g/d), C18:0 (7.30, 9.06 and 10.82 g/d), C20:5n-3 (4.82, 7.23 and 9.64 g/d) and C22:6n-3 (18.31, 27.47 and 36.62 g/d) while reduction in the proportion of linseed oil reduced the intake of C18:1n-9 (75.22, 75.07 and 74.92 g/d), C18:2n-6

(31.46, 31.16 and 30.86 g/d) and C18:3n-3 (65.29, 49.47 and 33.65 g/d respectively) (Table 5.3). However, total fatty acid intakes were similar in all treatments.

Table 5.3 DM, CP, fat and fatty acid intakes of experimental cattle.

Items	LSO+FO at 3% of total feed DM			SEM	P-value
	2:1 w/w	1:1 w/w	(1:2 w/w)		
DM intake (kg/d)					
Concentrate	3.58	3.58	3.58	-	-
Rice straw	2.13	2.13	2.13	-	-
Oil	0.18	0.18	0.18	-	-
Total	5.89	5.89	5.89	-	-
CP intake (g/d)					
Concentrate	505	505	505	-	-
Rice straw	44	44	44	-	-
Total	549	549	549	-	-
Fat intake (g/d)					
Concentrate	133	133	133	-	-
Rice straw	43	43	43	-	-
Oil	180	180	180	-	-
Total	356	356	356	-	-

LSO = linseed oil; FO = fish oil

Table 5.3 DM, CP, fat and fatty acid intakes of experimental cattle (cont.).

Items	LSO+FO at 3% 0f total feed DM			SEM	P-value
	2:1 w/w	1:1 w/w	1:2 w/w		
Fatty acid intake (g/d)					
C12:0	27.99[a]	27.81[b]	27.65[c]	0.001	<0.001
C14:0	12.42[c]	13.34[b]	14.24[a]	0.001	<0.001
C16:0	62.66[c]	63.84[b]	65.03[a]	0.001	<0.001
C18:0	5.48[c]	6.80[b]	8.12[a]	0.001	<0.001
C18:1n-9	56.42[a]	56.30[b]	56.19[c]	0.001	<0.001
C18:2n-6	23.60[a]	23.37[b]	23.15[c]	0.001	<0.001
C18:3n-3	48.97[a]	37.10[b]	25.24[c]	0.001	<0.001
C20:5n-3	3.62[c]	5.42[b]	7.23[a]	0.001	<0.001
C22:6n-3	13.73[c]	20.60[b]	27.47[a]	0.001	<0.001
Others	8.30[c]	8.58[b]	8.86[a]	0.001	<0.001
Total	263	263	263	0.008	0.500

LSO = linseed oil; FO = fish oil; SEM = standard error of the mean

Others = C8:0 + C15:0 + C20:1 + C21:0+ C23:0

[abc] Within a row means without a common superscript letter differ

5.4.3 Fatty acid profile in rumen content

At 2 h after feeding, cattle on 1:1 LSO+FO at 3% of feed DM had significantly higher ruminal proportion of C12:0 ($P<0.05$) than other cattle while cattle on 1:2 LSO+FO at 3% of feed DM contained highest ruminal proportion of C22:6n-3, followed by cattle on 1:1 LSO+FO at 3% of feed DM and cattle on 2:1 LSO+FO at 3% of feed DM respectively (Table 5.4). At 4 h post-feeding, the proportion of ruminal

C18:1n-9t was highest in cattle fed 1:1 LSO+FO at 3% of feed DM, followed by in cattle received 1:2 and 2:1 LSO+FO at 3% of feed DM respectively whereas the proportion of ruminal C20:5n-3 and C22:6n-3 was significantly higher ($P<0.05$) in cattle fed 1:2 LSO+FO at 3% of feed DM than those cattle on 1:1 and 2:1 LSO+FO at 3% of feed DM. However, at 6 h after feeding, there were no significant differences in the proportions of all ruminal fatty acids measured.

Table 5.4 Effect of different ratio of linseed oil in combination with fish oil supplementation on ruminal fatty acid profile in fistulated cattle (g/100g fatty acids).

Items	LSO+FO at 3% of total feed DM			SEM	P-value
	2:1 w/w	1:1 w/w	1:2 w/w		
Pre - feeding					
C12:0	5.54	5.91	6.80	0.183	0.193
C14:0	5.37	6.92	5.96	0.150	0.329
C16:0	32.38	32.01	31.86	0.292	0.380
C18:0	50.16	48.94	49.09	0.529	0.599
C18:1n-9	3.66	3.12	3.43	0.093	0.265
C18:2n-6	2.89	3.08	2.85	0.216	0.875

LSO = linseed oil; FO = fish oil; SEM = standard error of the mean

Table 5.4 Effect of different ratio of linseed oil in combination with fish oil supplementation on ruminal fatty acid profile in fistulated cattle (g/100g fatty acids) (cont.).

Items	LSO+FO at 3% of total feed DM			SEM	P-value
	2:1 w/w	1:1 w/w	1:2 w/w		
2h after feeding					
C12:0	4.80[b]	5.23[a]	4.79[b]	0.090	0.041
C14:0	4.82	5.66	5.93	0.473	0.181
C16:0	25.59	28.13	29.44	3.854	0.564
C18:0	7.68	8.07	7.17	2.044	0.871
C18:1n-9	6.69	6.06	6.57	0.954	0.773
C18:2n-6	1.28	1.13	0.84	0.143	0.120
C18:2n-6	7.41	5.72	5.30	1.139	0.260
C18:3n-3	4.43	4.29	4.71	0.115	0.399
t11-C18:1	29.27	28.78	22.04	4.419	0.285
c9,t11-C18:2	1.94	0.42	1.3	2.640	0.800
t10,c12-C18:2	0.86	0.43	2.28	0.718	0.155
C20:5n-3	1.31	1.57	1.11	0.328	0.410
C22:6n-3	3.92[c]	4.51[b]	8.52[a]	0.323	0.045

LSO = linseed oil; FO = fish oil; SEM = standard error of the mean

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

Table 5.4 Effect of different ratio of linseed oil in combination with fish oil supplementation on ruminal fatty acid profile in fistulated cattle (g/100g fatty acids) (cont.).

Items	LSO+FO at 3% of total feed DM			SEM	P-value
	2:1 w/w	1:1 w/w	1:2 w/w		
4h after feeding					
C12:0	5.10	5.25	4.82	1.193	0.908
C14:0	5.97	5.13	5.63	0.844	0.573
C16:0	30.04	27.19	31.73	4.023	0.506
C18:0	8.62	6.80	8.23	2.435	0.683
C18:1n-9	5.38	5.33	4.67	0.469	0.316
C18:2n-6	4.53	2.75	1.00	1.552	0.204
C18:3n-3	4.11	3.56	3.71	0.170	0.181
t11-C18:1	26.95^{c}	36.43^{a}	31.04^{b}	2.441	0.039
c9,t11-C18:2	1.31	2.02	1.39	1.131	0.740
c9,c11-C18:2	1.38	0.00	0.00	1.383	0.500
t10,c12-C18:2	2.53	0.00	0.00	2.530	0.500
t9,t11-C18:2	0.00	0.00	0.62	0.620	0.500
C20:5n-3	0.26^{c}	0.55^{b}	0.83^{a}	0.114	0.049
C22:6n-3	4.00^{c}	4.99^{b}	6.33^{a}	0.185	0.040

LSO = linseed oil; FO = fish oil; SEM = standard error of the mean

^{abc} Within a row means without a common superscript letter differ.

Table 5.4 Effect of different ratio of linseed oil in combination with fish oil supplementation on ruminal fatty acid profile in fistulated cattle (g/100g fatty acids) (cont.).

Items	LSO+FO at 3% of total feed DM			SEM	P-value
	2:1 w/w	1:1 w/w	1:2 w/w		
6h after feeding					
C12:0	4.79	5.08	4.24	0.355	0.187
C14:0	6.05	6.22	4.47	1.413	0.416
C16:0	29.39	31.32	33.18	1.425	0.158
C18:0	8.91	7.45	7.92	1.661	0.624
C18:1n-9	3.35	3.95	3.96	0.286	0.186
C18:2n-6	4.50	3.34	2.75	0.747	0.189
C18:3n-3	0.59	0.47	0.36	0.480	0.856
t11-C18:1	37.13	35.92	37.26	4.074	0.967
C20:5n-3	1.58	1.57	1.54	0.231	0.969
C22:6n-3	3.71	4.68	4.24	1.181	0.661

LSO = linseed oil; FO = fish oil; SEM = standard error of the mean

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

5.4.4 Ruminal Fermentation

Feeding all ratios of LSO+FO did not influence ruminal pH, NH_3-N, VFAs and A:P ratio at all h after feeding.

Table 5.5 Effect of different ratio of linseed oil in combination with fish oil supplementation on pH, ammonia nitrogen (mg/100 ml) and volatile fatty acids (mol/100mol) in fistulated cattle.

Items	LSO+FO at 3% of total feed DM			SEM	P-value
	2:1 w/w	1:1 w/w	1:2 w/w		
Pre - feeding					
pH	6.87	6.89	6.87	0.052	0.988
NH_3N	11.68	12.44	11.20	0.272	0.358
Acetic acid	64.70	64.60	64.80	0.486	0.986
Propionic acid	22.90	22.40	22.47	0.310	0.773
Butyric acid	12.40	12.90	12.73	0.173	0.529
A:P ratio	5.21	5.03	5.18	0.107	0.619
2h after feeding					
pH	6.54	6.51	6.62	0.017	0.223
NH_3N	22.82	23.64	21.98	1.042	0.826
Acetic acid	66.22	63.96	66.80	1.804	0.805
Propionic acid	23.05	24.87	22.79	1.458	0.831
Butyric acid	10.73	11.27	10.41	0.471	0.795
A:P ratio	2.97	2.58	2.99	0.294	0.776

LSO = linseed oil; FO = fish oil; A:P ratio = acetate: propionate ratio

SEM = standard error of the mean

Table 5.5 Effect of different ratio of linseed oil in combination with fish oil supplementation on pH, ammonia nitrogen (mg/100 ml) and volatile fatty acids (mol/100mol) in fistulated cattle (cont.).

Items	LSO+FO at 3% of total feed DM			SEM	P-value
	2:1 w/w	1:1 w/w	1:2 w/w		
4 h after feeding					
pH	6.46	6.33	6.41	0.037	0.484
NH_3N	8.13	8.12	8.38	0.254	0.895
Acetic acid	64.53	66.90	65.18	1.169	0.732
Propionic acid	23.92	21.73	24.19	1.052	0.644
Butyric acid	11.55	11.37	10.63	0.298	0.525
A:P ratio	2.76	3.09	2.71	0.170	0.669
6h after feeding					
pH	6.50	6.44	6.31	0.063	0.572
NH_3N	6.71	5.46	6.23	0.413	0.522
Acetic acid	66.95	65.03	64.19	0.565	0.322
Propionic acid	21.15	23.20	24.09	0.285	0.096
Butyric acid	11.89	11.77	11.72	0.281	0.965
A:P ratio	3.17	2.81	2.70	0.051	0.114

LSO = linseed oil; FO = fish oil; A:P ratio = acetate: propionate ratio

SEM = standard error of the mean

5.4.5 Degradability of DM CP NDF and ADF

The readily soluble fraction (*a*) and the potentially degradability fraction (*b*) of concentrate and rice straw DM were unaffected by all ratios of LSO+ FO at 3% of total feed DM. As a result, the calculated effective DM degradability was also unaffected (P>0.05) (Table 5.6). However, the dry matter degradation of rice straw at out flow rate 0.02 /h tended to reduce when 1:1 w/w LSO+FO was supplied (P=0.069) (Table 5.6).

Different ratios of LSO+FO had no effect on crude protein degradation of concentrate at all out flow rate (P>0.05) (Tabel 5.7), however, the rate constant of potential degradation tended to reduce (P=0.073) when 1:1 w/w LSO+FO was added to the diet. (Table 5.7).

Supplementation of 1:1 w/w LSO+FO reduced ADF potential degradability of rice straw (P<0.05) when compared to 2:1 w/w and 1:2 w/w LSO+FO (Table 5.8), however, no significant differences were found on ADF and NDF effective degradability at all out flow rates (Table 5.8).

Table 5.6 Effect of different ratio of linseed oil in combination with fish oil supplementation on dry matter degradability (DMD) of concentrate and rice straw in fistulated cattle.

Item	LSO+FO at 3% of total feed DM			SEM	P-Value
	2:1 w/w	1:1 w/w	1:2 w/w		
Dry matter degradability of concentrate					
a	24.73	22.50	22.23	2.754	0.442
b	43.53	43.20	43.50	2.098	0.997
a + *b*	68.28	65.70	65.73	2.342	0.648
c, per h	0.195	0.146	0.209	0.025	0.126
dg, 0.02/h	0.63	0.64	0.63	0.010	0.878
dg, 0.05/h	0.59	0.58	0.59	0.010	0.882
dg, 0.08/h	0.55	0.54	0.56	0.010	0.868
Dry matter degradability of rice straw					
a	16.07	15.03	16.27	0.552	0.676
b	50.33	49.50	47.87	1.764	0.855
a + *b*	66.40	64.53	64.13	1.372	0.794
c, per h	0.017	0.021	0.024	0.002	0.457
dg, 0.02/h	0.43	0.40	0.43	0.002	0.069
dg, 0.05/h	0.30	0.29	0.30	0.003	0.388
dg, 0.08/h	0.26	0.24	0.26	0.003	0.366

LSO = linseed oil; FO = fish oil; SEM = standard error of the mean; *a* = the intercept of the degradation curve at time zero; *b* = the potential degradability of the component; *c* = the rate constant for the degradation of '*b*'.

Table 5.7 Effect of different ratio of linseed oil in combination with fish oil supplementation on crude protein degradability (CPD) of concentrate in fistulated cattle.

Item	LSO+FO at 3% of total feed DM			SEM	P-Value
	2:1 w/w	1:1 w/w	1:2 w/w		
Crude protein degradability of concentrate					
a	22.39	22.47	23.17	2.004	0.131
b	55.98	55.80	52.47	1.125	0.202
a + *b*	78.27	78.27	75.63	0.659	0.360
c, per h	0.308	0.280	0.324	0.032	0.073
dg, 0.02/h	0.76	0.74	0.75	0.010	0.756
dg, 0.05/h	0.74	0.72	0.73	0.008	0.600
dg, 0.08/h	0.73	0.70	0.71	0.008	0.521

LSO = linseed oil; FO = fish oil; SEM = standard error of the mean;

a = the intercept of the degradation curve at time zero;

b = the potential degradability of the component;

c = the rate constant for the degradation of '*b*'.

Table 5.8 Effect of different ratio of linseed oil in combination with fish oil supplementation on neutral detergent fiber degradability (NDFD) and acid detergent fiber (ADFD) of rice straw in fistulated cattle.

