ACCUMULATION OF CONJUGATED LINOLEIC ACID IN BEEF AND COW'S MILK THROUGH SUPPLEMENTATION OF SOYBEAN OIL, WHOLE COTTONSEED OR RUMEN PROTECTED CONJUGATED LINOLEIC ACID

Khukhuan Chullanandana

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

Suranaree University of Technology

Academic Year 2007

การเสริมน้ำมันถั่วเหลือง เมล็ดฝ้าย หรือ Rumen protected conjugated linoleic acid (RP-CLA) ต่อการสะสมของ conjugated linoleic acid (CLA) ในเนื้อ และน้ำนม

นางสาวคู่ขวัญ จุลละนันทน์

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรดุษฎีบัณฑิต

สาขาวิชาเทคโนโลยีการผลิตสัตว์

มหาวิทยาลัยเทคโนโลยีสุรนารี

ปีการศึกษา 2550

ACCUMULATION OF CONJUGATED LINOLEIC ACID IN BEEF AND COW'S MILK THROUGH SUPPLEMENTATION OF SOYBEAN OIL, WHOLE COTTONSEED OR RUMEN PROTECTED CONJUGATED LINOLEIC ACID

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คู่ขวัญ จุลละนันทน์ : การเสริมน้ำมันถั่วเหลือง เมล็ดฝ้าย หรือ Rumen protected conjugated linoleic acid ต่อการสะสมของ conjugated linoleic acid ในเนื้อ และน้ำนม (ACCUMULATION OF CONJUGATED LINOLEIC ACID IN BEEF AND COW'S MILK THROUGH SUPPLEMENTATION OF SOYBEAN OIL, WHOLE COTTONSEED OR RUMEN PROTECTED CONJUGATED LINOLEIC ACID) อาจารย์ที่ปรึกษา : รองศาสตราจารย์ ดร.วิศิษฐิพร สุขสมบัติ, 142 หน้า.

วัตถุประสงค์ของงานวิจัยนี้คือ การศึกษาแหล่งของไขมันที่มีปริมาณกรดลิโนเลอิกสูง โดยเฉพาะอย่างยิ่งน้ำมันถั่วเหลือง เมล็ดฝ้าย หรือ Rumen protected conjugated linoleic acid (RP-CLA) เสริมในอาหารโคขุนและโครีดนม เพื่อพิจารณาผลต่อการสะสมกรดไขมันและ CLA ในเนื้อและน้ำนม

การทดลองที่ 1 โคขุนจำนวน 18 ตัว น้ำหนักตัวเฉลี่ย 241±24 กิโลกรัม และอายุเฉลี่ยประมาณ 1 ปี ใช้แผนการทดลองแบบบล็อกสมบูรณ์ กลุ่มทดลอง คือ 1) กลุ่มควบคุม 2) เสริมน้ำมันถั่วเหลือง 170 กรัมต่อวัน และ 3) เสริมน้ำมันจากเมล็ดฝ้าย 170 กรัมต่อวันในอาหารโคขุน ผลการทดลองพบว่า การเสริมน้ำมันถั่วเหลือง ทำให้ C18:2 *cis*-9, *trans*-11 CLA เพิ่มขึ้น 116 เปอร์เซ็นต์ ในกล้ามเนื้อสันนอก (*longissimus dorsi* muscle) ($P<0.01$) และในกล้ามเนื้อสะโพก (*semimembranosus* muscle) เพิ่มขึ้น 240 เปอร์เซ็นต์ ($P<0.01$) อย่างไรก็ตามการเสริมทั้งน้ำมันถั่วเหลืองและเมล็ดฝ้ายไม่ส่งผลกระทบต่อการจริญเติบโต และลักษณะซาก นอกจากนี้ ค่าความเป็นกรด-ด่าง แอมโมเนีย ไนโตรเจน โปรโตซัว และความเข้มข้นกรดไขมันระเหยได้ พบว่า ไม่แตกต่างกันอย่างมีนัยสำคัญทางสถิติ เมื่อเสริมน้ำมันถั่วเหลืองและเมล็ดฝ้าย การเสริมน้ำมันถั่วเหลืองในอาหารเพียงกลุ่มเดียวเท่านั้นสามารถตรวจพบ C18:2 *cis*-9, *trans*-11 CLA ใน rumen digesta เปรียบเทียบกับกลุ่มควบคุมและกลุ่มเสริมเมล็ดฝ้าย การศึกษาครั้งนี้ชัดเจนว่าการเสริมน้ำมันถั่วเหลืองในอาหารโคขุนดีกว่าเมล็ดฝ้ายในการสะสม CLA ในเนื้อ

การทดลองที่ 2 ใช้โครีดนมลูกผสมโฮลสไตน์ฟรีเชี่ยนจำนวน 24 ตัว จำนวนวันการให้นมเฉลี่ย 126±45 วัน ใช้แผนการทดลองแบบบล็อกสมบูรณ์ กลุ่มทดลองคือ 1) กลุ่มควบคุม 2) เสริมน้ำมันถั่วเหลือง 150 กรัมต่อวัน และ 3) เสริม Rumen protected conjugated linoleic acid (RP-CLA) 150 กรัมต่อวันในอาหารโครีดนม ผลการทดลอง พบว่า การเสริมน้ำมันถั่วเหลืองทำให้ C18:2 *cis*-9, *trans*-11 CLA ในน้ำนมเพิ่มขึ้น 65 เปอร์เซ็นต์ ($P<0.01$) ส่วนกลุ่ม RP-CLA สามารถเพิ่ม C18:2 *trans*-10, *cis*-12 CLA ในน้ำนม ($P<0.01$) เมื่อเปรียบเทียบกับกลุ่มควบคุม อย่างไรก็ตามการเสริมทั้งน้ำมันถั่วเหลืองและ RP-CLA สามารถเพิ่ม total CLA ในน้ำนม ($P<0.01$) นอกจากนี้ยังพบว่า ปริมาณน้ำนมและองค์ประกอบน้ำนมไม่แตกต่างกันอย่างมียสำคัญ

ทางสถิติระหว่างกลุ่มทดลอง ยกเว้นกลุ่มที่เสริม RP-CLA ทำให้เปอร์เซ็นต์ไขมันนมและปริมาณไขมันนมลดลง ($P<0.01$) ค่าความเป็นกรด-ด่าง แอมโมเนียไนโตรเจน โปรโตซัว และความเข้มข้นกรดไขมันระเหยได้ไม่แตกต่างกันอย่างมีนัยสำคัญทางสถิติ เมื่อเสริมทั้งน้ำมันถั่วเหลืองและ RP-CLA ในอาหารโครีดนม กรดไขมันส่วนใหญ่ใน rumen digesta พบว่า ไม่แตกต่างกันอย่างมีนัยสำคัญทางสถิติระหว่างกลุ่มทดลอง อย่างไรก็ตามในกลุ่มเสริม RP-CLA ทำให้ CLA isomers ใน rumen digesta โดยเฉพาะ C18:2 *trans*-10, *cis*-12 CLA เพิ่มขึ้น เมื่อเปรียบเทียบกับกลุ่มอื่น การศึกษาครั้งนี้แนะนำว่าการเสริมน้ำมันถั่วเหลืองในอาหารโครีดนมดีกว่า RP-CLA ในการสะสม CLA ในน้ำนม

สาขาวิชา เทคโนโลยีการผลิตสัตว์ ลายมือชื่อนักศึกษา_____

ปีการศึกษา 2550 ลายมือชื่ออาจารย์ที่ปรึกษา_____

ลายมือชื่ออาจารย์ที่ปรึกษาร่วม_____

ลายมือชื่ออาจารย์ที่ปรึกษาร่วม_____

KHUKHUAN CHULLANANDANA : ACCUMULATION OF CONJUGATED LINOLEIC ACID IN BEEF AND COW'S MILK THROUGH SUPPLEMENTATION OF SOYBEAN OIL, WHOLE COTTONSEED OR RUMEN PROTECTED CONJUGATED LINOLEIC ACID. THESIS ADVISOR : ASSOC. PROF. WISITIPORN SUKSOMBAT, Ph.D., 142 PP.