Item	LSO+FO at 3% of total feed DM			SEM	P-Value
	2:1 w/w	1:1 w/w	1:2 w/w		
Neutral detergent fiber degradability of rice straw					
a	12.33	12.07	12.63	0.506	0.883
b	52.28	51.40	50.33	0.371	0.973
a + *b*	64.62	63.47	62.96	0.395	0.864
c, per h	0.020	0.018	0.017	0.001	0.693
dg, 0.02/h	0.37	0.38	0.40	0.003	0.128
dg, 0.05/h	0.24	0.26	0.26	0.003	0.269
dg, 0.08/h	0.20	0.21	0.21	0.003	0.103
Acid detergent fiber degradability of rice straw					
a	5.57	6.87	5.60	0.262	0.272
b	43.17[a]	37.90[b]	43.83[a]	0.416	0.046
a + *b*	48.73[a]	44.77[b]	49.48[a]	0.225	0.023
c, per h	0.059	0.058	0.045	0.001	0.079
dg, 0.02/h	0.37	0.35	0.35	0.005	0.261
dg, 0.05/h	0.28	0.26	0.25	0.004	0.182
dg, 0.08/h	0.23	0.22	0.21	0.003	0.103

LSO = linseed oil; FO = fish oil; SEM = standard error of the mean ;*a* = the intercept of the degradation curve at time zero; *b* = the potential degradability of the component; *c* = the rate constant for the degradation of '*b*'.

5.5 Discussion

5.5.1 Fatty Acid Profile in Ruminal Content

Linear increases in the ruminal proportion of C20:5n-3 and C22:6n-3 of cattle received fish oil at 4 h post-feeding reflected the higher intake of C20:5n-3 and C22:6n-3 from fish oil since fish oil contained high proportion of these 2 fatty acids (Table 5.2). Similar response was previously reported (Kim et al., 2008) and observed that supplementation of 2.3% and 6.9% fish oil linearly increased C20:5n-3 and C22:6n-3 (from 0.27 to 0.48 and 0.83 mg and from 0.14 to 0.39 and 1.01 mg respectively). Similarly, Palmquist and Griinari (2006) added 0, 0.33, 0.67 and 1.00% fish oil to dairy cows diets and observed a linear increase in the concentration of C20:5n-3 and C22:6n-3 in milk with increasing fish oil. However, Chow et al. (2004) reported that fish oil inclusion did not affect average lipolysis and release of the individual PUFA examined. After 24 h, release of C20:5n-3 and C22:6n-3 from TAG was only 0.7 (±0.073), comparable to data found by Dohme et al. (2003) when incubating between 1.7 and 22.8 mg of C20:5n-3 + C22:6n-3.The investigation of Chow et al. (2004) found that lipolysis of EPA C20:5n3 and C22:6n3 was dose independent, which also confirms findings of Dohme et al. (2003). Lipolysis of C20:5n-3 and C22:6n-3 was always lower than average lipolysis and lipolysis of C18:2n-6 and C18:3n-3 with values increasing respectively from 0.24 and 0.21 after 6 h to 0.77 and 0.74 after 24 h. (Chow et al.,2004)

In the current study, supplementation of 2:1, 1:1 and 1:2 w/w linseed oil in combination with fish oil at 3% of feed DM did not affect the ruminal proportion of C18:2n-6 and C18:3n-3 at all times after feeding. The intakes of C18 carbon atom were significant different, however, when observed no change in the fatty acid profile

in the rumen content. In *in vitro* study, Chow et al. (2004) showed that the apparent bio-hydrogenation of C18:2n-6 and C18:3n-3 was not affected by fish oil addition. Free EPA and DHA, however, were bio-hydrogenated to a lesser extent after 24 h compared to 6 h, and hydrogenation of C22:6n-3 was always lower than of C20:5n-3. For both 20:5n-3 and C22:6n-3, hydrogenation was dose dependent, with the lower level of FO inclusion generally subject to more extensive bio-hydrogenation. Lipolysis of C18 PUFA did not exceed 0.41 after 6 h, while apparent bio-hydrogenation reached more than 0.70. This confirms earlier findings that lipolysis is the rate limiting process (Chow et al., 2003). As with lipolysis, FO inclusion had no effect on apparent bio-hydrogenation of C18:3n-3 and C18:2n-6, and as a consequence, disappearance of these FA occurred to the same extent. Similar *in vitro* observations were also reported by Gulati et al. (1999) when incubating cottonseed supplemented with or without fish oil. *In vivo* experiments of AbuGhazaleh et al. (2002) showed no significant difference in ruminal C18:2n-6 content of animals on a diet containing extruded soybean or fish oil/extruded soybean. Similarly, Wachira et al. (2000) reported no difference in duodenal flow of C18:3n-3 when offering linseed or linseed/fish oil supplemented.

The concentration of C18:0 at all times after feeding in this study was similar among treatments. It was clearly that fish oil inhibited complete bio-hydrogenation to C18:0 and this effect is dose dependent. This is in line with *in vivo* observations of Wachira et al. (2000), reporting a significantly higher C18:0 duodenal flow in sheep fed linseed oil diet compared to linseed/fish oil. The current study found an increase in *t11*-C18:1 in the rumen at 4h post-feeding when supplemented 1:1 linseed oil in combination with fish oil at 3% of feed DM compared with 1:2 linseed oil+fish oil and 2:1 linseed oil+fish oil. Fish oil is a potent inhibitor of the conversion of *t11*-C18:1 to C18:0 and the combination of linseed and fish oils would

complementarily maximize *t11*-C18:1 production, the primary source of CLA in milk fat (Palmquist et al., 2005). The effect of fish oil on trans-18:1 is curvilinear, whereas the effect on *de novo* synthesis of fatty acids is linear. Fish oil replaced unsaturated C18 fatty acids of sunflower oil linearly and reduced the C18 fatty acid inhibition on *de novo* synthesis (Baumgard et al., 2000). Chow et al. (2004) showed that increasing of fish oil proportion in combination oil found a highly significant accumulation of *t11*-C18:1. In addition, Wachira et al. (2000) reported a 63% increase of duodenal flow of *trans* C18:1 when supplementing fish oil in sheep diets containing linseed, which is in accordance with the 54.9% increase of *t11*-C18:1 in *in vitro* study with 4% LO+FO. Comparably, Donovan et al. (2000) reported a continuous and gradual increase in milk *t11*-C18:1 and c9t11CLA with increasing proportions of EPA+DHA (0.3-5.78 g/100 g total dietary FA), which are in line with EPA + DHA proportions in substrates (0, 2.5 and 5 of EPA + DHA per 100 g total FA).

5.5.2 Ruminal Fermentation

Enriching cow diets with C18:3n-3, C20:5n-3 and C22:6n-3 to increase the long-chain fatty acid content of milk entails some problems. The benefits of adding PUFA-rich fat to the diets of cows may be limited by its negative effect on fermentation in the rumen, mainly through reduction of the cellulolytic activity of rumen microflora. Supplemental fat may also cause rumen acidosis. The most important problem in terms of the possibility of enriching milk with C20:5n-3 and C22:6n-3 is, however, the extensive bio-hydrogenation of these acids in the rumen. Bio-hydrogenation of polyunsaturated fatty acids in the rumen is reduced with high concentrate diets (Doreau and Ferlay, 1994), causing a lower conversion of *trans*-C18:1 isomers to C18:0. This response was independently shown to be associated with shifts in bacterial populations (Latham et al., 1972) and decreased pH (Kalscheur et al.,

1997). However, the current study found no significant difference in ruminal pH at all times after feeding when different ratios of combination oils were fed. Loor et al. (2004) suggested that ruminal pH would not have always been observed *in vivo* despite reduced bio-hydrogenation and might indicate that other unknown factors such as dietary starch amount and degradation rate, buffering capacity contributed to accumulation of bio-hydrogenation intermediates in the rumen with high-concentrate diets. The study from Keady and Mayne (1999) found supplemented fish oil at 0, 150, 300 and 450 g/d had no effect on ruminal pH and the similar result was also observed (Toral et al., 2009). Toral et al. (2009) supplemented combination oil containing fish oil at different levels and observed that the ruminal pH was not affected by oil supplementation which is in agreement with previous *in vivo* studies using different lipid sources, including fish and sunflower oils (Fievez et al., 2003; Beauchemin et al., 2007). In contrast, Shingfield et al. (2003) reported a higher pH when fish oil was included in the diet of cows, which was attributed to associated decreases in DM intake that were not observed in our study.

Ruminal ammonia nitrogen in this study was not significantly affected by oil supplements at all times post-feeding which is similar to the work of Gudla et al. (2012) who added fish oil in combination with other oils and reported no significant difference in ammonia nitrogen when compare to non-oil supplement. Similarly, Toral et al. (2009) fed fish oil at 3 g and 10 g per day in sheep and found no effect on ammonia nitrogen in the rumen when compare to control group. Keady and Mayne (1999) supplemented fish oil at 150g/d and 300 g/d and previously showed no significant difference in ruminal ammonia nitrogen concentration, however, when supplemented up to 450 g/d, ruminal ammonia nitrogen was increased. They suggested that the lack of a significant effect on the concentrations of either ammonia or those

VFA, originating from the deamination of some amino acids (valerate and branched-chain VFA).

Different proportions of fish oil and linseed oil in the present study did not affect ruminal volatile fatty acid concentration. Similar result was also reported (Toral et al., 2009). Previously, Doreau and Chilliard (1997) offered fish oil in one feed daily and concluded that the inclusion of 200 g fish oil had no effect on rumen fermentation patterns whereas inclusion of 400 g fish oil in one feed reduced the molar proportions of acetate and increased the molar proportion of propionate. At 6 h after feeding in the present study, the molar proportion of propionate tended to increase ($P = 0.096$) when cattle received high proportion of fish oil. According to Keandy and Mayne (1999), supplementation of fish oil at 150 and 300 g/d showed no effect on molar proportion of propionic acid but when supplemented at 450 g/d molar proportion of propionate was increased but molar proportion of acetate was reduced. A decreased ruminal acetate concentration is a common response to the addition of fish oil (Doreau and Chilliard, 1997; Fivez et al., 2003; Toral et al., 2009) or linoleic acid-rich sources to the diet (Zhang et al., 2008). This trend supports the hypothesis that polyunsaturated fatty acids may exert an inhibitory effect on acetate-producing bacteria (Toral et al., 2009). However, these bacteria are predominantly fibrolytic, and in the present experiment there was no effect of fish oil supplementation on fiber digestibility, which is consistent with the findings of other authors (Lee et al., 2008; Shingfield et al., 2010; Toral et al., 2009, 2010). Furthermore, Doreau and Chilliard (1997) reported a reduction in ruminal acetate concentration when fish oil was fed. A decrease in acetate concentration might contribute to a reduction in mammary *de novo* fatty acid synthesis, which requires acetate as a precursor. (Doreau and Chilliard, 1997).

5.5.3 Degradability of DM, CP, NDF and ADF

The dry matter degradations of concentrate and rice straw in the current study were unaffected by different ratios of combination oils which is in agreement with Keady and Mayne. (1999) who supplied fish oil from 150 g/d up to 450 g/d in steer and observed no significant differences in disappearances of DM, NDF and ADF. Annet et al. (2008) added fish oil at 40g/d in ewes and showed similar results that fish oil had no effect on degradations of DM, CP, NDF and ADF. However, fish oil at 40g/d tended to increase digestibility coefficient of ADF (P=0.08). The result was in consistence to the current study that 1:1 w/w LSO+FO significantly decreased potential degradability of rice straw (Table 5.8). Liu et al. (2012) documented that bacterial populations that are relevant for fiber digestion and bio-hydrogenation have been found to be sensitive to PUFA. Therefore, the impact of PUFA supplementation on ruminal bacteria should be made by examining specific bacterial species rather than the total number of bacteria. Furthermore, Abuelfatah et al. (2016) concluded that feeding linseed oil significantly decreased the population of *F. succinogenes*, *R. flavefaciens* and *R. albus*, which is the most important cellulolytic bacteria. When supplementation 1:1 w/w LSO+FO tended to decrease potential dry matter degradability at out flow rate 0.02 /h (Table 5.6) and the rate constant for the potential degradation of ADF component (Table 5.8). The tendency to decrease the rate constant might have compensated for the reduced rumen fiber digestion (Sutton et al., 1983; Van Nevel et al., 1993). Additionally, Yang et al. (2009) found that cows fed supplemental oil had considerably lower numbers of cellulolytic bacteria, which likely accounted for the lower ruminal digestibility of NDF (56% v. 51%) and ADF (53% v. 50%). The present study did not found the detrimental effect of different ratios of oil on dry matter and crude protein degradation which is in agreement with others (Lee et

al., 2008; Shingfield et al., 2010; Toral et al., 2009, 2010; Ferreira et al. 2014).

5.6 Conclusion

Supplementation of 1:2 w/w linseed oil in combination with fish oil at 3% of total feed DM significantly increased C20:5n3 and C22:6n3 in the rumen content ($P<0.05$) whereas 1:1 w/w linseed oil mixed with fish oil showed a significant increase in the concentration of *t11*-C18:1. The current study did not found differences in pH, ammonia nitrogen and volatile fatty acids among dietary treatments. However, at 6 h after feeding, 2:1 w/w linseed oil and fish oil tended to increase molar proportion of propionic acid. Feeding 1:1 w/w LSO+FO at 3% of total feed DM reduced ADFD ($P>0.05$) but had no significant differences in DMD, CPD and NDFD ($P>0.05$).

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CHAPTER VI

RUMINAL BIO-HYDROGENATION AND FERMENTATION IN RESPONSE TO DIFFERENT RATIO OF OIL RICH IN OMEGA-6 FATTY ACID AND FISH OIL IN FISTULATED CATTLE'S DIETS

6.1 Abstract

The objective of current experiment was to investigate the effects of different ratio of soybean oil and fish oil addition on ruminal bio-hydrogenation and fermentation in fistulated cattle. Three fistulated cattle were assigned into 3 treatments in a 3×3 Latin square design. All cattle were fed approximately 4 kg/d of 14% CP concentrate and 2.4 kg/d of rice straw. Treatments were: 1) supplemented soybean oil in combination with fish oil 2:1 w/w at 3% of total feed DM; 2) supplemented soybean oil in combination with fish oil 1:1 w/w at 3% of total feed DM; 3) supplemented soybean oil in combination with fish oil 1:2 w/w at 3% of total feed DM. Each period in the Latin square design lasted 21 d, with the first 7 d for adaptation. The results revealed that 2:1 w/w soybean oil in combination with fish oil at 3% of total feed DM significantly increased *t11*-C18:1 at 2 and 6 h post feeding and *c9, t11*-C18:2 at 2 and 4h post feeding. In addition, 2:1 w/w soybean oil mixed with fish oil significantly increased the concentration C20:5n-3 and C22:6n-3 in the rumen ($P<0.05$). The present study did not found differences in pH at all times post feeding but at 2h post

feeding the ruminal ammonia nitrogen was higher when supplemented high proportion of fish oil. High proportion of fish oil also significantly decreased the molar proportion of acetic acid but increased the molar proportion of propionic acid at 4h post feeding. The degradation of DM, CP, NDF and ADF was unaffected by oil addition.

6.2 Introduction

Conjugated linoleic acid (CLA) is one of the representative functional nutrients due to the beneficial human health such as tumor reduction (Ha et al., 1987), reducing risk factors for atherosclerosis (Lee et al., 1994), and enhancing immunity (Miller et al., 1994). The CLAs are naturally produced during the bio-hydrogenation of C18-polyunsaturated fatty acids such as C18:2n-6 or C18:3n-3 by ruminal microorganisms. The CLA in the ruminant body fat can also be synthesized through the process of desaturation of *t11*-C18:1 which is a major intermediate of ruminal hydrogenation (Bauman and Griinari, 2003). Increases in *t11*-C18:1 and *c9,t11*-C18:2 can be obtained by feeding PUFA rich oils/oilseeds including soybean (Ludden et al., 2009), sunflower (Noci et al., 2007) or linseed (He et al., 2011).

When the cattle receive oil or feed containing high fat, first step is lipolysis. Lipids extracted from the feed can be largely hydrolyzed by enzymes of rumen bacteria: *Anaerovibrio lipolytica* and *Butyrivibrio fibrisolvens* (Harfoot and Hazlewood, 1997). The next step in the metabolism of lipids in the rumen is hydrogenation of unsaturated 18-carbon fatty acids into C18:0. The rate of hydrogenation increases with the degree of unsaturation (Harfoot and Hazlewood, 1997). The ruminal bacteria involved in hydrogenation have been classified into two groups, A and B (Kemp and Lander, 1984). For complete hydrogenation of PUFA,

both groups of bacteria are usually necessary. Group A comprises a plurality of bacteria able to hydrogenate PUFA into *t11*-C18:1, however, group B mainly in hydrogenation of C18:1n-9 into C18:0.

In chapter 4, the result showed that supplementation of soybean oil in combination with fish oil increased the concentration of *t11*-C18:1 but reduced the concentration of C18:0 when compare to control and soybean oil alone cattle. Dohme et al. (2003) founded that fish oil lowered ruminal bio-hydrogenation when compare to soybean oil. It was hypothesized that fish oil inhibited the complete bio-hydrogenation of C18:2n-6 and C18:3n-3 derived from sources other than fish oil (Bauman et al., 1999; AbuGhazaleh et al., 2002; Whitlock et al., 2002). AbuGhazaleh et al. (2003a) concluded that supplementing dairy cows' diets with a combination of fish oil and other high linoleic oil sources was the most efficient dietary regimen for increasing milk *c9,t11*-C18:2.

However, unsaturated fatty acids are more toxic than saturated ones (Harfoot and Hazlewood, 1997) and a differential toxicity of different PUFA to rumen microorganisms has also been observed (Maia et al., 2007). Dietary supplementation with oils has given inconsistent results on ruminal fermentation, with detrimental consequences (Fievez et al., 2003). The results from supplementation of oil did not similar, it depend on the type and amount of oil supplemented (Wachira et al., 2000; Fievez et al., 2003; Doreau and Chilliard, 1997; Shingfield et al., 2008). Therefore, the aim of this study was to evaluate the effect of different ratio of oil rich in omega-6 fatty acid and fish oil on ruminal bio-hydrogenation and fermentation in fistulated cattle.

6.3 Materials and Methods

6.3.1 Animals and Feeding

All experimental procedures were conducted following the Ethical Principles and Guidelines for the Use of Animal issued by National Research Council of Thailand. Three fistulated cattle were assigned into 3 treatments in a 3×3 Latin square design. All cattle were fed approximately 4 kg/d of 14% CP concentrate and 2.4 kg/d of rice straw. Treatments were: 1) supplemented 2:1 w/w soybean oil (SBO) in combination with fish oil (FO) at 3%of feed DM 2) supplemented 1:1 w/w soybean oil (SBO) in combination with fish oil (FO) at 3%of feed DM; 3) supplemented 1:2 w/w soybean oil (SBO) in combination with fish oil (FO) at 3%of feed DM. All cattle also had free access to clean water and were individually housed in a free-stall unit and individually fed according to treatments. The experiment lasted for 63 days (3 periods) with 21 d in each period, the first 7 d of each period for adaptation to diets followed by 14 d for ruminal sample collection and *in sacco* disappearance trial.

6.3.2 Sample Collection

To evaluate fatty acids profile in rumen content and ruminal fermentation, the procedures of sample collection, preservation of samples and pH measurement were the same as in Chapter 3 (Section 3.3.3)

6.3.3 Laboratory Analyses

6.3.3.1 Feed chemical composition analysis

Sample collection of feeds and feed chemical composition analyses were the same as in Chapter 3 (Section 3.3.4.1)

6.3.3.2 Analysis of fatty acids in feed analysis

The procedures of fatty acid composition analysis were the same as in Chapter 3 (Section 3.3.4.2).

6.3.4.3 Analysis of fatty acids in ruminal analysis

Preparation and analysis of rumen fluid samples were the same as in Chapter 3 (Section 3.3.4.3).

6.3.3.4 Volatile fatty acids and ammonia nitrogen analysis

Ruminal volatile fatty acids (VFA) and ammonia N were determined in rumen fluid samples as in Chapter 3 (Section 3.3.4.4).

6.3.3.5 Degradability determination of DM CP NDF and ADF

Preparation of feed samples and determination of DM, CP, NDF and ADF degradabilities were the same as in Chapter 3 (Section 3.3.4.5).

6.3.4 Statistical Analysis

All data were analyzed as a 3x3 Latin squares design using ANOVA procedure of SAS (SAS, 1996). Significant differences among treatment were assessed by Duncan's new multiple range test. A significant level of $p<0.05$ was used (Steel and Torrie, 1980).

6.3.5 Experimental Site

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

6.3.6 Duration

The duration of the present experiment was from May to August 2016.

6.4 Result and Discussion

6.4.1 Chemical composition of experimental diet

The concentrate used in this study contained 89.4% of DM, 14.1% of CP and 3.4% of EE (Table 6.1) and the main components of fatty acids were C18:1n-9 (29.51 g/100 g of total fatty acids) C12:0 (22.74 g/100g of total fatty acids) and C16:0 (16.63 g/100g of total fatty acids) showed in Table 6.2. The roughage source of this experiment was rice straw containing 88.2% of DM, 2.0% of CP and 1.4% of fat. The major fatty acids were C16:0 (45.71 g/100g of total fatty acids) and C18:1n-9 (24.81 g/100g of total fatty acids) showed in Table 6.2. This study used source of omega 6 in combination with EPA and DHA sources. Soybean oil was the major source of C18:2n-6 (44.74 g/100 g of total fatty acids) and C18:1n-9 (33.87 g/100g of total fatty acids). The EPA and DHA source used in the current study was fish oil. The main fatty acids in fish oil were C22:6n-3 (30.74g/100g of total fatty acids) and C16:0 (28.02 g/100g of total fatty acids) showed in Table 6.2.

Table 6.1 Chemical composition of the experimental diets.

Items	Concentrate	Soybean oil	Fish oil	Rice straw
Dry matter	89.4	100	100	88.2
	..... % of DM.....			
Ash	8.1			18.3
Crude protein	14.1			2.0
Ether extract	3.4	100	100	1.4
Crude fiber	14.8			40.1
Neutral detergent fiber	40.4			76.3
Acid detergent fiber	22.8			53.8
Acid detergent lignin	4.2			17.5

[1]kg/100 kg concentrate: 30 dried cassava chip, 4 ground corn, 10 rice bran, 25 palm meal, 15 coconut meal, 6 dried distillers grains with solubles, 0.5 sodium bicarbonate, 6 molasses, 1 dicalciumphosphate (16%P), 1.5 urea, 0.5 salt and 0.5 premix. Premix: provided per kg of concentrate including vitamin A, 5,000 IU; vitamin D3, 2,200 IU; vitamin E, 15 IU; Ca, 8.5 g; P, 6 g; K, 9.5 g; Mg, 2.4 g; Na, 2.1 g; Cl, 3.4 g; S, 3.2 g; Co, 0.16 mg; Cu, 100 mg; I, 1.3 mg; Mn, 64 mg; Zn, 64 mg; Fe, 64 mg; Se, 0.45 mg.

Table 6.2 Fatty acid compositions (g/100 g of total fatty acids) of concentrate, rice straw and oils used in the experiment.

Fatty acids	Concentrate	Rice straw	Soybean oil	Fish oil
C12:0	22.74	6.37	0.43	2.16
C14:0	7.81	8.20	1.09	4.39
C16:0	16.63	45.71	13.74	28.02
C18:0	2.50	0.12	5.26	6.10
C18:1n-9	29.51	24.81	33.87	14.42
C18:2n-6	17.14	11.47	44.74	1.71
C18:3n-3	0.25	ND	0.35	0.93
C20:5n-3	ND	ND	ND	7.98
C22:6n-3	ND	ND	ND	30.47
Others	3.42	3.28	0.52	3.83

ND = Not detected.

Others = C8:0 + C15:0 + C20:1 + C21:0+ C23:0

6.4.2 Intake of main components and major fatty acids

The current study was designed to control feed intake by limiting feed offered to control the ratio of concentrate to roughage (60:40 w/w DM basis). The results showed no differences between treatments in concentrate, rice straw and total dry matter intakes as well as crude protein and fat intakes (Table 6.3).

For fatty acid consumption, 2:1 w/w soybean oil in combination with fish oil showed greater intake of C18:2n-6 (81.84 g/d) and C18:1n-9 (92.10 g/d). The 1:2 w/w soybean oil in combination with fish oil cattle consumed higher level of C22:6n-3

(36.56 g/d), C20:5n-3 (9.58 g/d), C16:0 (81.42 g/d) and C18:3n-3 (1.66 g/d) whereas the 1:1 w/w soybean oil/fish oil cattle ate fatty acids in the middle between 2:1 w/w soybean oil/fish oil and 1:2 w/w soybean oil/fish oil cattle. However, no difference between treatments in total fatty acid intake was observed (Table 6.3).

Table 6.3 DM, CP, fat and fatty acid intakes of experimental cattle.

Items	SBO+FO (2:1 w/w)	SBO+FO (1:1 w/w)	SBO+FO (1:2 w/w)	SEM	P-value
DM intake (kg/d)					
Concentrate	3.58	3.58	3.58	-	-
Rice straw	2.13	2.13	2.13	-	-
Oil	0.18	0.18	0.18	-	-
Total	5.89	5.89	5.89	-	-
CP intake (g/d)					
Concentrate	505	505	505	-	-
Rice straw	45	45	45	-	-
Total	550	550	550	-	-
Fat intake (g/d)					
Concentrate	132	132	132	-	-
Rice straw	38	38	38	-	-
Oil	180	180	180	-	-
Total	350	350	350	-	-

SBO = Soybean oil; FO = fish oil

Table 6.3 DM, CP, fat and fatty acid intakes of experimental cattle (cont.).

Items	SBO+FO (2:1 w/w)	SBO+FO (1:1 w/w)	SBO+FO (1:2 w/w)	SEM	P-value
fatty acids intake (g/d)					
C12:0	26.19^{c}	26.55^{b}	26.58^{a}	0.001	<0.001
C14:0	13.07^{c}	13.82^{b}	14.57^{a}	0.001	<0.001
C16:0	54.65^{c}	57.86^{b}	61.07^{a}	0.001	<0.001
C18:0	10.00^{c}	10.19^{b}	10.38^{a}	0.001	<0.001
C18:1n-9	73.45^{a}	69.08^{b}	64.70^{c}	0.001	<0.001
C18:2n-6	61.38^{a}	51.69^{b}	42.02^{c}	0.001	<0.001
C18:3n-3	0.98^{c}	1.11^{b}	1.25^{a}	0.001	<0.001
C20:5n-3	3.59^{c}	5.39^{b}	7.19^{a}	0.001	<0.001
C22:6n-3	13.71^{c}	20.57^{b}	27.42^{a}	0.001	<0.001
Others	6.54^{c}	7.28^{b}	8.03^{a}	0.001	<0.001
Total	263	263	263	0.008	0.500

SBO = Soybean oil; FO = fish oil; SEM = standard error of the mean

Others = C8:0 + C15:0 + C20:1 + C21:0+ C23:0

^{abc} Within a row means without a common superscript letter differ

6.4.3 Fatty acid profile in rumen content

At all h post-feeding, C22:6n-3 were increased with increasing FO addition and the highest was in 1:2 w/w SBO+FO cattle. At 2 after feeding, *t11*-C18:1and C18:2n-6c were significantly reduced in cattle fed high FO (1:1 and 1:2 w/w SBO+FO) while *c9,t11*-C18:2 was significantly decreased in cattle fed 1:2 w/w

SBO+FO. At 4 h after feeding, C18:0 were significantly increased with increasing FO addition. However, at 4 h after feeding, C18:2n-6 was only reduced in cattle fed 1:2 w/w SBO+FO, whereas it was similar in cattle 2:1 and 1:1 w/w SBO+FO. At 6 h post-feeding, C16:0 and C20:5n-3 were highest in cattle fed 1:2 SBO+FO, while *t11*-C18:1 was decreased with increasing FO. Other fatty acids were similar at all h after feeding.

Table 6.4 Effect of different ratio of soybean oil in combination with fish oil supplementation on fatty acid profile in fistulated cattle (g/100g fatty acids).

Fatty acids	**SBO+FO at 3% of total feed DM**			**SEM**	**P-Value**
	2:1 w/w	**1:1 w/w**	**1:2 w/w**		
Pre - feeding					
C12:0	12.81	12.23	12.41	0.177	0.516
C14:0	9.10	9.06	8.54	0.292	0.782
C16:0	34.41	33.97	34.40	0.282	0.145
C18:0	37.92	39.29	38.84	0.383	0.235
C18:1n-9	2.44	2.36	2.33	0.350	0.990
C18:2n-6	1.32	1.18	1.44	0.036	0.189
t11-C18:1	1.99	1.89	2.03	0.132	0.917

SBO = soybean oil; FO = fish oil; SEM = standard error of the mean

Table 6.4 Effect of different ratio of soybean oil in combination with fish oil supplementation on fatty acid profile in fistulated cattle (g/100g fatty acids) (cont.).

Fatty acids	SBO+FO at 3% of total feed DM			SEM	P-Value
	2:1 w/w	1:1 w/w	1:2 w/w		
2h after feeding					
C12:0	4.72	5.05	5.00	0.426	0.515
C14:0	4.46	5.76	5.34	0.539	0.174
C16:0	24.46	31.04	27.45	3.225	0.240
C18:0	7.28	8.03	7.53	0.631	0.858
C18:1n-9	4.60	3.78	5.63	0.576	0.194
C18:2n-6t	1.03^{b}	0.86^{b}	4.30^{a}	0.091	0.025
C18:2n-6	2.90^{a}	2.58^{ab}	2.41^{b}	0.046	0.047
C18:3n-3	0.59	0.66	0.61	0.194	0.487
t11-C18:1	39.16^{a}	28.60^{b}	29.69^{b}	1.782	0.032
c9,t11-C18:2	5.38^{a}	6.69^{a}	2.56^{b}	1.059	0.022
t10,c12-C18:2	2.53	1.19	1.19	0.460	0.775
C20:5n-3	0.69	0.71	0.68	0.371	0.446
C22:6n-3	2.20^{c}	5.05^{b}	7.61^{a}	0.677	0.048

SBO = soybean oil; FO = fish oil; SEM = standard error of the mean

^{abc} Within a row means without a common superscript letter differ.

Table 6.4 Effect of different ratio of soybean oil in combination with fish oil supplementation on fatty acid profile in fistulated cattle (g/100g fatty acids) (cont.).