SOYBEAN OIL/WHOLE COTTONSEED/RUMEN PROTECTED CONJUGATED LINOLEIC ACID/CONJUGATED LINOLEIC ACID/FATTY ACID/BEEF/MILK

The objective of this research was to study the sources of fat rich in linoleic acid, especially, soybean oil (SBO), whole cottonseed (WCS) or rumen protected conjugated linoleic acid (RP-CLA), supplemented in fattening cattle and lactating cow diets; and then determine fatty acid profiles and conjugated linoleic acids (CLA) accumulation in beef and milk.

Experiment I, eighteen fattening cattle, averaging 241±24 kg live weight (LW) and approximate 1 year old, were stratified and randomly assigned in a randomized complete block design. The treatments were divided into 3 groups: 1) control, 2) control plus 170 g SBO/d, and 3) control plus 170 g of oil from WCS/d in fattening cattle diets. The results showed that feeding SBO significantly increased ($P<0.01$) C18:2 *cis*-9, *trans*-11 CLA in *longissimus dorsi* muscle by 116% and in *semimembranosus* muscle by 240%. However, both SBO and WCS supplementation did not significantly affect their performances and carcass quality. Moreover, ruminal pH, ammonia N, total protozoa and VFA concentrations in rumen fluid were not significantly different when SBO and WCS were added. Only with the addition of SBO in diets could C18:2 *cis*-9, *trans*-11 CLA content in rumen digesta be detected, compared with control and

WCS supplemented groups Thus, it could be clearly concluded in the present study that the SBO addition to fattening cattle diets was superior to WCS in accumulation of CLA in beef.

Experiment II, twenty four crossbred Holstein Friesian lactating dairy cows that averaged 126±45 days in milk were stratified and randomly assigned in a randomized complete block design. The treatments were divided into 3 groups as well: 1) control, 2) control plus 150 g of SBO/d and 3) control plus 150 g of RP-CLA/d supplementation in lactating cow diets. The results demonstrated that the feeding of SBO significantly increased ($P<0.01$) C18:2 *cis*-9, *trans*-11 CLA in milk by 65%. RP-CLA group significantly increased ($P<0.01$) *trans*-10, *cis*-12 CLA concentration compared with control and SBO treatments. However, total CLA concentration was significantly increased ($P<0.01$) by SBO and RP-CLA additions. Moreover, there were no significantly differences in milk yield and milk composition among the treatment groups, except for milk percentage and fat yield that were significantly decreased ($P<0.01$) by RP-CLA supplementation. Moreover, the additions of SBO and RP-CLA did not significantly affect ruminal pH, ammonia N, total protozoa and VFA concentration. Most of fatty acids in rumen digesta were not significantly different by treatments. However, CLA isomers in rumen digesta particularly *cis*-9, *trans*-11 CLA were increased by RP-CLA, compared with other treatments. Therefore, this study suggests that SBO supplementation in lactating cow diets is better than RP-CLA in accumulation of CLA in dairy cows' milk.

School of Animal Production Technology Student's Signature_____

Academic Year 2007 Advisor's Signature_____

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ACKNOWLEDGEMENTS

First of all, I would like to express my sincere thanks to my thesis advisor, Assoc. Prof. Dr. Wisitiporn Suksombat for his invaluable help, continuous guidance and encouragement throughout the course of this research. In addition, I would also like to thank Assoc. Prof. Dr. Suthipong Uriyapongson and Asst. Prof. Dr. Pramote Paengkoum, my co-advisors, for their guidance and supports. I would also like to thank Dr. Pipat Lounglawan for helping and guidance of the thesis.

I would like to thank staff of dairy group, University farm and the Center of Scientific and Technological Equipment and my friends in Animal Production Technology, Suranaree University of Technology for helpful suggestion.

Finally, I most gratefully acknowledge my parents and my friends for all their support throughout the period of this research.

Khukhuan Chullanandana

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CHAPTER I

INTRODUCTION

In general, food products from ruminant are high in saturated fatty acids and low in polyunsaturated fatty acids. The consumption of saturated fatty acid may increase serum low-density lipoprotein (LDL) cholesterol level, which is risk factor for coronary heart disease (Tanaka, 2005). Therefore, increasing the proportion of healthy fatty acids in ruminant products is interesting, mainly milk and meat because they are major dietary sources of conjugated linoleic acid (CLA) for human. As the interested beneficial effects of CLA, particularly *cis*-9, *trans*-11 CLA and *trans*-10, *cis*-12 CLA, it has been reported that they include, inhibiting carcinogenesis (Belury, 2002), reducing atherosclerosis, enhancing the immune response and reducing the body fat mass (Bauman et al., 1999; McGuire and McGuire, 1999; Khanal and Dhiman, 2004; Wahle et al., 2004; Schmid et al., 2006; De La Torre et al., 2006). Moreover, Rizenthaler et al. (2001) reported that CLA requirement for human should more than 400 mg/d for good health, but in the present time human receive on the average less than 200 mg CLA/d. Thus, increasing CLA content in milk and meat are very interesting for good health.

Conjugated linoleic acids are fatty acids that are found naturally in foods derived from ruminant animals, first discovered by Pariza and his group when investigating the carcinogenic components of grilled beef (Wahle et al., 2004). Conjugated linoleic acid is a mixture of geometric and positional isomers of linoleic

acid with conjugated double bonds. Conjugated linoleic acids in ruminant fat are intermediates in the biohydrogenation of polyunsaturated fatty acids (PUFA) by ruminal microorganisms. The originate CLA from absorbed after escaping completed biohydrogenation in the rumen and the major source is endogenous synthesis of CLA from *trans*-11 C18:1 vaccenic acid by Δ^9 desaturase in tissue (Corl et al., 2001). Thus, supplementation sources of fat rich in linoleic acid such as plant oils may increase the proportion of CLA in milk (Leonardi et al., 2005; Loor et al., 2005; Zheng et al., 2005; Shingfield et al., 2006). Researchers have successfully increased the C18:2 *cis*-9, *trans*-11 CLA content of muscle lipids by source of plant oils (Engle et al., 2000; Mir et al., 2002; Mir et al., 2003; Noci et al., 2005, Noci et al., 2007) and oilseeds (Bolte et al., 2002). Furthermore, previous researches reported that supplementation of rumen protected conjugated linoleic acid (RP-CLA) (protected from rumen biohydrogenation) increased CLA concentration in milk (Kelly et al., 1998; Perfield et al., 2002; Perfield et al., 2004; Piperova et al., 2004; Castaneda-Gutierrez et al., 2005).

This study interested in sources of fat rich in linoleic acid, especially, soybean oil, whole cottonseed and rumen protected conjugated linoleic acid and effects of supplementation in cattle diets on fatty acid profiles and CLA accumulation in beef and milk. The first part of this study was to supplement with soybean oil (SBO) and whole cottonseed (WCS) in fattening cattle's diets. The second part added soybean oil (SBO) and rumen protected conjugated linoleic acid (RP-CLA) in Crossbred Holstein Friesian cow's diets.

1.1 Research Hypothesis

1.1.1 Supplementation of soybean oil and whole cottonseed in fattening cattle's diets may increase conjugated linoleic acids accumulation in beef.

1.1.2 Supplementation of soybean oil and rumen protected conjugated linoleic acid in crossbred Holstein Friesian cow's diets may improve milk production and milk composition through increasing conjugated linoleic acids accumulation in milk.

1.1.3 Supplementation of soybean oil and whole cottonseed in fattening cattle's diets and supplementation of soybean oil and rumen protected conjugated linoleic acid in crossbred Holstein Friesian cow's diets may increase conjugated linoleic acids in rumen digesta and had no negative effect on rumen ecology.

1.2 Research Objectives

1.2.1 To study the effect of soybean oil and whole cottonseed on fatty acid profiles and conjugated linoleic acid accumulation in beef.

1.2.2 To study the effect of soybean oil and rumen protected conjugated linoleic acid on fatty acid profiles and conjugated linoleic acid accumulation in milk.

1.2.3 To study the changes in fatty acid profiles and rumen ecology when soybean oil and whole cottonseed were supplemented in fattening cattle's diets and supplementation of soybean oil and rumen protected conjugated linoleic acid in crossbred Holstein Friesian cow's diets.

1.3 Scope of the Study

These researches intended to study the effects of soybean oil and whole cottonseed supplementation in fattening cattle's diets on change in fatty acids and accumulation of conjugated linoleic acids in beef. In addition, effects of soybean oil and rumen protected conjugated linoleic acid supplementation in crossbred Holstein Friesian cow's diets on change in fatty acids and accumulated conjugated linoleic acids in milk.

1.4 Expected Results

1.4.1 Higher level of conjugated linoleic acid accumulation in beef may occur when soybean oil and whole cottonseed were supplemented in fattening cattle's diets.

1.4.2 Higher level of conjugated linoleic acid accumulation in milk may occur when soybean oil and rumen protected conjugated linoleic acid were supplemented in crossbred Holstein Friesian cow's diets.

1.4.3 Some change in fatty acids and rumen ecology may occur when soybean oil, whole cottonseed and rumen protected conjugated linoleic acid were supplemented.

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CHAPTER II

LITERATURE REVIEW

2.1 Structure of Conjugated linoleic acid

Conjugated linoleic acid (CLAs) are series of group of geometric and positional isomers of linoleic acid. Linoleic acid, an 18-carbon unsaturated fatty acid with two double bonds, is either in the '*cis*' or the '*trans*' configuration (Wahle et al., 2004). For a comparison structure of the *cis*-9, *trans*-11 and *trans*-10, *cis*-12 isomers of CLA with linoleic acid, see Figure 2.1.

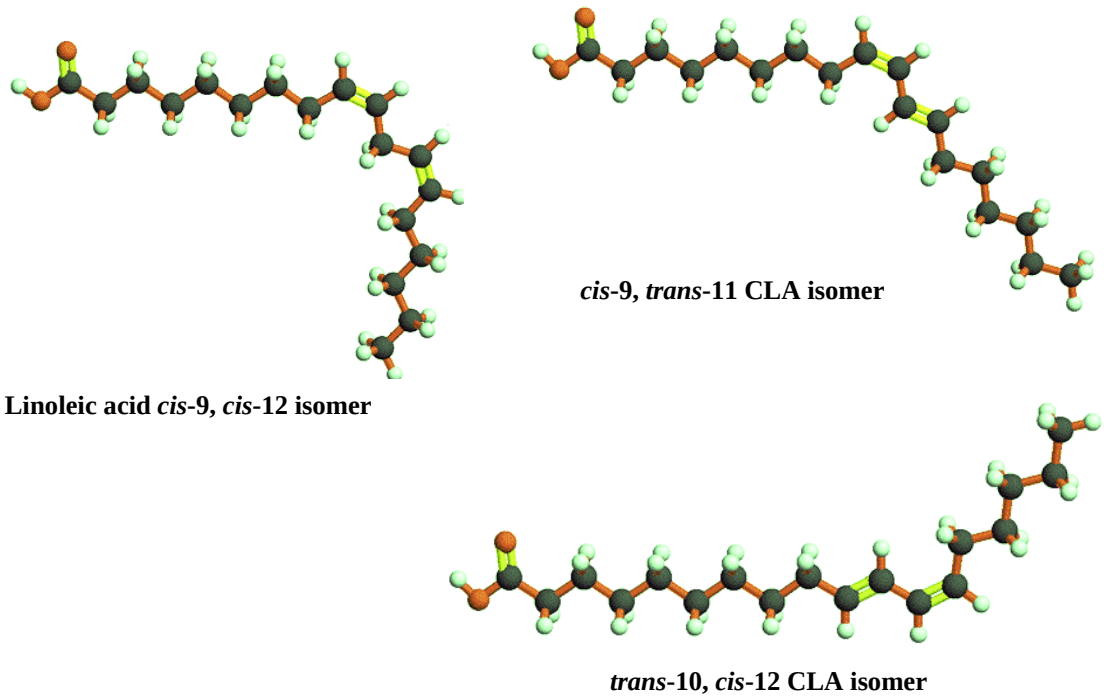

Figure 2.1 Structure of linoleic acid and two isomers of conjugated linoleic acid (CLA)

Source : http://www.raw-milk-facts.com/CLA_T3.html

2.2 Biosynthesis of CLA

CLA found in milk and meat of ruminant originate from two sources mainly from bacterial isomerisation or/and biohydrogenation of polyunsaturated fatty acids (PUFA) in the rumen and from the desaturation of *trans*-fatty acids in the adipose tissue and mammary gland. Thus, the uniqueness of CLA in food products derived from ruminants relates to the incomplete biohydrogenation of dietary unsaturated fatty acids in rumen (Bauman et al., 1999; Khanal and Dhiman, 2004; Schmid et al., 2006).

Rumen biohydrogenation of CLA

Lipids in ruminant feed are derived from forages, grains and oil supplements. The lipid content in ruminant diets are approximately 3-7 percentage of dry matter. Fatty acid profiles of some feeds in ruminant are presented in Table 2.1. They contain linoleic acid (C18:2) and/or linolenic acid (C18:3), which are the major fatty acids found in many feeds.

Table 2.1 Fatty acid profile of common ruminant feeds

Feed	**----Fatty acid, % of total reported fatty acids----**							
	C14:0	**C16:0**	**C16:1**	**C18:0**	**C18:1**	**C18:2**	**C18:3**	**others**
Pasture								
Grass	0.5	19.2	0.2	1.6	2.2	20.4	55.9	0.0
Clover	0.5	22.9	0.3	3.4	3.6	21.1	48.2	0.0
Grass + legume	1.5	20.0	1.2	2.6	4.2	18.9	51.6	0.0
Silage								
Grass	5.4	24.0	0.6	2.9	6.3	14.5	46.2	0.0
Corn	1.1	15.2	0.5	3.5	18.9	40.9	6.1	13.8
Hay alfalfa	1.2	22.9	0.4	4.0	4.9	18.1	23.5	25.0
Concentrates								
Barley	0.0	27.6	0.9	1.5	20.5	43.3	4.3	1.9

Table 2.1 Fatty acid profile of common ruminant feeds (Cont.)

Feeds	**----Fatty acid, % of total reported fatty acids----**							
	C14:0	**C16:0**	**C16:1**	**C18:0**	**C18:1**	**C18:2**	**C18:3**	**others**
Corn	0.0	16.3	0.0	2.6	30.9	47.8	2.3	0.0
Oats	0.0	22.1	1.0	1.3	38.1	34.9	2.1	0.5
Wheat	0.0	20.0	0.7	1.3	17.5	55.8	4.5	0.2
By product								
Gluten meal	0.0	17.2	0.9	0.8	26.7	53.0	1.4	0.0
Distillers grains	0.0	15.6	0.0	2.7	24.2	54.5	1.8	1.2
Plant seed/oils								
Soybean	0.0	11.0	0.0	3.8	23.3	54.5	5.9	1.5
Extruded soybean	0.0	14.5	0.0	3.8	19.5	53.2	9.1	0.0
Extruded cottonseed	0.0	23.4	0.5	2.2	16.5	57.4	0.0	0.0

Table 2.1 Fatty acid profile of common ruminant feeds (Cont.)

Feeds	**----Fatty acid, % of total reported fatty acids----**							
	C14:0	**C16:0**	**C16:1**	**C18:0**	**C18:1**	**C18:2**	**C18:3**	**others**
Sunflower	0.0	4.0	0.0	5.4	21.2	69.4	0.0	0.0
Peanut	0.0	12.3	0.0	3.2	51.5	30.2	0.0	2.8
Linseed	0.0	6.5	0.0	4.0	22.7	15.4	51.4	0.0
Fish oil	8.0	22.0	11.0	3.0	21.0	2.0	1.0	32.0
Animal tallow	3.2	24.8	5.3	14.5	45.9	5.9	0.3	0.0

Source : Dhiman et al. (2005a)

The lipid content in forages contains glycolipids and phospholipids which the major of fatty acids are unsaturated fatty acid such as linoleic acid (C18:2) and linolenic acid (C18:3). Lipid content in oilseeds are triglycerides which contain linoleic acid and olelic acid (*cis*-9 C18:1) as major fatty acids. When ruminants consume feeds, lipids of feeds are subjected to two major processes in the rumen. The first step, the plant lipids or triglycerides are esterified and quickly hydrolyzed to free fatty acid by microbial lipases. The second step, the unsaturated free fatty acids are rapidly hydrogenated to saturated fatty acid end products by microorganism in the rumen.