Fatty acids	SBO+FO at 3% of total feed DM			SEM	P-Value
	2:1 w/w	1:1 w/w	1:2 w/w		
4h after feeding					
C12:0	4.43	4.73	4.34	0.588	0.734
C14:0	4.50	5.84	4.29	0.673	0.175
C16:0	25.57	24.87	28.93	1.701	0.142
C18:0	6.70^{c}	7.92^{b}	8.14^{a}	0.126	0.017
C18:1n-9	5.78	4.91	4.62	0.560	0.222
C18:2n-6	6.17^{a}	1.55^{b}	1.89^{b}	0.808	0.043
C18:3n-3	0.37	0.76	0.33	0.185	0.141
t11-C18:1	36.56	40.15	39.60	2.437	0.523
c9,t11-C18:2	6.86^{a}	3.90^{b}	0.94^{c}	1.581	0.434
C20:5n-3	0.09^{b}	0.38^{ab}	0.59^{a}	0.187	0.564
C22:6n-3	2.97^{c}	4.99^{b}	6.33^{a}	0.662	0.043

SBO = soybean oil; FO = fish oil; SEM = standard error of the mean

^{abc} Within a row means without a common superscript letter differ.

Table 6.4 Effect of different ratio of soybean oil in combination with fish oil supplementation on fatty acid profile in fistulated cattle (g/100g fatty acids) (cont.).

Fatty acids	SBO+FO at 3% of total feed DM			SEM	P-Value
	2:1 w/w	1:1 w/w	1:2 w/w		
6h after feeding					
C12:0	4.90	4.68	3.48	1.486	0.555
C14:0	4.93	5.25	5.31	0.770	0.824
C16:0	27.77^{b}	31.20^{ab}	34.43^{a}	1.392	0.045
C18:0	7.96	7.32	8.73	0.821	0.312
C18:1n-9	3.80	4.07	4.94	0.659	0.290
C18:2n-6	0.88	0.98	1.04	0.068	0.166
t11-C18:1	46.56^{a}	41.04^{b}	34.06^{c}	0.575	0.002
C20:5n-3	1.23^{b}	1.42^{ab}	1.65^{a}	0.083	0.044
C22:6n-3	1.97^{c}	4.04^{b}	6.36^{a}	0.087	0.037

SBO = soybean oil; FO = fish oil; SEM = standard error of the mean

^{abc} Within a row means without a common superscript letter differ.

6.4.4 Ruminal Fermentation

There were no significant differences in ruminal pH at all h post-feeding and in ruminal NH_3-N at 4 and 6 h after feeding, however, NH_3-N concentration was significantly increased in cattle fed 1:2 w/w SBO+FO at 2 h post-feeding (Table 6.5). At 2 h after feeding, there were no significant differences in molar proportions of acetate, propionate and butyrate, however, acetate: propionate ratio was significantly increased in cattle fed 1:1 w/w SBO+FO when compared to cattle fed 2:1 and 1:2 w/w SBO+FO. At 4 h post-feeding, molar proportion of butyrate was similar in all treatments whereas molar proportion of acetate was significantly decreased but molar proportion of propionate was significantly increased in cattle fed 1:2 SBO+FO resulting in significant degreased acetate:propionate ratio. The molar proportion of propionate was significantly increased in cattle fed 1:2 SBO+FO at 6 h after feeding (Table 6.5).

Table 6.5 Effect of different ratio of soybean oil in combination with fish oil supplementation on pH, ammonia nitrogen (mg/100 ml) and volatile fatty acids (mol/100mol) in fistulated cattle.

Item	SBO+ FO at 3% of total feed DM			SEM	P-value
	2:1 w/w	1:1 w/w	1:2 w/w		
Pre - feeding					
pH	6.73	6.72	6.74	0.350	0.140
NH_3N	9.95	10.78	9.36	2.035	0.961
Acetic acid	65.57	64.39	66.93	0.682	0.463
Propionic acid	20.53	22.07	21.27	0.355	0.392
Butyric acid	13.94	13.54	11.73	0.804	0.583
A:P ratio	3.22	2.94	3.15	0.056	0.303
2 h after feeding					
pH	6.42	6.33	6.35	0.039	0.677
NH_3N	20.95^{b}	24.47^{b}	30.28^{a}	0.483	0.031
Acetic acid	61.57	65.50	62.15	0.322	0.115
Propionic acid	27.56	25.86	28.23	0.221	0.134
Butyric acid	10.87	9.64	9.62	0.491	0.583
A:P ratio	2.23^{b}	2.49^{a}	2.20^{b}	0.012	0.044

SBO = soybean oil; FO = fish oil; A:P ratio = acetate: propionate ratio

SEM = standard error of the mean

^{abc} Within a row means without a common superscript letter differ

Table 6.5 Effect of different ratio of soybean oil in combination with fish oil supplementation on pH, ammonia nitrogen (mg/100 ml) and volatile fatty acids (mol/100mol) in fistulated cattle (cont.).

Item	SBO+FO at 3% of total feed DM			SEM	P-value
	2:1 w/w	1:1 w/w	1:2 w/w		
4h after feeding					
pH	6.00	6.07	6.07	0.040	0.724
NH_3N	10.99	11.20	9.96	0.384	0.497
Acetic acid	67.53[a]	67.74[a]	61.70[b]	0.373	0.039
Propionic acid	23.19[b]	23.26[b]	27.08[a]	0.357	0.072
Butyric acid	9.28	9.00	11.22	0.349	0.200
A:P ratio	2.92[a]	2.93[a]	2.29[b]	0.038	0.032
6h after feeding					
pH	5.99	6.08	5.94	0.105	0.867
NH_3N	7.47	8.09	7.05	1.137	0.934
Acetic acid	67.16	68.74	64.27	0.781	0.247
Propionic acid	22.35[b]	22.09[b]	25.41[a]	0.198	0.045
Butyric acid	10.49	10.31	9.17	0.663	0.721
A:P ratio	2.69	3.13	2.56	0.054	0.092

SBO = soybean oil; FO = fish oil; A:P ratio = acetate: propionate ratio

SEM = standard error of the mean

[abc] Within a row means without a common superscript letter differ

6.4.5 Degradability of DM CP NDF and ADF

Dry matter degradation of concentrate and rice straw were calculated from the sum of dry matter degradability at time zero and the potential degradability of dry matter component in time incubation. Two keys were the main value to estimate the degradability of feed. In the current study, the intercept of the degradation curve at time zero and the potential degradability of the component were unaffected by different proportion of SBO+FO (Table 6.6). Similarly, the rate constant of the potential degradability was not significantly influenced (P>0.05) by oil addition. Consequently, supplementation of variuos proportion of SBO and FO had no effects on dry matter degradability of concentrate and rice straw at all out flowrates (P>0.05).

For crude protein degradability of concentrate (Table 6.7), supplementation of SBO+FO at all ratios had no effects on the intercept of the degradation curve at time zero, the potential degradability of the component and crude protein degradability of concentrate at all out flowrates (P>0.05).

Supplementation of different ratios of SBO+FO had no effect on neutral detergent fiber degradability and acid detergent fiber degradability of rice straw (P>0.05) as showed in Table 6.8.

Table 6.6 Effect of different ratio of soybean oil in combination with fish oil supplementation on dry matter degradability (DMD) of concentrate and rice straw in fistulated cattle.

Item	SBO+FO at 3% of total feed DM			SEM	P-Value
	2:1 w/w	1:1 w/w	1:2 w/w		
Dry matter degradability of concentrate					
a	24.85	25.76	25.27	0.738	0.885
b	41.80	42.28	42.70	1.408	0.564
a + *b*	66.65	68.05	67.97	1.911	0.633
c, per h	0.222	0.230	0.236	0.027	0.133
dg, 0.02/h	0.64	0.66	0.65	0.019	0.847
dg, 0.05/h	0.54	0.56	0.55	0.015	0.341
dg, 0.08/h	0.50	0.52	0.50	0.012	0.200
Dry matter degradability of rice straw					
a	16.23	15.20	15.00	0.320	0.139
b	45.45	45.63	43.47	0.817	0.581
a + *b*	61.68	60.83	58.47	0.541	0.249
c, per h	0.025	0.024	0.026	0.001	0.858
dg, 0.02/h	0.39	0.40	0.38	0.007	0.267
dg, 0.05/h	0.28	0.29	0.27	0.003	0.445
dg, 0.08/h	0.24	0.24	0.23	0.002	0.441

SBO = soybean oil; FO = fish oil; SEM = standard error of the mean; *a* = the intercept of the degradation curve at time zero; *b* = the potential degradability of the component; *c* = the rate constant for the degradation of '*b*'

Table 6.7 Effect of different ratio of soybean oil in combination with fish oil supplementation on crude protein degradability (CPD) of concentrate in fistulated cattle.

Item	SBO+FO at 3% of total feed DM			SEM	P-Value
	2:1 w/w	1:1 w/w	1:2 w/w		
Crude protein degradability of concentrate					
a	28.33	29.36	32.05	1.606	0.677
b	47.23	50.73	43.00	1.704	0.367
a + *b*	75.56	80.10	75.05	2.956	0.773
c, per h	0.254	0.285	0.313	0.034	0.181
dg, 0.02/h	0.72	0.74	0.75	0.024	0.872
dg, 0.05/h	0.68	0.71	0.68	0.010	0.429
dg, 0.08/h	0.65	0.69	0.65	0.008	0.304

SBO = soybean oil; FO = fish oil; SEM =standard error of the mean;

a = the intercept of the degradation curve at time zero;

b = the potential degradability of the component;

c = the rate constant for the degradation of '*b*'

Table 6.8 Effect of different ratio of soybean oil in combination with fish oil supplementation on neutral detergent fiber degradability (NDFD) and acid detergent fiber degradability (ADFD) of rice straw in fistulated cattle.

Item	SBO+FO at 3% of total feed DM			SEM	P-Value
	2:1 w/w	1:1 w/w	1:2 w/w		
Neutral detergent fiber degradability of rice straw					
a	4.30	2.23	2.85	0.787	0.622
b	64.03	60.27	61.35	1.724	0.703
a + *b*	68.33	62.50	64.20	1.395	0.393
c, per h	0.023	0.028	0.027	0.001	0.477
dg, 0.02/h	0.36	0.37	0.38	0.005	0.682
dg, 0.05/h	0.23	0.24	0.23	0.004	0.552
dg, 0.08/h	0.17	0.18	0.17	0.004	0.650
Acid detergent fiber degradability of rice straw					
a	4.30	5.20	8.10	0.623	0.227
b	57.70	56.98	62.17	1.331	0.394
a + *b*	62.00	62.17	70.27	1.371	0.202
c, per h	0.034	0.034	0.031	0.004	0.945
dg, 0.02/h	0.43	0.43	0.45	0.005	0.428
dg, 0.05/h	0.30	0.31	0.31	0.003	0.500
dg, 0.08/h	0.30	0.31	0.31	0.003	0.636

SBO = soybean oil; FO = fish oil; SEM = standard error of the mean; *a* = the intercept of the degradation curve at time zero; *b* = the potential degradability of the component; *c* = the rate constant for the degradation of '*b*'

6.5 Discussion

6.5.1 Fatty Acid Profile in Ruminal Content

Overall supplementation of soybean oil in combination with fish oil at 2:1 w/w had significantly greater *t11*-C18:1 in ruminal content than those other ratios. Generally, *t11*-C18:1 was the product of incomplete bio-hydrogenation of C18:2n-6 in the rumen (Kepler et al., 1970). Thus, high level of C18:2n-6 supplementation resulted in greater *t11*-C18:1 in the rumen. Similarly, the result from Jalč et al. (2007) showed that supplemented oil rich in C18:2n-6 mixed with fish oil at 1:1, 3:1 and 5:1, the concentration of *t11*-C18:1 and *c9,t11*-C18:2 in the rumen was linearly increased. Abughazaleh et al. (2003b) reported that supplemented fish oil with high source of C18:2n-6 increased the concentration of *t11*-C18:1 in ruminal digesta when compare to supplemented fish oil with C18:0, C18:1n-9 and C18:3n-3 sources. Under normal ruminal conditions, bio-hydrogenation of C18:2n-6 and C18:3n-3 fatty acids to *t11*-C18:1 formation as an intermediate (Wilde and Dawson, 1966; Harfoot and Hazlewood, 1997). The C18:2n-6 interferes with its own bio-hydrogenation when present in ruminal contents at higher concentrations. Beam et al. (2000) reported that the overall rate of bio-hydrogenation of C18:2n-6 was 14.3% /h, but declined by 1.2% /h for each percentage unit increase in C18:2n-6 added to the substrate. AbuGhazaleh et al. (2002) reported that fish oil can also cause incomplete bio-hydrogenation of C18:2n-6. Higher intake of C18:2n-6 in soybean oil in combination with fish oil at 2:1 w/w cattle increased the concentration of C18:2n-6 in the rumen content in the present study. Similar result was previously reported by Chow et al. (2004) who stated that when increased the proportion of fish oil to sunflower oil the concentration of C18:2n-6 was linearly decreased in *in vitro* experiment, however, at 24 h after incubation

C18:2n-6 concentration was similar. Adding high proportion of fish oil into the diets increased the concentration of ruminal C20:5n-3 and C22:6n-3. The present study showed that greater intake of C20:5n-3 and C22:6n-3 significantly increased the concentration of C20:5n-3 and C22:6n-3 at 2 and 4 h after feeding. Kim et al. (2008) supplemented 2.3% and 6.9% fish oil and found that the concentration of C20:5n-3 and C22:6n-3 were linearly increased when compare to none supplemented fish oil. However, the secondary fatty acid component in fish oil namely C16:0 remained high concentration in the rumen even at 6 h after feeding. Similarly, Kitessa et al. (2001) supplemented protected tuna oil and tuna oil and found an increase in C16:0 concentration in the rumen. In addition, Loor et al. (2005) supplemented fish oil 2.5% of total feed DM, sunflower oil 5% of total feed DM and linseed oil 5% of total feed DM in Holstein cows and reported that fish oil cow had higher concentration of C16:0 when compare to sunflower oil and linseed oil cows.

6.5.2 Ruminal Fermentation

Ruminal microorganisms require an ideal environment for development, including a temperature between 38°C and 40°C and a pH of 5.5-7.0 (Hoover, 1986). The factor with the greatest influence on rates of bio-hydrogenation of unsaturated fatty acids was rumen pH (Kalscheur et al., 1997; Beam et al., 2000; Jenkins and Adams, 2002). Ruminal bio-hydrogenation may be sensitive to changes in microbial populations induced by specific dietary fatty acids even if ruminal pH is not reduced (Loor and Herbein, 2003). In the present study, there were no significant differences in ruminal pH among treatments. Similarly, Toral et al. (2009) who supplemented different ratios of sunflower oil and fish oil, and found no difference between treatment in ruminal pH. Similar results had also been reported (Fievez et al., 2003; Beauchemin et al., 2007) which they suggested that the pH was not affected by

oil supplementation and in agreement with previous *in vivo* studies using different lipid sources, including fish and sunflower oils. In contrast, Shingfield et al. (2003) reported a higher pH when fish oil was included in the diet of cows, which was attributed to associated decreases in dry matter intake. However, Messana et al. (2013) reported that in animals receiving the highest dietary lipid content (60 g/kg), rumen pH decreased quadratically ($P<0.001$) with an increase in the lipid content. Latham et al. (1972) showed that low rumen pH resulted in lower levels of lipolytic activity and bio-hydrogenation of unsaturated FA in ruminal fluid. Most rumen microbes are sensitive to low pH conditions as acidity in the rumen impact microbial growth and enzymes activities (Martin et al., 2002; Jenkins et al., 2008). AbuGhazaleh and Jacobson (2007) and Fuentes et al. (2009) suggested that higher ruminal pH favors the formation of *t11*-C18:1 and *c9,t11*-C18:2. The higher proportion of *c9,t11*-C18:2 and *t11*-C18:1 at higher pH was also observed by Troegeler-Meynadier et al. (2003) and Fuentes et al. (2008). The decreased linoleic and linolenic acids in low pH cultures might have been due to a lowered bio-hydrogenation activity by culture microbes because low rumen pH has been shown to have a negative effect on microbial growth (Martin and Jenkins, 2002).