The *cis*-9, *trans*-11 CLA isomer is the first intermediate from biohydrogenation of linoleic acid by linoleated isomerase, which produced by *Butyrivibrio fibrisolvens* and other bacterial species. Some of *cis*-9, *trans*-11 CLA isomer is quickly reduced to *trans*-11 C18:1 vaccenic acid or C18:0 stearic acid, which are available for absorption by the small intestine. Rumen biohydrogenation of α-linolenic acid (*cis*-9, *trans*-12, *cis*-15 octadecatrienoic acid) as the predominant from isomerization reaction product is followed by reduction of the *cis*-double bonds to *trans*-11 vaccenic acid. Therefore, the *cis*-9, *trans*-11 CLA and *trans*-11 vaccenic acid are intermediates from completely biohydrogenation which escaped from the rumen and are absorbed by intestine and incorporated into milk fat and meat (Figure 2.2) (Bauman et al., 1999; Dhiman et al., 2005a)

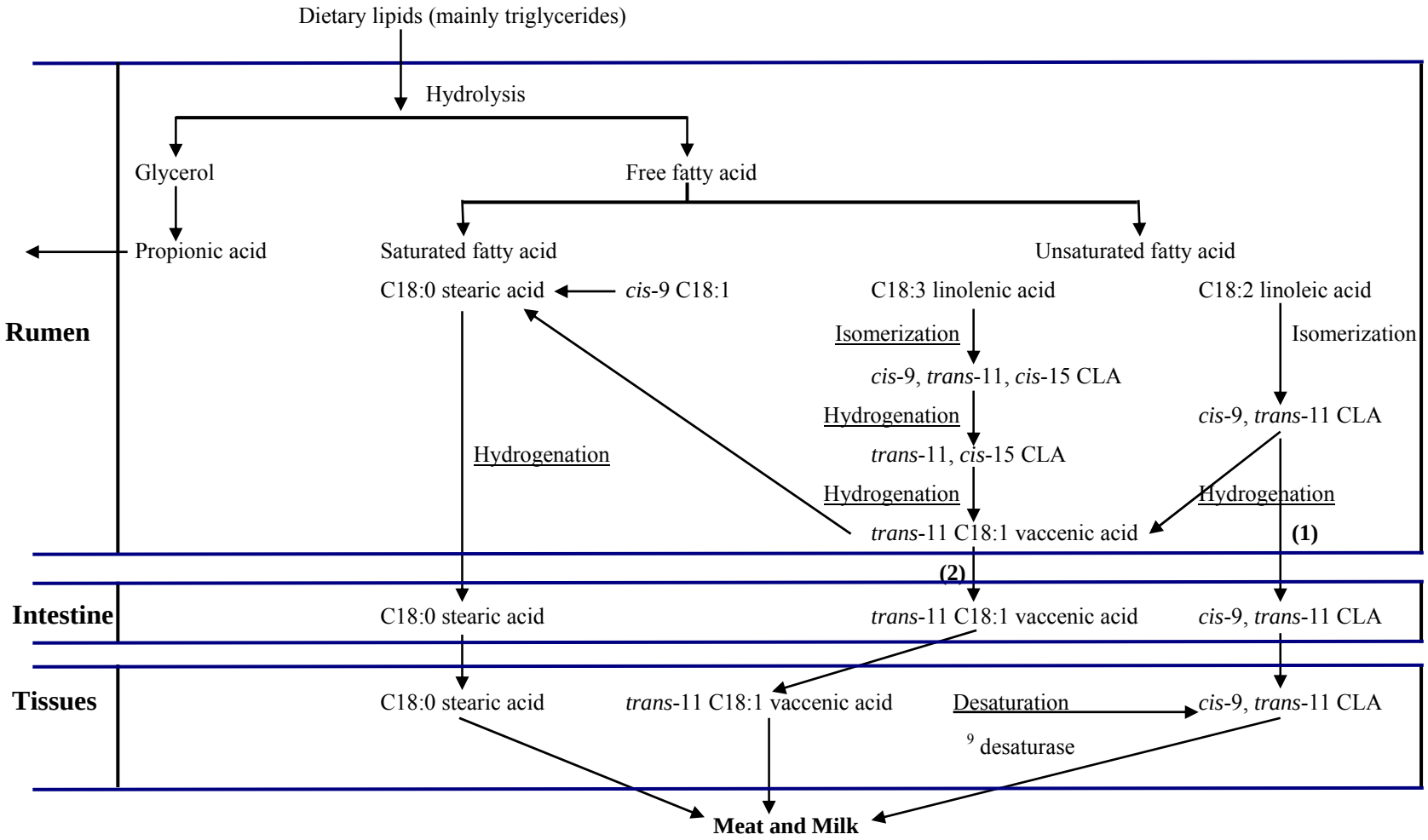

Figure 2.2 Lipid metabolism in the rumen and the origins of conjugated linoleic acid in ruminant products
Source : Tanaka (2005)

Endogenous synthesis of CLA

The tissue synthesis of CLA occurs in the mammary gland and meat by Δ^9-desaturase enzyme, which only a some of *cis*-9, *trans*-11 CLA escapes biohydrogenation in the rumen and that the major portion of *cis*-9, *trans*-11 CLA in milk and meat comes from endogenous synthesis via a pathway involving the desaturation of *trans*-11 vaccenic acid by Δ^9-desaturase enzyme (Griinari et al., 2000; Corl et al., 2001). The Δ^9-desaturase reduction introduces a *cis*-double bond between carbons 9 and 10 of fatty acid. Corl et al. (2001) reported that endogenous synthesis was estimated to account for 78% of the total *cis*-9, *trans*-11 CLA in milk fat by Δ^9-desaturase.

2.3 Potential beneficial effect of CLA in health

The beneficial physiological effects of CLA in animals and human includes 1) an inhibition of carcinogenesis; 2) a prevention of cholesterol-induced atherosclerosis (reduction of low-density lipoprotein (LDL) concentration and ratio of LDL : high-density lipoprotein (HDL)); 3) a reduction of body fat accumulation (reduced whole body fat); 4) an enhancement of the immune response; 5) a growth promotion (increased body protein); 6) an improvement of diabetes (the normalization of impaired glucose tolerance) and 7) an improvement of bone metabolism (Tanaka, 2005).

. **CLA and Carcinogenesis**

CLA is a potent cancer preventive agent in animal models. Ip et al. (1999) reported that feeding butter fat CLA to rats reduced mammary epithelial mass by 22%, decreased the size of TEB population (terminal end bud) by 30%, suppressed the proliferation of TEB cells by 30% and inhibited mammary tumor yield by 53%

($P<0.05$). Increasing dietary levels of vaccenic acid and *cis*-9, *trans*-11 CLA also decreased mammary carcinogenesis in rat (Corl, et al., 2003). Mechanisms of anticarcinogenic effect included cell proliferation, alterations in the components of the cell cycle and induction of apoptosis (Belury, 2002). The mechanism of anticancer effects of CLA is not clear and possible mechanism may include its ability of proliferation reduction of cancer cells, increased in apoptotic cell death, inhibition of angiogenesis or increased in oxidative stress (Kapoor et al., 2005)

CLA and Atherosclerosis

A high cholesterol level in the plasma has been ranked as the greatest risk factors in the development of chronic heart disease. Nicolosi et al. (1997) found that the reduction of LDL cholesterol in the plasma when hamsters received CLA and also inhibited antherosclerosis. Nicolosi et al. (2004) suggested that mid-oleic acid sunflower oil reduces risk factors such as lipoprotein cholesterol and oxidative stress associated with early atherolsclerosis greater than the typical high-linolenic sunflower oil in hypercholesterolemic hamsters. The mechanisms involved in the antiatherosclerotic and lipid-lowering effects of CLA, include their role on peroxisome proliferators-activated receptors, sterol regulatory element-binding proteins. Peroxisome proliferators-activated receptors are ligand-activated nuclear receptors regulating the expression of genes in adipose tissue and induces expression of genes that promote lipid storage including lipoprotein lipase that is critical in the removal of TG-rich lipoproteins (Bhattacharya et al., 2006).

CLA and body composition

The American Journal of Clinical Nutrition reported that daily consumption of CLA helped overweight adults to lose a significant portion of body fat (Adams, 2004).

Pariza et al. (2001) and Evans et al. (2002) reviewed that dietary CLA decreases adiposity in animal models, for example, feeding 1-1.5% CLA (mixed *cis*-9, *trans*-11 and *trans*-10, *cis*-12) in rodent diets reduced body fat and increased lean body mass compared with control animals. Moreover, it reduced lipoprotein lipase activity, which would in turn reduce fatty acid uptake by adipocytes. Ostrowska et al. (1999) reported that dietary CLA supplementation increased lean tissue deposition and decreased fat deposition in pigs. The mechanism by which CLA leads to a decrease in fat deposition is shown in Figure 2.3. Potential antiobesity mechanisms of CLA include decreased preadipocyte proliferation and differentiation into mature adipocytes, decreased fatty acid and triglyceride synthesis, and increased energy expenditure, lipolysis and fatty acid oxidation (Evans et al., 2002). Park et al. (1997) reported that dietary CLA reduced body fat and enhanced lean body mass compared with controls, the rate-limiting enzyme in beta-oxidation and carnitine palmitoyltransferase (CPT) activity was enhanced in CLA fed animals. CLA may involve in the activity of key enzyme in fat metabolism, both fat storage and mobilization/oxidation. The CLAs reduce body fat in animals not only by altering the key enzymes of lipid storage, mobilization and oxidation but also by reducing adipocyte proliferation and differentiation and stimulating apoptosis in pre-adipocytes (Wahle et al., 2004)

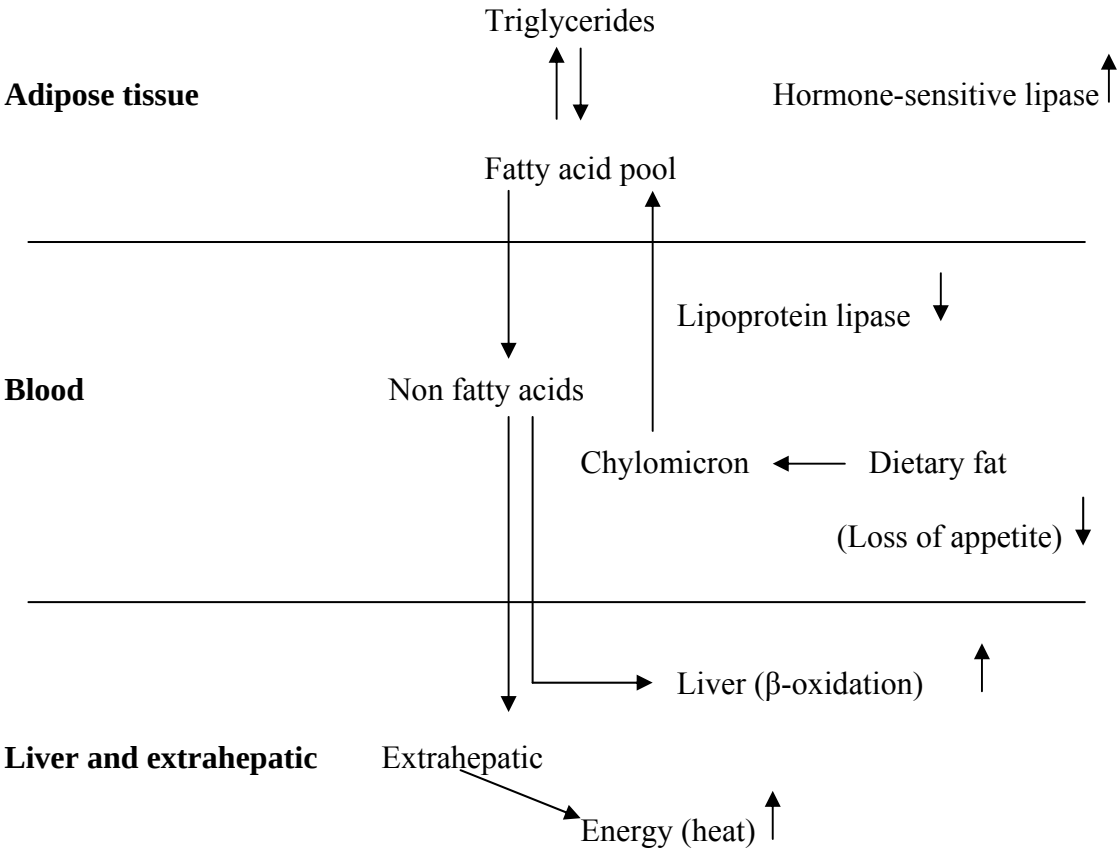

Figure 2.3 Conjugated linoleic acid and lipid metabolism

Source : Tanaka (2005)

2.4 Effect of plant oils and oilseeds on performance, carcass and conjugated linoleic acid accumulation in beef

Addition of plant oils in beef cattle diets did not affect performances and carcass quality such as carcass weight, dressing percentage, fat thickness and marbling score compared with control treatment (Table 2.2). However, Engle et al. (2000) reported that dry matter intake (DMI) and average daily gain (ADG) were decreased ($P<0.01$) and also decreased ($P<0.01$) by 6.9% and 10%, respectively in carcass weight and marbling score when steers received 4% SBO in diet. In contrast, fed high-oil corn diet to steers increased ($P<0.05$) marbling score by 9%.

Effects of plant oil on fatty acid content in beef are presented in Table 2.3. This table showed that the addition of plant oils to diet increased CLA content in beef. For example, Noci et al. (2005) showed that *cis*-9, *trans*-11 CLA content in intramuscular fat were increased ($P<0.01$) by 45.6% and 109.7% when fed 5.5 and 11.5% sunflower oil (SFO). Similarly, Mir et al. (2002) reported that CLA content in muscle lipid was increased by 339% when 6% SFO was fed. Engle et al. (2000) fed 4% SBO to steer diets and found that CLA content in *longissimus* muscle was increased ($P<0.05$) by 45%. Moreover, Garcia et al. (2003) reported that *cis*-9, *trans*-11 CLA was increased ($P < 0.05$) by 135% in subcutaneous fat when heifers were fed high fat diet. Addition of 6% SFO in concentrate increased ($P<0.05$) CLA content in *longissimus* muscle. However, Dhiman et al. (2005b) reported that when steers were fed 2% and 4% SBO, linoleic acid, *cis*-9, *trans*-11 CLA and saturated fatty acids in *longissimus* muscle were similar.

Table 2.2 Effect of dietary treatment of supplementation on performance and carcass characteristics of feedlot cattle

References	Treatments	Items						
		DMI	ADG	G : F	Carcass weight	Dressing percentage	Fat thickness	Marbling score
Griswold et al.	Control	11.8	1.45	0.12	341	58.6	-	4.66
(2003)	4% SBO	11.5	1.40	0.12	334	58.0	-	4.40
	C:F = 60:40 +4% SBO	11.4	1.56	0.14	337	58.3	-	4.71
	C:F = 60:40 +8% SBO	10.8	1.38	0.13	329	57.1	-	4.32
Mir et al.	Control	8.40	1.20	0.14	-	-	-	-
(2002)	6% SFO	8.60	1.33	0.15	-	-	-	-
Beaulieu et al.	Control	8.80	1.40	0.13	317.8	64.2	1.37	1,139.0
(2002)	5% SBO	9.40	1.60	0.14	316.4	62.9	1.31	1,172.0
Engle et al.	Control	9.61[e]	1.60[e]	0.17	334[e]	58.9	-	6.0[e]
(2000)	4% SBO	8.61[d]	1.41[d]	0.16	311[d]	57.9	-	5.4[d]

[a,b,c] Means within row with different superscripts differ (P<0.05); [d,e,f] Means within row with different superscripts differ (P<0.01)
DMI = Dry matter intake, ADG = Average daily gain, G:F = Gain per feed ratio, SBO = Soybean oil, C:F = Concentrate per forage, SFO = Sunflower oil

Table 2.3 Effect of diet on muscle tissue fatty acid composition of beef

References	Treatments	Items			
		LA (C 18:2)	**CLA**	**SFA**	**UFA**
Dhiman et al. (2005b)	Control	6.00	0.23	39.40	60.60
	2% SBO	6.80	0.29	39.80	60.20
	4% SBO	7.40	0.31	40.20	59.80
Noci et al. (2005)	Control	4.16[g]	0.43[g]	45.57	54.43
	5.5% SFO	4.70[h]	0.63[h]	44.94	55.06
	11.5% SFO	5.44[i]	0.91[i]	44.60	55.40
Griswold et al. (2003)	Control (C:F = 80:20)	-	0.31	45.40	54.60
	Control+ 4% SBO	-	0.25	49.80	50.20
	C:F = 60:40 +4% SBO	-	0.28	47.30	52.70
	C:F = 60:40 +8% SBO	-	0.31	45.50	54.50

Table 2.3 Effect of diet on muscle tissue fatty acid composition of beef (Cont.)