Ammonia nitrogen uses for the efficiency of amino acid synthesis and microbial growth. Ruminal ammonia nitrogen was increased when high proportion of fish oil was supplemented in this study, which was similar to the finding of Keady and Mayne. (1999). Keady and Mayne (1999) supplemented fish oil up to 450 g/d and found an increase in ruminal ammonia nitrogen concentration. They suggested that the lack of a significant effect on the concentrations of either ammonia or those VFA originating from the deamination of some amino acids (valerate and branched-chain VFA). However, Gudla et al. (2012) added soybean oil in combination with fish oil

and observed no significant difference in ruminal ammonia nitrogen when compare to non-oil supplement. Recently, Ferreira et al. (2014) reported that animals receiving diets with 40 g/kg DM of soybean oil exhibited lower ruminal ammonia concentrations in comparison to the control treatment. This finding may be attributed to a lower ruminal CP digestion by animals in this treatment, which is compatible with lower CP digestibility in the total digestive tract. The ruminal ammonia concentration linearly increased with the increase of fish oil blend levels in the diet. If greater substitutions of fish oil blend for soybean oil reduced ruminal microbial growth, it can also be said that an increased ruminal ammonia concentration was due to lower utilization of ammonia available in the rumen for microbial growth.

Supplementation of higher fish oil proportion in this study found that the ruminal proportion of acetic acid was decreased whereas the proportion of propionic acid was increased which was similar to the report from Keady and Mayne. (1999) who found that supplementation of fish oil from 150 g/d up to 450 g/d linearly increased the ruminal concentration of propionic acid. Doreau and Chilliard (1997) offered fish oil in one feed daily and concluded that the inclusion of 200 g fish oil had no effect on rumen fermentation patterns whereas inclusion of 400 g fish oil in one feed reduced the molar proportions of acetate and increased the molar proportions of propionate. Decreasing of ruminal acetate concentration is a common response to the addition of fish oil (Doreau and Chilliard, 1997; Fivez et al., 2003; Toral et al., 2009) or linoleic acid-rich sources to the diet (Zhang et al., 2008). This trend supports the hypothesis that polyunsaturated fatty acids may exert an inhibitory effect on acetate-producing bacteria (Toral et al., 2009). Toral et al., 2016 supplemented fish oil and sunflower oil and showed significant increased molar proportion of propionic acid when compare to the control group. Jalč et al. (2009) supplemented different ratios of

oil rich in omega 6 in combination with fish oil and found that oil supplemented group had greater propionic acid when compare to the control group while acetic acid decreased. Zhang et al. (2008) incubated C18:2n-6 in sheep and showed increased the molar proportion of propionic acid and decreased the molar proportion of acetic acid. This suggests that acetate-producing bacteria, such as *Fibrobacter succinogenes* and *Ruminococcus flavefaciens*, which are considered to be predominant cellulolytic bacteria in the rumen, may have been more inhibited by PUFA (Maia et al., 2007; Zhang et al., 2008). From a physiological point of view, a shift in the rumen microbial communities may result in changes in bio-hydrogenation and, consequently, in the milk or beef FA profile (Palmquist et al., 2005). Furthermore, a decrease in acetate concentration might contribute to a reduction in mammary or tissue *de novo* fatty acid synthesis, which requires acetate as a precursor (Doreau and Chilliard, 1997).

6.5.3 Degradability of DM CP NDF and ADF

Supplementation of combination oil at all ratios had no effects on DM, CP, NDF and ADF degradability of concentrate and rice straw which was similar to the investigation by Ferreira et al. (2014). They supplied soybean oil in combination with fish oil at 2.5 g/kg FO+37.5 g/kg SBO, 5 g/kg FO+35 37.5 g/kg SBO and 7.5 g/kg FO+32.5 g/kg SBO and observed that the ruminal digestibility of NDF was unaffected by treatments, moreover, the apparent digestibility of DM, OM, NDF and NFC in the total digestive tract was also not affected by treatments. Fievez et al. (2003) reported that fish oil did not alter *in vivo* NDF digestibility although rumen degradability of hay NDF after 48 h *in sacco* incubation was lower and no differences were observed after 6 h *in sacco* incubation. Longer rumen retention tended to reduce rumen outflow rates, and it is consistent with the lower dry matter intake. A decrease in dry matter intake of ruminants has been associated with low digestibility (Steen et

al., 1998), however, the fish oil induced depression in dry matter intake was observed despite an apparent increase in disappearance of DM. The observation of Lee et al. (2008), Shingfield et al. (2010) and Toral et al. (2009, 2010) suggested that the supply of up to 7.5 g/kgDM of fish oil and 32.5 g/kgDM of soybean oil does not compromise the NDF digestibility in diets with high concentrate diets (Evandro Maia Ferreira et al., 2016). However, soybean oil was the source of linoleic acid and Hristov et al. (2005) reported that linoleic acid is toxic to ruminal protozoa. Moreover; Oldick and Firkins (2000) observed a linear decrease in ruminal protozoa with increasing degree of unsaturation of dietary fats. In their study, a marked decrease in protozoa and cellulolytic bacteria numbers were observed when oils were supplemented. These effects are possibly associated with direct inhibition and/or coating action of the unsaturated fatty acids on microorganisms. Nevertheless, Jalc et al. (2007) indicated that fatty acids (C18:1n-9, C18:2n-6 and C18:3n-3) supplementation at a dose of 35 g/kg (w/w) to a mixed diet containing 80% lucerne and 20% barley did not show any effect on DM, NDF and ADF degradation.

6.6 Conclusion

Soybean oil mixed with fish oil 1:2 w/w significantly increased the concentrations of C20:5n-3 and C22:6n-3 in the rumen ($P<0.05$). However, feeding 1:1 w/w SBO+FO compromised the concentrations of *t11*-C18:1, C20:5n-3 and C22:6n-3. This study did not found a difference in pH at all times post feeding, but at 2h post feeding, the ruminal ammonia nitrogen was higher when supplemented high proportion of fish oil. Supplemented high proportion of fish oil significantly decreased the molar proportion of acetic acid but increased the molar proportion of propionic

acid at 4h post feeding. The degradation of DM, CP, NDF and ADF was unaffected (P>0.05) by various ratios of oil addition.

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CHAPTER VII

RUMINAL BIO-HYDROGENATION AND FERMENTATION IN RESPONSE TO DIFFERENT LEVEL OF COMBINATION OILS RICH IN OMEGA-6 FATTY ACID, OMEGA-3 FATTY ACID AND FISH OIL IN FISTULATED CATTLE'S DIETS

7.1 Abstract

The aim of this study was to investigate the effects of different level of combination oil supplementation on ruminal fermentation in fistulated cattle. Three fistulated cattle were assigned into 3 treatments in a 3×3 Latin square design. All cattle were fed approximately 4 kg/d of 14% CP concentrate and 2.4 kg/d of rice straw. Treatments were: 1) supplemented 1:1:1 (w/w/w) soybean oil, linseed oil and fish oil at 2% of total feed DM ; 2) supplemented 1:1:1 (w/w/w) soybean oil, linseed oil and fish oil at 3% of total feed DM; 3) supplemented 1:1:1 (w/w/w) soybean oil, linseed oil and fish oil at 4% of total feed DM. Each period in the Latin square design lasted 21 d, with the first 7 d for adaptation. The results found that supplemented 4% combination oil significantly decreased the concentration of C18:0 and increased *t11*-C18:1, C20:5n-3 and C22:6n-3 in rumen content at all times after feeding. No significant difference in ruminal pH, however, addition of 4% combination oil to the diet increased molar proportion of propionic acid meanwhile molar proportion of

acetic acid decreased at 2, 4 and 6 h after feeding. Ammonia nitrogen was greater at 2, 4 and 6 h after feeding when 4% of combination oil was added. Nevertheless, addition of 4% combination oil tended to reduce ADF degradability at 0.05 and 0.08 /h out flowrate (P=0.07), however, no significant difference in DM, CP and NDF degradability was observed (P>0.05).

7.2 Introduction

Recently, lipids in addition to supplying dietary energy to ruminants, can modify the fat composition of their products particularly to improve quality of products such as plant oils has been reported to be a good strategy for increasing milk *c9,t11*-C18:2 (CLA) levels in goats (Mele et al., 2008; Bernard et al., 2009; Martínez Marín et al., 2011). Additionally, multiple studies have attempted to increase the concentration of 20:5n-3 and 22:6n-3 in ruminant milk by adding fish oil to the diet, but the apparent transfer rate of these FA from diet to milk is relatively low (Kitessa et al., 2001b; Loor et al., 2005; Toral et al.,2010). However, as a rumen bio-hydrogenation modulator, fish oil yields large increases in milk *c9,t11*-C18:2 and *t11*-C18:1 concentrations, particularly when combined with plant oils either in goats, cows, or sheep (Gagliostro et al., 2006; Shingfieldet al., 2006; Toral et al., 2010).

The results from Chapter 5 and 6 found that the optimum ratio between oil rich in omega 3 or omega 6 and fish oil was 1:1 w/w and supplemented at 3% of total feed DM or 30 g/kgDM. However, Gomez-Cortês et al. (2008) suggested that oil supplemented to a concentrate-rich diet at 60 g/kgDM of oil did not affect *in vitro* ruminal fermentation. Earlier studies on the addition of lipids to ruminant diets as an energy source raised concerns about detrimental effects of fatty acids on ruminal

fermentation (Jenkins, 1993). The rumen ecosystem consist of a highly diverse collection of anaerobic microbes with the majority (70-80% of the microbial matter in the rumen) attached to feed particles in the digesta (McAllister et al., 1994). Rumen bacteria play the main role in lipid metabolism in the rumen (Harfoot and Hazlewood, 1997; Jenkins et al., 2008). Lipids are extensively hydrolyzed in the rumen, rendering fatty acids that have bacteriostatic and bacteriocidal effects. Among them, unsaturated fatty acids are more antimicrobial than saturated ones (Harfoot and Hazlewood, 1997), and a differential toxicity of different PUFA to rumen microorganisms has also been observed (Maia et al., 2007). Dietary supplementation with oils has given inconsistent results on ruminal fermentation, with detrimental consequences (Fievez et al., 2003).

The present study selected the suitable results from Chapter 5 and 6 and assigned to mix oil rich in omega 3, omega 6 and fish oil at the optimum ratio. Therefore, the aim of this study was to evaluate the optimum level of combination oil on ruminal bio-hydrogenation and fermentation in fistulated cattle.

7.3 Materials and Methods

7.3.1 Animals and Feeding

All experimental procedures were conducted following the Ethical Principles and Guidelines for the Use of Animal issued by National Research Council of Thailand. Three fistulated cattle were assigned into 3 treatments in a 3×3 Latin square design. All cattle were fed approximately 4 kg/d of 14% CP concentrate and 2.4 kg/d of rice straw. Treatments were: 1) supplemented 1:1:1 w/w/w soybean oil (SBO), linseed oil (LSO) and fish oil (FO) at 2% of feed DM 2) supplemented 1:1:1 w/w/w soybean oil (SBO), linseed oil (LSO) and fish oil (FO) at 3%of feed DM; 3)

supplemented 1:1:1 w/w/w soybean oil (SBO), linseed oil (LSO) and fish oil (FO) at 4% of feed DM. All cattle also had free access to clean water and were individually housed in a free-stall unit and individually fed according to treatments. The experiment lasted for 63 days (3 periods) with 21 d in each period, the first 7 d of each period for adaptation to diets followed by 14 d for ruminal sample collection and *in sacco* disappearance trial.

7.3.2 Sample Collection

To evaluate fatty acids profile in rumen content and ruminal fermentation, the procedures of sample collection, preservation of samples and pH measurement were the same as in Chapter 3 (Section 3.3.3).

7.3.3 Laboratory Analyses

7.3.3.1 Feed chemical composition analysis

Sample collection of feeds and feed chemical composition analyses were the same as in Chapter 3 (Section 3.3.4.1).

7.3.3.2 Analysis of fatty acids in feed

The procedures of fatty acid composition analysis were the same as in Chapter 3 (Section 3.3.4.2).

7.3.4.3 Analysis of fatty acids in ruminal digesta

Preparation and analysis of rumen fluid samples were the same as in Chapter 3 (Section 3.3.4.3).

7.3.3.4 Volatile fatty acid and ammonia nitrogen analyses

Ruminal volatile fatty acids (VFA) and ammonia N were determined in rumen fluid samples as in Chapter 3 (Section 3.3.4.4).

7.3.3.5 Degradability determination of DM, CP, NDF and ADF

Preparation of feed samples and determination of DM, CP, NDF and ADF degradabilities were the same as in Chapter 3 (Section 3.3.4.5).

7.3.4 Statistical Analysis

All data were analyzed as a 3x3 Latin squares design using ANOVA procedure of SAS (SAS, 1996). Significant differences among treatment were assessed by Duncan's new multiple range test. A significant level of $P<0.05$ was used (Steel and Torrie, 1980).

7.3.5 Experimental Site

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

7.3.6 Duration

The duration of the present experiment was from January to March 2017.

7.4 Results

7.4.1 Chemical composition of experimental diet

The concentrate used in this experiment contained 89.8% of dry matter, 14.2% of crude protein and 3.2% of fat and rice straw contained 89.7% of dry matter, 1.8% of crude protein and 1.2% of fat showed in Table 7.1.

Combination oils were the sources of omega 6, omega 3 and fish oil. Soybean oil, the source of Omega 6, contained high amount of C18:2n-6 (44.74% of total fatty acids) whereas Linseed oil, the source of Omega 3, contained 53.67% of total fatty acids C18:3n-3. Fish oil contained 30.38% DHA and 7.77% EPA of total

fatty acid showed in Table 7.2, although it also contained other sources of main fatty acids in particular C16:0 (28.22% of total fatty acids) and C18:1n-9 (14.42% of total fatty acids).

Table 7.1 Chemical composition of the experimental diets.

Items	Concentrate	SBO	LSO	FO	Rice straw
Dry matter	89.8	100	100	100	89.7
		..... % of DM.....			
Ash	7.9				19.1
Crude protein	14.2				1.8
Ether extract	3.2	100	100	100	1.2
Crude fiber	14.3				40.2
Neutral detergent fiber	40.1				76.4
Acid detergent fiber	20.5				51.4
Acid detergent lignin	4.2				17.2

[1]kg/100 kg concentrate: 30 dried cassava chip, 4 ground corn, 10 rice bran, 25 palm meal, 15 coconut meal, 6 dried distillers grains with solubles, 0.5 sodium bicarbonate, 6 molasses, 1 dicalciumphosphate (16%P), 1.5 urea, 0.5 salt and 0.5 premix. Premix: provided per kg of concentrate including vitamin A, 5,000 IU; vitamin D3, 2,200 IU; vitamin E, 15 IU; Ca, 8.5 g; P, 6 g; K, 9.5 g; Mg, 2.4 g; Na, 2.1 g; Cl, 3.4 g; S, 3.2 g; Co, 0.16 mg; Cu, 100 mg; I, 1.3 mg; Mn, 64 mg; Zn, 64 mg; Fe, 64 mg; Se, 0.45 mg.