References	Treatments	Items			
		LA (C 18:2)	**CLA**	**SFA**	**UFA**
Mir et al. (2002)	Control	1.18	0.27[a]	-	-
	6% SFO	1.19	1.29[b]	-	-
	Control	1.52	0.28[a]	-	-
	6% SFO	1.95	1.19[b]	-	-
	Control	1.66[a]	0.29[a]	-	-
	6% SFO	2.23[b]	1.22[b]	-	-
Beaulieu et al. (2002)	Control	4.33	0.35	-	-
	5% SBO	4.44	0.34	-	-
	Control	3.91	0.32	-	-
	5% SBO	3.93	0.36	-	-
	Control	6.32	0.33	-	-
	5% SBO	7.00	0.37	-	-

Table 2.3 Effect of diet on muscle tissue fatty acid composition of beef (Cont.)

References	Treatments	Items			
		LA (C 18:2)	**CLA**	**SFA**	**UFA**
Engle et al. (2000)	Control	5.51	0.20[a]	47.40	52.60
	4.0% SBO	6.60	0.29[b]	48.50	51.50

[a,b,c] Means within row with different superscripts differ (P<0.05); [g,h,i] Means within row with different superscripts differ (P<0.001)
LA = Linoleic acid C 18:2, CLA = conjugated linoleic acid, *c*-9, *t*-11 = *cis*-9, *trans*-11 CLA, *t*-10, *c*-12 = *trans*-10, *cis*-12 CLA, SFA = Saturated fatty acid, PUFA = Polyunsaturated fatty acid, ND = not detect
SBO = Soybean oil, C:F = Concentrate per forage, SFO = Sunflower oil

Effects of oilseeds supplementation on performance and carcass quality in beef cattle are presented in Table 2.4. The performance pattern such as dry matter intake (DMI), average daily gain (ADG) and Gain:Feed ratio were similar in all treatments. However, Gibb et al. (2004) reported that adding whole sunflower seed in fattening diets linearly increased DMI (P=0.05), ADG (P=0.01) and Gain:Feed ratio (P=0.01), but when rolled sunflower seed was substituted to whole sunflower seed in the diets, DMI, ADG and Gain:Feed ratio were not affected (8.55 vs. 8.30 kg/d; 1.36 vs. 1.31 kg; 0.157 vs. 0.158, respectively). Huerta-Leidenz et al. (1991) reported that the feeding levels of whole cottonseed at 15% and 30% showed no effect on DMI, ADG and Gain:Feed ratio. Table 2.4 showed that carcass weight, lean yield and dressing percentage were similar across treatments. Similarly, Madron et al. (2002) reported that carcass weight (average 603±11.6 kg) and hot carcass weight, dressing percentage, yield grade and quality grade were not affected by feeding extruded full fat soybeans compared with other treatments. Gibb et al. (2004) showed no difference in dressing percentage and backfat, but carcass weight was linearly increased (P=0.03).

Effects of supplementation of oilseeds in diets on fatty acid in beef are presented in Table 2.5. Conjugated linoleic acid (CLA) content in beef was increased (P<0.05) by 44% and *trans*-11 vaccenic acid was increased (P<0.05) by 25% when feeding extruded soybeans compared with raw soybeans (McNiven et al., 2004). This suggested ruminal biohydrogenation of the main fatty acid of soybean as linoleic acid (C18:2), resulting in higher levels of *trans*-C18:1 (a precursor of CLA). The production of CLA in the tissues would have increased, resulting in higher levels of CLA in tissues. Levels of CLA, and *trans*-11 vaccenic acid were lower the meat from raw soybean fed

steers. This shows that biohydrogenation in the rumen was more complete for the untreated soybean versus the heated soybean treatments. The process of roasting appeared to protect the C18:2 from hydrogenation, compared with extrusion, resulting in higher levels of C18:2 in the meat and lower levels of CLA and its precursor *trans*-C18:1 (McNiven et al., 2004).

Madron et al. (2002) reported that concentration of *cis*-9, *trans*-11 CLA was increased ($P<0.01$) by 17% when high extruded full fat soybean was fed compared with control treatment. Increase in CLA was attributed to polyunsaturated fatty acid especially linoleic acid, which is a key substrate in rumen biohydrogenation. Theoretically, this would increase ruminal production of CLA as well as ruminal production of *trans*-11 C18:1 for the endogenous synthesis of CLA, but the observed increase in the CLA content in muscle lipid was relatively small. Moreover, Gibb et al. (2004) showed that when sunflower seed form whole and rolled sunflower seed were fed, *cis*-9, *trans*-11 CLA and *trans*-10, *cis*-12 CLA in subcutaneous fat were increased compared with control treatment, while linoleic acid was higher ($P<0.01$) in 14% whole sunflower seed compared with other treatments.

Table 2.4 Effect of oilseeds supplementation on performance and carcass characteristics of feedlot cattle

References	Treatments	Items						
		DMI	**ADG**	**G : F**	**Carcass weight**	**Dressing percentage**	**Fat thickness**	**LM area**
Gibb et al. (2004)[1]	Control	7.42	0.96	0.129	338.2	58.3	10.7	84.8
	9% WSS	8.19	1.22	0.149	360.4	59.0	11.3	87.3
	14% WSS	8.55	1.36	0.160	368.0	59.7	13.7	93.7
	14% RSS	8.30	1.31	0.156	362.0	59.1	13.7	84.2
Huerta-Leidenz et al. (1991)	Control	7.89	0.95	0.12	223.9	-	-	-
	15% WCS	8.97	1.15	0.12	234.3	-	-	-
	30% WCS	8.45	1.03	0.12	231.2	-	-	-

[1]Linear effect of including whole SS in the diet (0, 9 or 14%) (DMI : P=0.02; ADG : P=0.01; G:F : P=0.03), WSS = whole sunflower seed, RSS = rolled sunflower seed
DMI = Dry matter intake, ADG = Average daily gain, G : F = Gain : Feed ratio, LM area = *longissimus* muscle area
WCS = whole cottonseed

Table 2.5 Effect of oilseeds supplementation on fatty acid of feedlot cattle

References	Treatments	Items (% of fatty acid)			
		C18:1 *trans*-11 vaccenic acid	**C 18:2 Linoleic acid**	**C 18:2 *cis*-9, *trans*-11 CLA**	**C 18:2 *trans*-10, *cis*-12 CLA**
Gibb et al. (2004)	Control	-	1.15[d]	0.78[a]	0.03[d]
	14% WSS	-	1.84[e]	0.92[b]	0.08[e]
	14% RSS	-	1.40[f]	0.91[b]	0.07[f]
McNiven et al. (2004)	Megalac	1.75[ab]	2.80[a]	0.33[a]	0.008
	Extrude soybean	2.11[c]	2.81[a]	0.46[b]	0.003
	Raw soybean	1.69[a]	2.94[a]	0.32[a]	<0.001
	Roasted soybean	1.85[b]	4.06[b]	0.35[a]	0.03
Madron et al. (2002)	Control	1.33[d]	1.61[d]	0.66[d]	-
	Low ESB	1.42[e]	1.67[de]	0.69[d]	-
	High ESB	1.71[f]	1.91[e]	0.77[e]	-

[a,b,c] Means within row with different superscripts differ (P<0.05); [d,e,f] Means within row with different superscripts differ (P<0.01)
WSS = whole sunflower seed, RSS = rolled sunflower seed, Low ESB = low extrude soybean, High ESB = high extrude soybean

2.5 Effect of plant oils and rumen protected conjugated linoleic acid (RP-CLA) on performance, milk yield, milk composition and conjugated linoleic acid content accumulation in milk

Dairy cows milk fat is a rich source of CLA. The CLA content can be influenced by many factors. Diet is the most important factor affecting the CLA content of milk, especially, plant oils. Plant oils contain different fatty acid compositions. Plant oils are rich in linoleic acid may increase CLA content in milk fat. Moreover, rumen protected conjugated linoleic acid (RP-CLA) is also increase CLA content in milk fat.