Table 7.2 Fatty acid compositions (g/100 g of total fatty acids) of concentrate, rice straw and oils used in the experiment.

Fatty acids	Concentrate	Rice straw	Soybean oil	Linseed oil	Fish oil
C12:0	22.74	6.35	0.43	2.91	2.17
C14:0	7.81	8.22	1.09	0.35	4.39
C16:0	16.63	45.72	13.74	22.76	28.22
C18:0	2.50	0.11	5.26	0.22	6.14
C18:1n-9	29.51	24.78	33.87	14.90	14.42
C18:2n-6	17.14	11.40	44.74	2.73	1.70
C18:3n-3	0.25	ND	0.35	53.67	0.93
C20:5n-3	ND	ND	ND	ND	7.77
C22:6n-3	ND	ND	ND	ND	30.38
Others	3.42	3.39	0.52	2.48	3.86

ND = Not detected.

Others = C8:0 + C15:0 + C20:1 + C21:0+ C23:0

7.4.2 Intake of main components and major fatty acids

The present study was designed to control the ratio of concentrate to roughage at 60:40 (DM basis) and to restrict feed intake. Thus, the concentrate DM intake was 3.58 kg/d and the DM intake of rice straw was 2.15 kg/d, giving a total DM intake of 5.73 kg/d. This total DM intake was used to calculate the supply of combination oil at 2%, 3% and 4% of total feed DM (120, 180 and 240 g/d). The inclusion of combination oil in the diet resulted in a significant increase in total DM

and fat intake. A linear increase in total fat intake with increasing oil level related to high intakes of individual fatty acid and of total fatty acids.

Table 7.3 DM, CP, fat and fatty acid intakes of experimental cattle.

Items	SBO+LSO+FO (1:1:1 w/w/w)			SEM	P-value
	2%	3%	4%		
DM intake (kg/d)					
Concentrate	3.59	3.59	3.59	-	-
Rice straw	2.15	2.15	2.15	-	-
Oil	0.12^{c}	0.18^{b}	0.24^{a}	0.001	0.001
Total	6.06^{c}	6.12^{b}	6.18^{a}	0.001	0.001
CP intake (g/d)					
Concentrate	510	510	510	-	-
Rice straw	39	39	39	-	-
Total	549	549	549	-	-
Fat intake (g/d)					
Concentrate	115	115	115	-	-
Rice straw	26	26	26	-	-
Oil	120^{c}	180^{b}	240^{a}	0.001	0.001
Total	261^{c}	321^{b}	381^{a}	0.001	0.001

SBO = Soybean oil; LSO= Linseed oil; FO = fish oil

^{abc} Within a row means without a common superscript letter differ

Table 7.3 DM, CP, fat and fatty acid intakes of experimental cattle (cont.).

Items	SBO+LSO+FO (1:1:1 w/w/w)			SEM	P-value
	2%	3%	4%		
Fatty acids intake (g/d)					
C12:0	22.49^{c}	23.31^{b}	24.14^{a}	0.001	<0.001
C14:0	10.07^{c}	10.95^{b}	11.82^{a}	0.001	<0.001
C16:0	42.61^{c}	52.32^{b}	62.03^{a}	0.001	<0.001
C18:0	5.66^{c}	7.40^{b}	9.14^{a}	0.001	<0.001
C18:1n-9	49.20^{c}	58.68^{b}	68.15^{a}	0.001	<0.001
C18:2n-6	31.73^{c}	39.11^{b}	46.49^{a}	0.001	<0.001
C18:3n-3	16.70^{c}	24.95^{b}	33.19^{a}	0.001	<0.001
C20:5n-3	2.33^{c}	3.50^{b}	4.66^{a}	0.001	<0.001
C22:6n-3	9.11^{c}	13.67^{b}	18.23^{a}	0.001	<0.001
Others	5.66^{c}	6.69^{b}	7.72^{a}	0.001	<0.001
Total	195^{c}	241^{b}	286^{a}	0.001	<0.001

SBO = Soybean oil; LSO = Linseed oil; FO = fish oil;

SEM = standard error of the mean;

Others = C8:0 + C15:0 + C20:1 + C21:0 + C23:0;

^{abc} Within a row means without a common superscript letter differ

7.4.3 Fatty acid profile in rumen content

At all times after feeding, supplementation of combination oil at 3 and 4% of total feed DM significantly decreased the concentration of C18:0 when compare to 2%. However, C18:1n-9t, C20:5n-3 and C22:6n-3 were significantly increased in 4% combination oil cattle. At 2 h after feeding, 3 and 4 % combination oil tended to reduce C18:1n-9c. At 4 h after feeding 3 and 4% combination oil cattle had higher ruminal C16:0 content than 2% combination oil cattle. The concentration of C12:0, C14:0, C18:2n-6, C18:3n-3 and CLA in the rumen were not affected by oil supplement at all times.

Table 7.4 Effect of different level of combination oil supplementation on ruminal fatty acid profile in fistulated cattle (g/100g fatty acids).

Fatty acids	SBO+LSO+FO (1:1:1 w/w/w)			SEM	P-Value
	2%	3%	4%		
Pre - feeding					
C12:0	12.89	12.43	12.79	0.834	0.797
C14:0	8.15	8.74	9.15	0.563	0.295
C16:0	34.65	34.24	34.89	1.062	0.775
C18:0	38.03	37.96	37.39	1.393	0.842
C18:1n-9	2.58	2.77	2.46	1.128	0.945
C18:2n-6	1.69	1.65	1.59	0.254	0.881
t11-C18:1	2.01	2.20	1.71	0.531	0.610

SBO = soybean oil; LSO = Linseed oil; FO = fish oil;

Table 7.4 Effect of different level of combination oil supplementation on ruminal fatty acid profile in fistulated cattle (g/100g fatty acids) (cont.).

Fatty acids	SBO+LSO+FO (1:1:1 w/w/w)			SEM	P-Value
	2%	3%	4%		
2h after feeding					
C12:0	15.65	14.07	13.31	0.772	0.557
C14:0	6.99	7.10	6.97	0.057	0.656
C16:0	16.25	16.96	16.71	0.216	0.552
C18:0	16.32^{a}	13.31^{b}	6.86^{c}	0.185	0.004
C18:1n-9	8.16	6.28	3.78	0.388	0.085
C18:2n-6	1.74	1.66	2.33	0.255	0.587
C18:3n-3	0.68	0.41	0.52	0.040	0.203
t11-C18:1	17.03^{c}	20.06^{b}	22.44^{a}	0.081	0.002
c9,t11-C18:2	6.94	6.72	6.36	0.105	0.274
t10,c12-C18:2	2.35	2.28	2.22	0.030	0.396
C20:5n-3	0.30^{b}	0.32^{ab}	0.33^{a}	0.002	0.050
C22:6n-3	7.54^{c}	11.14^{b}	18.18^{a}	0.268	0.007

SBO = soybean oil; LSO = Linseed oil; FO = fish oil;

SEM = standard error of the mean

^{abc} Within a row means without a common superscript letter differ.

Table 7.4 Effect of different level of combination oil supplementation on ruminal fatty acid profile in fistulated cattle (g/100g fatty acids) (cont.).

Fatty acids	SBO+LSO+FO (1:1:1 w/w/w)			SEM	P-Value
	2%	3%	4%		
4h after feeding					
C12:0	11.35	11.95	9.75	0.742	0.561
C14:0	6.88	6.63	6.75	0.294	0.938
C16:0	17.82^{b}	19.41^{a}	19.64^{a}	0.053	0.008
C18:0	14.85^{a}	7.43^{b}	4.76^{b}	0.307	0.010
C18:1n-9	5.12	7.01	5.34	0.792	0.690
C18:2n-6	2.98^{b}	4.37^{a}	3.81^{a}	0.059	0.020
C18:3n-3	0.96	0.85	0.55	0.081	0.300
t11-C18:1	23.87^{b}	24.78^{b}	29.45^{a}	0.263	0.022
c9,t11-C18:2	3.92	4.11	3.59	0.190	0.608
C20:5n-3	0.22^{c}	0.29^{b}	0.64^{a}	0.003	0.001
C22:6n-3	11.63^{b}	13.15^{b}	15.71^{a}	0.210	0.030

SBO = soybean oil; LSO = Linseed oil; FO = fish oil;

SEM = standard error of the mean

^{abc} Within a row means without a common superscript letter differ.

Table 7.4 Effect of different level of combination oil supplementation on ruminal fatty acid profile in fistulated cattle (g/100g fatty acids) (cont.).

Fatty acids	SBO+LSO+FO (1:1:1 w/w/w)			SEM	P-Value
	2%	3%	4%		
6 h after feeding					
C12:0	15.73	12.70	11.90	0.969	0.408
C14:0	8.11	7.35	7.38	0.252	0.511
C16:0	18.26	19.91	19.67	0.257	0.199
C18:0	13.45[a]	8.44[b]	6.51[b]	0.326	0.024
C18:1n-9	5.85	3.81	2.83	0.573	0.293
C18:2n-6	1.86	2.38	3.05	0.254	0.353
t11-C18:1	21.06[b]	25.97[a]	25.91[a]	0.328	0.039
C20:5n-3	0.30[b]	0.32[ab]	0.33[a]	0.002	0.049
C22:6n-3	15.38[c]	19.92[b]	21.61[a]	0.089	0.002

SBO = soybean oil; LSO = Linseed oil; FO = fish oil;

SEM = standard error of the mean

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

7.4.4 Ruminal Fermentation

High level of combination oil reduced acetic acid molar proportion ($P<0.05$) but increased propionic acid molar proportion at all times when compare to 2% combination oil ($P<0.05$). A: P ratio was significantly decreased by combination oil addition, however, ruminal pH was unaffected by oil supplementation. Addition of 4% combination oil resulted in higher ruminal concentration of NH_3-N at all h post-feeding.

Table 7.5 Effect of different level of combination oil supplementation on ruminal pH, ammonia nitrogen (mg/100 ml) and volatile fatty acids (mol/100 mol) in fistulated cattle.

Item	SBO+LSO+FO (1:1:1 w/w/w)			SEM	P-value
	2%	3%	4%		
Pre feeding					
pH	6.65	6.62	6.58	0.092	0.549
NH_3N	16.59	14.10	15.35	1.902	0.437
Acetic acid	65.45	66.29	64.99	0.696	0.272
Propionic acid	22.31	21.96	21.88	0.885	0.836
Butyric acid	12.24	11.75	13.12	0.486	0.139
A:P ratio	2.93	3.02	2.08	0.135	0.749
2 h after feeding					
pH	6.51	6.47	6.41	0.045	0.724
NH_3N	20.74^{b}	22.82^{ab}	27.38^{a}	0.411	0.050
Acetic acid	74.65^{a}	68.36^{b}	67.37^{b}	0.426	0.043
Propionic acid	16.80^{b}	23.00^{a}	23.17^{a}	0.473	0.042
Butyric acid	8.55	8.64	9.48	0.180	0.268
A:P ratio	4.49^{a}	2.98^{b}	2.81^{c}	0.114	0.049

SBO = soybean oil; LSO = Linseed oil; FO = fish oil;

A:P ratio = acetate: propionate ratio

SEM = standard error of the mean

^{abc} Within a row means without a common superscript letter differ.

Table 7.5 Effect of different level of combination oil supplementation on ruminal pH, ammonia nitrogen (mg/100 ml) and volatile fatty acids (mol/100mol) in fistulated cattle (cont.).

Item	SBO+LSO+FO (1:1:1 w/w/w)			SEM	P-value
	2%	3%	4%		
4h after feeding					
pH	6.34	6.25	6.19	0.057	0.641
NH_3N	15.02^{b}	17.01^{ab}	21.99^{a}	0.519	0.045
Acetic acid	71.32^{a}	66.02^{b}	63.30^{c}	0.133	0.001
Propionic acid	18.19^{b}	24.06^{a}	25.09^{a}	0.663	0.043
Butyric acid	10.49	9.92	11.60	0.719	0.678
A:P ratio	3.92^{a}	2.77^{b}	2.52^{b}	0.133	0.048
6h after feeding					
pH	6.61	6.51	6.48	0.089	0.844
NH_3N	21.15^{c}	26.55^{b}	38.59^{a}	0.484	0.008
Acetic acid	75.25^{a}	69.89^{b}	70.92^{b}	0.504	0.035
Propionic acid	16.76^{b}	20.92^{a}	20.05^{a}	0.425	0.034
Butyric acid	7.99	9.18	9.03	0.339	0.447
A:P ratio	4.49^{b}	3.35^{a}	3.55^{a}	0.141	0.033

SBO = soybean oil; LSO = Linseed oil; FO = fish oil;

A:P ratio = acetate: propionate ratio

SEM = standard error of the mean

^{abc} Within a row means without a common superscript letter differ.

7.4.5 Degradability of DM, CP, NDF and ADF

Supplementation of SBO+LSO+FO at 2% up to 4% of total feed DM had no effects on the intercept of the degradation curve at time zero, the potential degradability of the component, the rate constant of the potential degradability of the component and dry matter degradability coefficients of concentrate and rice straw at all out flow rates (P>0.05). (Table 7.6).

The degradation of crude protein in concentrate (Table 7.7) was unaffected by different levels of combination oil addition to fistulated cattle's diet. No changes in the potential degradability of crude protein component all h of incubation.

Addition of combination oil to the diet did not significantly influence (P>0.05) on the neutral detergent fiber degradability of rice straw (Table 7.8), however, 4% of total feed DM oils (SBO+LSO+FO) increased the intercept of the degradation curve at time zero (P<0.05) but tended to reduce the potential degradability of the ADF component (P=0.087). Mixed oils at 4% of total feed DM tended to decrease the rate constant for the degradation (P = 0.056). There is a tendency toward a reduction in ADF degradability coefficients of rice straw at out flow rate 0.05/h (P= 0.073) and 0.08/h (P=0.072).

Table 7.6 Effect of different level of combination oil supplementation on dry matter degradability (DMD) of concentrate and rice straw in fistulated cattle.

Item	SBO+LSO+FO (1:1:1 w/w/w)			SEM	P-Value
	2%	3%	4%		
Dry matter degradability of concentrate					
a	23.57	21.93	21.55	0.329	0.221
b	58.15	60.13	64.20	2.171	0.597
a + *b*	81.72	82.07	85.75	2.036	0.713
c, per h	0.151	0.151	0.149	0.003	0.187
dg, 0.02/h	0.65	0.64	0.67	0.029	0.918
dg, 0.05/h	0.56	0.55	0.55	0.014	0.908
dg, 0.08/h	0.52	0.50	0.49	0.017	0.738
Dry matter degradability of rice straw					
a	7.40	8.60	8.16	0.346	0.493
b	47.83	44.13	40.87	1.906	0.472
a + *b*	55.23	52.73	49.03	2.189	0.596
c, per h	0.027	0.023	0.028	0.003	0.742
dg, 0.02/h	0.33	0.33	0.32	0.010	0.881
dg, 0.05/h	0.23	0.22	0.22	0.008	0.941
dg, 0.08/h	0.18	0.18	0.18	0.007	0.917

SBO = soybean oil; LSO = linseed oil; FO= fish oil; SEM = standard error of the mean; *a* = the intercept of the degradation curve at time zero; *b* = the potential degradability of the component; *c* = the rate constant for the degradation of '*b*'

Table 7.7 Effect of different level of combination oil supplementation on crude protein degradability (CPD) of concentrate in fistulated cattle.