Effects of plant oils and rumen protected conjugated linoleic acid (RP-CLA) on milk production, milk composition, and fatty acid profiles in milk are presented in Table 2.6. DMI and milk yield were not affected when plant oils were added. In contrast, Shingfield et al. (2006) showed that supplementation of fish oil (FO) and sunflower oil (SFO) in dairy diet, significantly decreased ($P<0.05$) DMI. Leonardi et al. (2005) noted that addition of corn oil (CO) to dairy diets increased ($P<0.01$) milk yield by 5.8%. Milk composition such as milk fat and milk protein percentage were significantly decreased ($P<0.05$) when supplemented with FO and SFO (Shingfield et al., 2006). Similarly, Zheng et al. (2005) reported that when supplemented cottonseed oil (CSO), soybean oil (SBO) and CO to diets, milk fat percentage was lowest when cow received SBO compared with other treatments. Leonardi et al. (2005) reported that milk protein percentage was decreased ($P<0.01$) by CO supplemented to diets. However, Dhiman et al. (2000) suggested that addition of SBO did not affect in DMI and milk yield, while supplementation of 2 and 4% SBO

reduced (P<0.01) milk fat percentage but did not affect milk protein, milk lactose and solid not fat percentage.

Addition of plant oils and RP-CLA on fatty acids in milk are presented in Table 2.7. The *trans*-11 C18:1, C18:2 linoleic acid, *cis*-9, *trans*-11 CLA and total CLA were increased by plant oils supplementation (Shingfield et al., 2006; Leonardi et al., 2005). For example, Shingfield et al. (2006) reported that supplementation of FO and SFO in diets significantly increased (P<0.01) C18:2 linoleic acid and total CLA by 41.9% and 594%, respectively. *Trans*-11 C18:1, C18:2 linoleic acid and *cis*-9, *trans*-11 CLA were increased (P<0.01) by 105.%, 22.4% and 97.9%, respectively by addition of CO in diet (Leonardi et al., 2005). Moreover, Zheng et al. (2005) reported that when SBO was supplemented to diet, *trans*-11 C18:1 was highest (P<0.01) compared with other treatments, while *cis*-9 and *trans*-11 content in milk fat was increased (P<0.01) by 97.1% when cow received SBO. Similar to Dhiman et al. (2000) who reported that CLA content in milk fat was increased (P<0.01) by 77% and 187.8%, respectively when 2% and 4% SBO were supplemented.

Table 2.6 Dry matter intake, milk composition and milk production by lactating cows influenced by supplementation of different plant oils

References	Treatments	DMI (kg/d)	Milk yield (kg/d)	Milk fat (%)	Milk protein (%)	Milk lactose (%)
Shingfield et al. (2006)	Control	21.9[e]	27.1	4.6[e]	3.61[b]	4.50
	4.5 % FSO	17.4[d]	26.4	2.9[d]	3.33[a]	4.55
Leonardi et al. (2005)	Control	26.7	44.6[h]	3.38	3.08[h]	4.86
	1.5% CO	26.9	47.2[g]	3.28	3.01[g]	4.84
Loor et al. (2005)	2.5% FO	17.1	27.2	2.56	2.97	4.77
	5% LO	17.2	24.4	2.75	3.18	4.71
	5% SFO	19.3	26.5	2.62	3.50	4.68
Zheng et al. (2005)	Control	24.1	34.4	3.44[f]	3.11	5.04
	500 g of CSO	23.8	35.0	3.34[ef]	3.14	5.03
	500 g of SBO	24.0	34.9	3.05[d]	3.09	5.00
	500 g of CO	23.9	34.8	3.18[e]	3.11	4.89

Table 2.6 Dry matter intake, milk composition and milk production by lactating cows influenced by supplementation of different plant oils (Cont.)

References	Treatments	DMI (kg/d)	Milk yield (kg/d)	Milk fat (%)	Milk protein (%)	Milk lactose (%)
Dhiman et al. (2000)	Control	20.6	27.4	3.44[e]	3.53	4.99
	0.5% SBO	21.7	27.9	3.60[e]	3.50	4.98
	1 % SBO	20.6	28.3	3.56[e]	3.44	4.98
	2 % SBO	19.7	28.3	2.80[d]	3.47	4.96
	4 % SBO	21.1	28.5	2.93[d]	3.59	5.00

[a,b,c] Means within row with different superscripts differ (P<0.05); [d,e,f] Means within row with different superscripts differ (P<0.01)
C 18:1 *t*-11 = vaccenic acid; LA = Linoleic acid C 18:2; CLA = conjugated linoleic acid; *c*-9, *t*-11 = *cis*-9, *trans*-11 CLA; *t*-10, *c*-12 = *trans*-10, *cis*-12 CLA
FSO = 45 g of a mixture (1:2, wt/wt) of fish oil 15 g and sunflower oil 30 g/kg of DM (FSO); CO = corn oil; FO = fish oil; LO = linseed oil; SFO = sunflower oil; CSO = cottonseed oil; SBO = soybean oil

Table 2.7 Contents of fatty acids in milk fat from dairy cows influenced by supplementation of different plant oils

References	Treatments	Items (% of fatty acid)				
		C18:1 *t*-11 Vaccenic acid	C 18:2 Linoleic acid	*c*-9, *t*-11 CLA	*t*-10, *c*-12 CLA	Total CLA
Shingfield et al. (2006)	Control	-	2.05[d]	-	-	0.50[d]
	4.5 % FSO	-	2.91[e]	-	-	3.47[e]
Leonardi et al. (2005)	Control	0.86[d]	5.04[d]	0.45[d]	0.01	-
	1.5% CO	1.77[e]	6.17[e]	0.89[e]	0.02	-
Loor et al. (2005)	2.5% FO	6.58	-	2.29	-	2.73
	5% LO	5.43	-	2.36	-	2.84
	5% SFO	4.22	-	1.83	-	2.40
Zheng et al. (2005)	Control	1.18[d]	2.60[a]	0.35[a]	0.01	-
	500 g of CSO	1.99[e]	3.43[b]	0.60[b]	0.02	-
	500 g of SBO	2.39[f]	3.87[b]	1.02[c]	0.01	-
	500 g of CO	2.03[e]	2.95[a]	0.69[b]	0.01	-

Table 2.7 Contents of fatty acids in milk fat from dairy cows influenced by supplementation of different plant oils (Cont.)