Item	SBO+LSO+FO (1:1:1 w/w/w)			SEM	P-Value
	2%	3%	4%		
Crude protein degradability of concentrate					
a	17.97	15.50	19.06	1.228	0.575
b	64.25	59.20	53.60	1.415	0.174
a + *b*	82.23	74.70	72.66	1.981	0.316
c, per h	0.119	0.113	0.111	0.003	0.246
dg, 0.02/h	0.66	0.68	0.70	0.017	0.165
dg, 0.05/h	0.62	0.60	0.64	0.012	0.508
dg, 0.08/h	0.60	0.57	0.60	0.015	0.665

SBO = soybean oil; LSO = linseed oil; FO= fish oil;

SEM = standard error of the mean;

a = the intercept of the degradation curve at time zero;

b = the potential degradability of the component;

c = the rate constant for the degradation of '*b*'

Table 7.8 Effect of different level of combination oil supplementation on neutral detergent fiber degradability (NDFD) and acid detergent fiber degradability (ADFD) of rice straw in fistulated cattle.

Item	SBO+LSO+FO (1:1:1 w/w/w)			SEM	P-Value
	2%	3%	4%		
Neutral detergent fiber degradability of rice straw					
a	6.43	6.40	6.31	1.228	0.991
b	35.37	35.97	39.53	1.415	0.536
a + *b*	41.80	42.37	45.84	1.981	0.647
c, per h	0.076	0.097	0.078	0.003	0.434
dg, 0.02/h	0.36	0.36	0.37	0.017	0.962
dg, 0.05/h	0.30	0.30	0.29	0.012	0.993
dg, 0.08/h	0.26	0.26	0.25	0.015	0.916
Acid detergent fiber degradability of rice straw					
a	3.13^{b}	3.47^{b}	5.96^{a}	0.106	0.004
b	52.97	46.27	61.10	2.290	0.087
a + *b*	56.10	49.74	67.06	2.183	0.056
c, per h	0.089^{a}	0.062^{a}	0.032^{b}	0.003	0.013
dg, 0.02/h	0.40	0.35	0.34	0.012	0.317
dg, 0.05/h	0.31	0.26	0.21	0.008	0.073
dg, 0.08/h	0.25	0.21	0.17	0.007	0.072

SBO = soybean oil; LSO = linseed oil; FO= fish oil; SEM = standard error of the mean;*a* = the intercept of the degradation curve at time zero; *b* = the potential degradability of the component; *c* = the rate constant for the degradation of '*b*'

7.5 Discussion

7.5.1 Fatty Acid Profile in Ruminal Content

The result from the current study observed that 4% combination oil of total feed DM addition significantly reduced ruminal concentration of C18:0 at all h after feeding. This can be attributed to high level of fish oil addition. Similar result was also found (Kim et al., 2008) when 2.3% up to 6.9% fish oil were added to the cattle diet resulting in decreased C18:0 flow into duodenal from 115.1 mg to 59.9 mg. Chow et al. (2004) also reported that supplementation of 0, 2 and 4% of fish oil in combination with linseed oil or sunflower oil linearly decreased concentration of C18:0 at 6 and 24 h *in vitro* incubation while *t11*-C18:1 concentration was significantly increased. Similarly, Wachira et al. (2000) supplemented fish oil in sheep diet and also observed a significant increase in duodenal flow of *t11*-C18:1. In addition, AbuGhazaleh and Jenkins (2004) observed several changes in the ruminal batch culture fatty acid profile when DHA was supplemented. Addition of 1, 2, 3, or 4% DHA increased *t11*-C18:1 and inhibited the bio-hydrogenation of oleic and linoleic acids and all level of DHA significantly decreased stearic acid in cultures by 24 h. The increase in *t11*-C18:1 was the product of ruminal bio-hydrogenation by group A bacteria. Kemp and Lander (1984) classified bacteria into two groups by functional change in the fatty acids. Both groups are capable of isomerization of C18:3n-3 and bio-hydrogenation to *t11,c15*-C18:2. The latter FA can be hydrogenated by group B bacteria resulting in *t15* or *c15*-C18:1, which will not be further hydrogenated, or by group A bacteria leading to *t11*-C18:1. Isomerization and bio-hydrogenation of C18:2n-6 to *t11*-C18:1, however, are solely affected by group A bacteria. Finally, *t11*-C18:1, derived from C18:3n-3 or C18:2n-6 can be hydrogenated

to C18:0 by group B bacteria (Harfoot and Hazlewood, 1997). Wachira et al. (2000) had indicated that fish oil might inhibit group B bacteria. AbuGhazaleh et al. (2002) suggested that an altering of these bacteria consequently caused an inhibition of the bacterial enzyme responsible for the final bio-hydrogenation step. The accumulation of *t11*-C18:1 in the rumen could explain the higher concentration of *c9,t11*-CLA in milk fat (Donovan et al., 2000) from cows receiving fish oil supplemented.

The greater intakes of C20:5n-3 and C22:6n-3 significantly increased the concentrations of 20:5n-3 and C22:6n-3 in the rumen content. It is clearly that lipolysis rates of C20:5n-3 and C22:6n-3 were lower as suggested by Chow et al. (2004), thus when high levels of fish oil were supplied, ruminal concentrations of both fatty acids were increased. Similarly, Doreau and Chilliard (1997) infused fish oil into the rumen and reported that more C22:6n-3 was detectable in duodenal contents of cows infused with fish oil (0.51%) than control (0.10%). Moreover, Loor et. al. (2005) injected C22:6n-3 into the rumen and found that C20:5n-3 and C22:6n-3 flow into duodenum was higher than control cattle. Dohme et al. (2003) reported *in vitro* rates of C22:6n-3 bio-hydrogenation that lipolysis and bio-hydrogenation both occurred in ruminal batch cultures, but that increasing levels of fish oil decreased the percentage of both lipolysis and bio-hydrogenation at 24 h. In addition, Sterk et al. (2012) supplemented DHA+Linseed oil and found an increase in the concentration of DHA flow into omasum when compare to those supplemented with crushed linseed, extruded whole linseed and formaldehyde- treated linseed. Donovan et al. (2000) supplemented 0, 1, 2 and 3% fish oil to lactating dairy cows and found a linear increase in EPA and DHA in milk. Similarly, Palmquist et al. (2006) added 0, 0.33, 0.67 and 1.00% fish oil to the diet of dairy and observed a linear increase in the concentration of C20:5n-3 and C22:6n-3 in milk with increasing fish oil addition.

However, Chow et al. (2004) reported that fish oil inclusion did not affect average lipolysis and release of the individual PUFA examined.

The increased ruminal C16:0 concentration at 4h after feeding when cattle received high level of fish oil reflected higher intake of C16:0 as a result of reasonable C16:0 content (28.22 % of total fatty acid) in fish oil. Kitessa et al. (2001a) supplemented protected tuna oil or tuna oil and also found an increase in C16:0 concentration in the rumen. Similarly, Loor et al. (2005) supplemented 2.5% of total feed DM fish oil, 5% of total feed DM sunflower oil and 5% of total feed DM linseed oil in Holstein cows and reported that fish oil cow had higher concentration of C16:0 when compared to sunflower oil and linseed oil cows.

Ruminal C18:2n-6 and C18:3n-3 were unaffected by all level of combination oil supplementation at all h after feeding in the present study. Similar result was also reported (Chow et al., 2004). They reported that lipolysis and bio-hydrogenation of C18:2n-6 and C18:3n-3 were not influenced by fish oil inclusion. In addition, there was no breakdown of C18 FA during incubation and the proportion of esterified or free C18:2n-6 and C18:3n-3 in total C18 FA did not differ significantly. Gulati et al. (1999) observed that FO inclusion had no effect on apparent bio-hydrogenation of C18:3n-3 and C18:2n-6, and consequently, disappearance of these FA occurred to the same extent. *In vivo* experiments of AbuGhazaleh et al. (2002) also showed no significant difference in ruminal C18:2n-6 content of animals on a diet containing extruded soybean or FO/extruded soybean. Similarly, Wachira et al. (2000) reported no difference in duodenal flow of C18:3n-3 when offering linseed or linseed/FO.

7.5.2 Ruminal Fermentation

The rumen ecosystem consists of a highly diverse collection of anaerobic microbes with the majority (70-80% of the microbial matter in the rumen) attached to feed particles in the digesta (McAllister et al., 1994). Rumen bacteria play the main role in lipid metabolism in the rumen (Harfoot and Hazlewood, 1997; Jenkins et al., 2008). Ruminal pH was one factor to control ruminal bio-hydrogenation. AbuGhazaleh and

Jacobson (2007) reported less disappearance of C18 unsaturated FA in cultures under low pH conditions. Additionally, Van Nevel and Demeyer (1996) observed a drop in C18:2n-6 disappearance from rumen cultures as pH changed from 6.8 to 5.2. Latham et al. (1972) showed that switching lactating dairy cows from a high to a low forage diet, which promoted low rumen pH, resulted in lower levels of lipolytic activity and BH of unsaturated FA in ruminal fluid. Most rumen microbes are sensitive to low pH conditions as acidity in the rumen impact microbial growth and enzymes activities (Martin et al., 2002; Jenkins et al., 2008). However, in the current study, supplementation combination oil up to 4% of total feed DM had no effect on ruminal pH. Toral et al. (2009) showed similar result to the present study. Toral et al. (2009) supplemented sunflower oil in combination with fish oil and found no significant difference in ruminal pH. The ruminal pH of 6.7 favors the formation of *t11*-C18:1 and c9, *t11*-C18:2. Under this condition, *t11*-C18:1 and c9,*t11*-C18:2 were produced at higher amount than *t10*-C18:1, *t10,c12*- C18:2 (Hou et al., 2011). AbuGhazaleh and Jacobson (2007) and Fuentes et al. (2009) suggested that higher ruminal pH favors the formation of *t11*-C18:1 and c9,*t11*-C18:2. The higher proportion of c9,*t11*-C18:2 and *t11*-C18:1 at higher pH was also observed by Troegeler-Meynadier et al. (2003) and Fuentes et al. (2008). The decreased linoleic

and linolenic acids in low pH cultures might have been due to a lowered bio-hydrogenation activity by culture microbes because low rumen pH has been shown to have a negative effect on microbial growth (Martin and Jenkins, 2002).

In the present study, supplementation of 4% combination oil significantly increased concentration of ruminal ammonia nitrogen at all h after feeding which is similar to Keandy and Mayne (1999) who supplied 0 to 450g fish oil and reported that the concentration of ruminal ammonia nitrogen was linearly increased. The effect of lipid supplementation on ruminal fermentation relies mainly on 3 factors: first, the type of oil (Wachira et al., 2000), second, the level of oil inclusion in the diet (Shingfield et al., 2008) and third, the dietary forage: concentrate ratio. The present study controlled 2 factors including type of oil and dietary concentrate: roughage ratio. The increased level of oil resulted in increased ruminal ammonia nitrogen. Fuentes et al. (2009) and Ramos et al. (2009) suggested that the higher ruminal ammonia nitrogen might be due to higher protein digestibility under high pH and/or the higher protein degradability in forage relative to concentrates. Gomez-Cortés et al. (2008) and Zhang et al. (2008) also observed that addition oil rich in C18:2n-6 in sheep increased ruminal ammonia nitrogen in the rumen fluid.

In previous studies, addition of fish oil to the diet has often been reported to result in an increase in molar proportion of propionate and a decrease in molar proportion of acetate (Doreau and Chilliard, 1997; Keady and Mayne, 1999; Wachira et al., 2000; Fievez et al., 2003) which is similar to the present study that supplementation of 4% combination oil significantly increased molar proportion of propionate but decreased molar proportion of acetate at all h after feeding. Higher oil consumption affected cellulolytic bacteria and produced lower acetate (Maia et al., 2007; Zhang et al., 2008). Wallace et al. (2006) observed a decrease in concentration

of acetate and suggested that fish oil supplementation favored, although transiently, *Butyrivibrio* strains producing butyrate by the acetyl-CoA/butyryl-CoA transferase pathway. Gonthier et al. (2004) supplying 3 to 4% of additional linseed oil in the diet have been shown to increase molar proportion of propionate at the expense of acetate. Jalč et al., (2007) supplementing oil rich in omega 6 in combination with fish oil showed the greater propionic acid while acetic acid decreased.

7.5.3 Degradability of DM, CP, NDF and ADF

The various levels of mixed oils did not change DM, CP and NDF degradability of concentrate and rice straw, which is similar to the observation of Aemiro et al. (2017) who added sources of DHA in the sheep's diets at 0, 50, 100 and 150 g/kg/d and reported that DM, OM, NDF and ADF degradability coefficients were unaffected by oil addition. Yang et al. (2009) supplemented 4% LSO, 4% SBO and 4% LSO+SBO (1:1 w/w) and found no effects on proteolytic bacteria, cellulolytic bacteria and protozoa populations. (Dohme et al., 2001) revealed that relatively high amounts of EPA and DHA might escape rumen hydrogenation. Fievez et al. (2003) investigated the daily amount of FO represented 4.2% (w/w) of the DMI of hay and concentrate and observed that FO supplementation did not reduce *in vivo* NDF digestion or NDF degradability after 6 h of *in sacco* incubation. This is consistent with *in vitro* results (Sutton et al., 1975; Doreau, 1992; Choi et al., 1998 and Keady and Mayne, 1999), suggesting no effect of FO treatment on ruminal OM or ADF disappearance. Nevertheless, rumen fiber degradation was reduced after 48 h of *in sacco* incubation which is in agreement with the present study. This study found the detrimental tendency on ADF degradability when high level of combination oil was supplied. Similarly, Yang et al. (2009) reported considerably lower number of cellulolytic bacteria when oil was fed and the observation by Hu et al. (2007) found corresponding

lower ruminal digestibility of NDF (56% v. 51%) and ADF (53% v. 50%) when supplied higher oils. Yang et al. (2009) concluded that oil supplementation up to 4% of diet DM to dairy cows decreased ruminal fermentation leading to lower VFA concentrations. Populations of ruminal microorganisms also were affected by LSO and SBO, with total protozoa and cellulolytic bacteria being reduced, and total proteolytic bacteria being increased by oils. However, Annet at al. (2008) fed fish oil to lamb and found a tendency towards an increase in the digestibility of ADF (P=0.08) in the total diet. FO infusion into the rumen increased degradation of fiber in dairy cows (Doreau and Chilliard, 1997). Ivan et al. (2012) reported an increase in *R. flavefaciens* population in dairy cattle. The different findings can be attributed to the different level and concentration of PUFA in the rumen. The growth of *R. flavefaciens* increased when PUFA in the rumen were at a low level, but decreased when these acids were fed higher levels (Zhang et al., 2008). Additionally, Ebrahimi (2012) reported increases in the population of *R. albus* when cattle and goats were fed PUFA.

7.6 Conclusion

It can be concluded in the present study that supplemented 4% combination oil significantly decreased the concentration of C18:0 and increased t11-C18:1, C20:5n-3 and C22:6n-3 in rumen content at all times after feeding. No significant difference in ruminal pH was found, however, addition of 4% combination oil to the diet increased molar proportion of propionic acid meanwhile molar proportion of acetic acid decreased at 2, 4 and 6 h after feeding. Ammonia nitrogen was greater at 2, 4 and 6 h after feeding when 4% of combination oil was added. Nevertheless, addition of 4% combination oil tended to reduce ADF degradability at 0.05 and 0.08 /h out flow rate.

7.7 References

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CHAPTER VIII

OVERALL CONCLUSIONS AND IMPLICATION

The series of the present studies aim to obtain health beneficial fatty acids or their isomers in the ruminal content so that they will be absorbed and ruminant's tissues can uptake these fatty acids or their isomers for deposition or synthesis and subsequently retain in milk or meat products. The series of these studies commence from the 1st experiment that was conducted to determine whether ruminal concentrations of *t11*-C18:1, the CLA synthesized precursor, and omega-3 fatty acids were increased by different forms of LSO and combination with fish oil. The result clearly revealed that the ruminal concentrations of *t11*-C18:1 and C22:6n-3 was increased while of C18:0 was decreased by LSO+FO addition. All of the oil treatments imposed had no effects on ruminal pH, *in sacco* DMD, CPD, NDFD, ADFD; and propionate and butyrate proportion when compare to non-supplemented control.

The 2nd trial was carried out to evaluate whether ruminal concentrations of *t11*-C18:1, *c9,t11*-C18:2 and omega-6 fatty acids were increased by SBO, FO and SBO+FO supplementation. The result clearly demonstrated that the ruminal concentrations of *t11*-C18:1 and *c9,t11*- C18:2 were significantly increased by SBO and SBO+FO application while the concentration of C18:0 was reduced by FO and SBO+FO supplementation.

The 3rd experiment was conducted in accordance with the result from experiment 1 to investigate whether the ruminal concentrations of *t11*-C18:1 and omega-3 fatty acids were favorably changed by different ratios of LSO+FO addition.

The result clearly indicated that the ruminal concentration of *t11*-C18:1 was increased by 1:1 w/w linseed oil mixed with fish oil whereas the ruminal concentrations of C20:5n-3 and C22:6n-3 were increased by the addition of 1:2 w/w linseed oil in combination with fish oil.

The 4th experiment was carried out in accordance with the result from experiment 2 to determine whether the ruminal concentration of *t11*-C18:1 and omega-3 fatty acids was positively enhanced by different ratios of SBO+FO supplementation. The result clearly showed that feeding 1:1 w/w SBO+FO compromised the ruminal concentrations of *t11*-C18:1, C20:5n-3 and C22:6n-3. The degradation of DM, CP, NDF and ADF was unaffected by various ratios of oil addition in this study.

The last experiment was designed to combine the best result from experiment 3 and experiment 4 and to evaluate the optimum level of combination oil enhancing the ruminal production of *t11*-C18:1, C18:3n-3, C20:5n-3 and C22:6n-3. The result clearly found that 4% combination oil (1:1:1 w/w SBO+LSO+FO) significantly increased the ruminal concentration of *t11*-C18:1, C20:5n-3 and C22:6n-3 at .all h after feeding.

It can be clearly concluded in the present studies that the health beneficial fatty acids and their isomers can be obtained by the addition of mixed oils. The enhancements of ruminal *trans* vaccenic acid (the precursor for CLA synthesis), C18:3n-3, DHA and EPA can be achieved by addition of an equal ratio of SBO+LSO+FO. The higher level was fed, the higher concentration of these fatty acids was obtained. The supplementation of different types, ratios and levels of oils had no or negligible influences on ruminal fermentation and nutrient degradation.

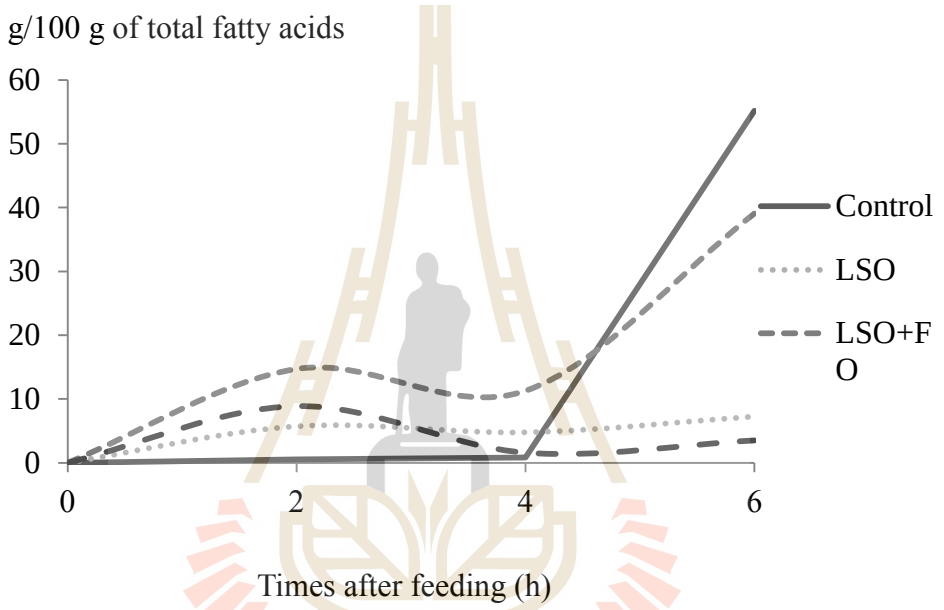
It can be recommended from the results of these studies that 1:1:1 w/w SBO+LSO+FO addition produced reasonable ruminal concentration of *t11*-C18:1, C20:5n-3 and C22:6n-3. These findings can be used as guideline to improve quality of

animal's products. The optimum level of oils supplement is one of many factors that improve animal performance, particularly growth rate, carcass quality, milk yield and composition. Therefore, to manipulate feeding approach to improve health beneficial fatty acids without or less negative effects, further researches investigating in production trials are advisable.

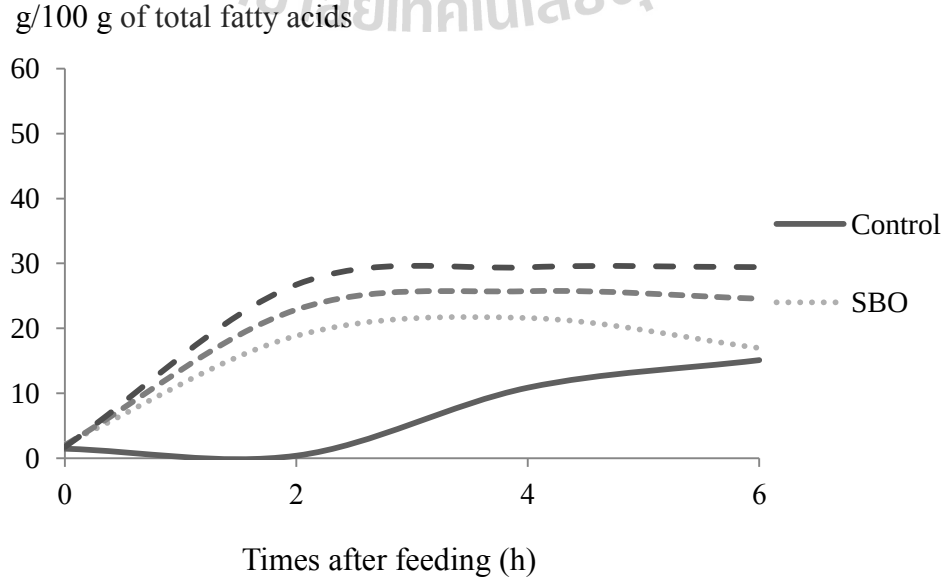
APPENDIX

Overall of *t11*-C18:1 produced in the rumen of cattle due to oil supplementation

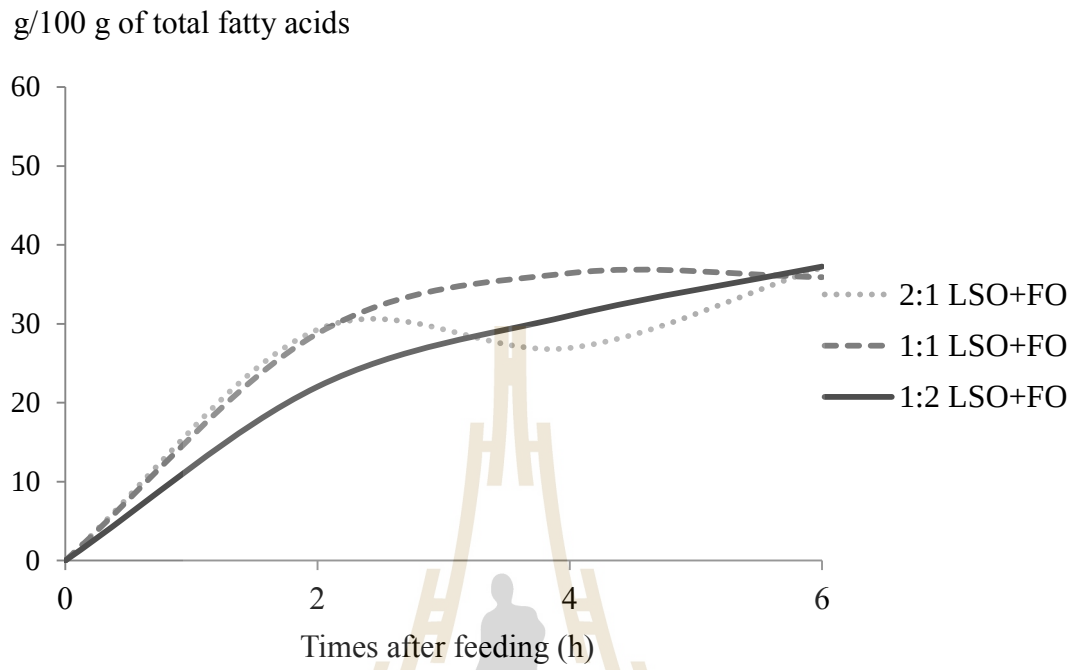
Experiment 1



Experiment 2



Experiment 3



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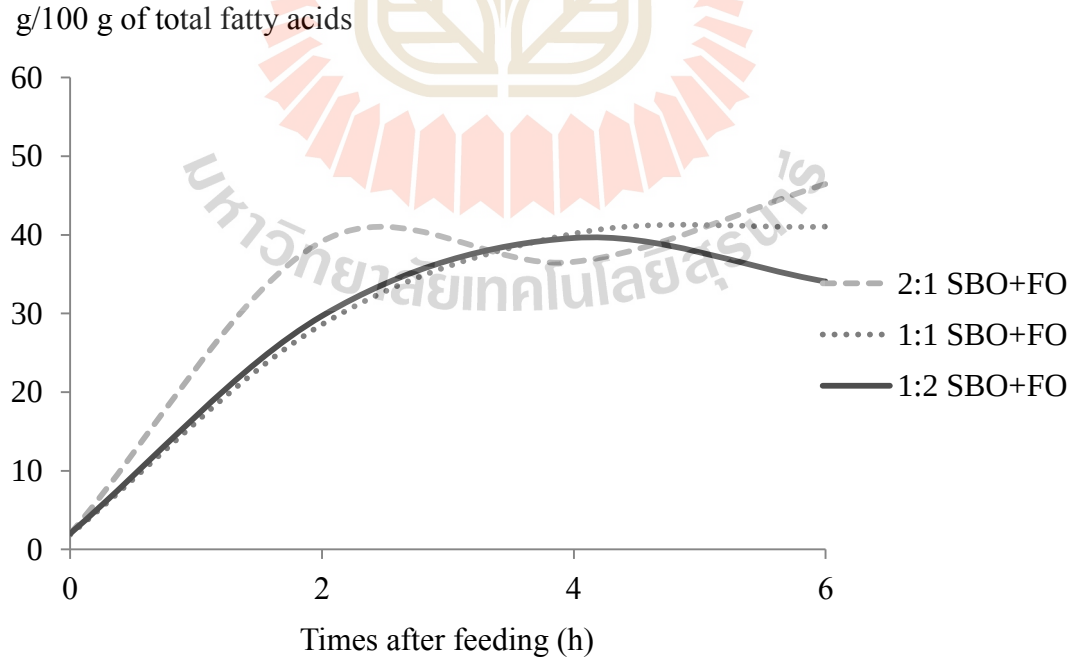
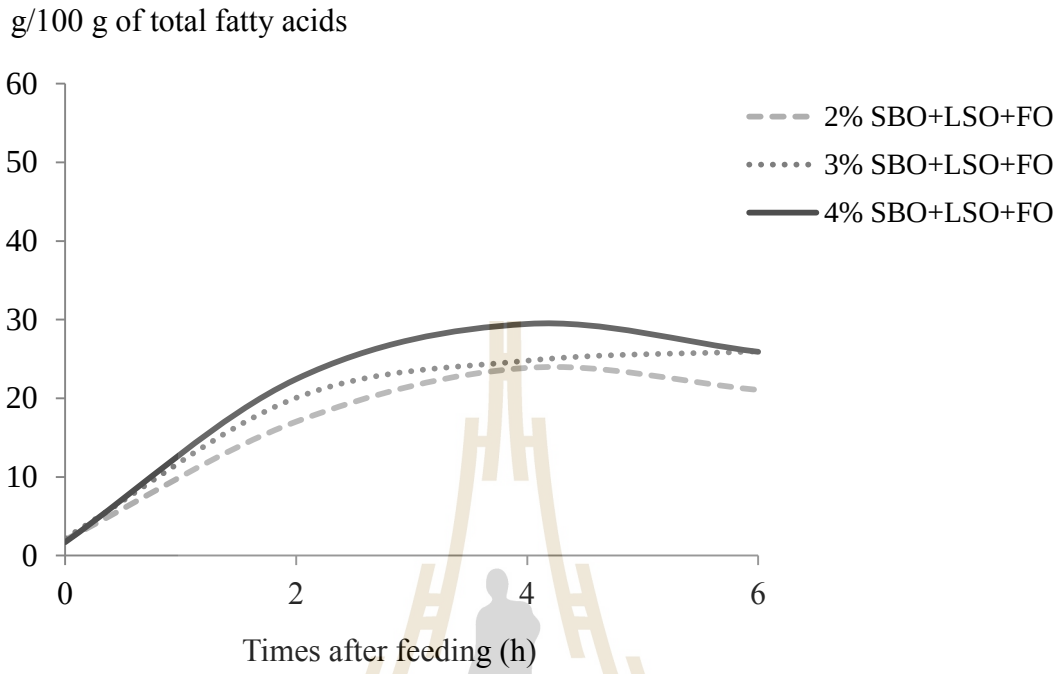


Experiment 5



BIOGRAPHY

Mr. Chayapol Meeprom was born on 5th November 1989 in Surin province, Thailand. He finished his primary school from Muang Surin School, Surin province and then he finished his secondary school from Surawittayakarn School at the same province. He graduated Bachelor of Science in Animal Production Technology, Institute of Agricultural Technology, Suranaree University of Technology in 2010.

He received Master of Science in Animal Production Technology, Institute of Agricultural Technology, Suranaree University of Technology in 2012. In 2013, he continued to study Doctor of Philosophy in Animal Production Technology, Institute of Agricultural Technology, Suranaree University of Technology.