References	Treatments	Items (% of fatty acid)				
		C18:1 *t*-11 Vaccenic acid	**C 18:2 Linoleic acid**	***c*-9, *t*-11 CLA**	***t*-10, *c*-12 CLA**	**Total CLA**
Dhiman et al. (2000)	Control	-	2.80	-	-	4.8[c]
	0.5% SBO	-	2.80	-	-	7.1[d]
	1 % SBO	-	2.80	-	-	8.5[d]
	2 % SBO	-	3.30	-	-	13.8[f]
	4 % SBO	-	3.00	-	-	18.1[g]

[a,b,c] Means within row with different superscripts differ (P<0.05); [d,e,f] Means within row with different superscripts differ (P<0.01)
C 18:1 *t*-11 = vaccenic acid, LA = Linoleic acid C 18:2, CLA = conjugated linoleic acid, *c*-9, *t*-11 = *cis*-9, *trans*-11 CLA, *t*-10, *c*-12 = *trans*-10, *cis*-12 CLA
FSO = 45 g of a mixture (1:2, wt/wt) of fish oil 15 g and sunflower oil 30 g/kg of DM (FSO), CO = corn oil, FO = fish oil, LO = linseed oil, SFO = sunflower oil
CSO = cottonseed oil, SBO = soybean oil

Effects of RP-CLA on performance, milk yield and milk composition are presented in Table 2.8. Supplementation of RP-CLA in form of calcium salts CLA (Ca-CLA) and formaldehyde-protected CLA (FP-CLA) decreased ($P<0.01$) milk fat percentage by 38.4% and 54.3%, respectively. Moreover, DMI, milk yield and milk protein percentage were not affected by RP-CLA supplementation (De Veth et al., 2005). Similar to Castaneda-Gutierrez et al. (2005) who noted that adding RP-CLA (including 32 and 63 g/d CLA isomers) in diet decreased ($P<0.01$) milk fat percentage by 10.2% and 19.4%, respectively while milk yield, milk protein and lactose percentage did not change. Moore et al. (2004) reported that supplementation of RP-CLA (including 62, 125 and 187 g/d CLA isomers) did not affect DMI, milk yield and milk composition, while milk fat percentage was decreased ($P<0.05$) by 27.4% and 32.2%, respectively, when cows received 125 and 187 g CLA isomers/d.

Similarly, Giesy et al. (2002) and Perfield et al. (2002) reported that when RP-CLA was supplemented in diets, milk fat synthesis in lactation period was reduced. Perfield et al. (2004) reported that RP-CLA reduced in milk fat percentage and milk fat yield, while DMI and milk yield were not affected. This reduction in milk fat yield was due to decrease in fatty acids originating from both *de novo* fatty acid synthesis and uptake of preformed fatty acids from circulation. However, reduction of *de novo* synthesis increased proportion of perform fatty acids in milk fat. Similar to Piperova et al. (2004) who showed that milk fat percentage was decreased ($P<0.01$) by 25% when cow received Ca-CLA (13 g CLA/d).

Effects of addition of RP-CLA in cow diets on fatty acids in milk are presented in Table 2.9. All researches in this table showed that when cow received RP-CLA, *trans*-10, *cis*-12 CLA and *cis*-9, *trans*-11 CLA in milk fat were increased and total fatty

acids were also increased. In contrast, Bernal-Santos et al. (2003) and Moore et al. (2004), reported that *cis*-9, *trans*-11 CLA in milk fat was similar in all treatments. Castaneda-Gutierrez et al. (2005) reported that supplementation of 63 g Ca-CLA/d increased *cis*-9, *trans*-11 CLA and *trans*-10, *cis*-12 CLA in milk fat by 11.8% and 1,425%, respectively. Perfield et al. (2002) reported that *trans*-10, *cis*-12 CLA in milk fat was increased by 300%, respectively when Ca-CLA was added.

Moreover, Moore et al. (2004), noted that RP-CLA supplementation linearly increased ($P<0.01$) total CLA content in milk fat, with the highest dose increasing CLA levels > 5-fold. Similarly, Piperova et al. (2004) reported that total CLA content was increased ($P<0.01$) by 59.6% and increased ($P<0.01$) *trans*-10, *cis*-12 CLA by 163.6%. Perfield et al. (2004) showed that *cis*-9, *trans*-11 CLA and *trans*-10, *cis*-12 CLA in milk fat were increased by RP-CLA supplementation. Infusion of *trans*-10, *cis*-12 CLA, linearly increased ($P<0.01$) *trans*-10, *cis*-12 CLA in milk fat (Viswanadha et al., 2003). Chouinard et al. (1999) reported that increasing CLA supplementation was linearly increased ($P<0.01$) in C18:2 linoleic acid and *trans*-10, *cis*-12 CLA in milk fat.

In this presented, when cow received RP-CLA, milk fat was decreased while *trans*-10, *cis*-12 concentration was increased. The result demonstrated that the *trans*-10, *cis*-12 CLA isomer inhibited milk fat synthesis in dairy cows. The increasing *trans*-10, *cis*-12 CLA in milk fat when RP-CLA supplementation reduced the mammary gland's lipogenesis (rates of acetate incorporation into fatty acids) and decreased the expression of genes encoding enzyme (mRNA abundance of acetyl CoA carboxylase) involved in the uptake and transport of circulating fatty acids *de novo* fatty acid synthesis, desaturation of fatty acids and formation of triglycerides (Baumgard et al., 2000; 2001; Piperova et al., 2000).

Table 2.8 Effects of rumen protected conjugated linoleic acid (RP-CLA) supplementation on milk production and composition in lactating cows

References	Treatments	DMI (kg/d)	Milk yield (kg/d)	Milk fat (%)	Milk protein (%)	Milk lactose (%)
De Veth et al. (2005)	Control	23.6	21.9	3.61[e]	3.16	-
	10 g *t*-10,*c*-12 CLA (Ca-CLA)	23.1	20.6	2.61[d]	3.38	-
	10 g *t*-10,*c*-12 CLA (FP-CLA)	23.3	19.5	2.34[d]	3.48	-
Castaneda-Gutierrez et al. (2005)	Control	21.7	43.4	3.82[f]	2.85	4.74
	31.6 g CLA (Ca-CLA)	21.3	43.8	3.43[d]	2.81	4.77
	63.2 g CLA (Ca-CLA)	20.5	43.8	3.08[e]	2.79	4.70
Moore et al. (2004)	Control	17.9	33.4	4.57[b]	4.02	4.67
	62 g CLA (Ca-CLA)	16.4	33.7	3.79[ab]	3.49	4.53
	125 g CLA (Ca-CLA)	18.2	35.5	3.32[a]	3.76	4.64
	187 g CLA (Ca-CLA)	16.0	34.3	3.10[a]	3.68	4.60
Perfield et al. (2004)	Control	30.6	40.5	3.23[e]	2.55	-
	54 g (AP-CLA)	31.6	42.6	2.37[d]	2.51	-
	138 g (LE-CLA)	30.4	42.7	2.34[d]	2.58	-

Table 2.8 Effects of rumen protected conjugated linoleic acid (RP-CLA) supplementation on milk production and composition in lactating cows (Cont.)

References	Treatments	DMI (kg/d)	Milk yield (kg/d)	Milk fat (%)	Milk protein (%)	Milk lactose (%)
Piperova et al. (2004)	Control	23.5	37.8	3.39[e]	3.05	4.88
	13 g CLA (Ca-CLA)	23.5	35.2	2.54[d]	3.03	4.83
Bernal-Santos et al.	Control	23.4	44.3	3.60[e]	2.77	4.47
(2003)	30.4 g CLA (Ca-CLA)	23.9	47.1	3.15[d]	2.74	4.73
Viswanadha et al.	Control	21.4	25.3	4.17	3.32	-
(2003)	2 g *t*-10,*c*-12 CLA	24.4	34.6	3.53	3.32	-
Linear, P<0.01	4 g *t*-10,*c*-12 CLA	21.1	28.0	3.29	3.26	-
	6 g *t-10,c*-12 CLA	22.0	28.6	2.93	3.20	-
Giesy et al. (2002)	Control	25.7	42.3	3.45	3.17	4.76
Linear	8.13 g CLA (Ca-CLA)	26.4	43.5	2.97	3.17	4.83
	16.25 g CLA (Ca-CLA)	26.4	47.9	2.96	3.17	4.73
	32.50 g CLA (Ca-CLA)	27.0	44.0	2.46	3.15	4.66

Table 2.8 Effects of rumen protected conjugated linoleic acid (RP-CLA) supplementation on milk production and composition in lactating cows (Cont.)

References	Treatments	DMI (kg/d)	Milk yield (kg/d)	Milk fat (%)	Milk protein (%)	Milk lactose (%)
Perfield et al. (2002)	Control	22.8	30.4	3.80[e]	3.13	4.74
	30.4 g CLA (Ca-CLA)	22.6	30.8	2.90[d]	3.16	4.72
Chouinard et al. (1999)	Control	22.5	21.5	2.81	3.31	-
	30.6 g CLA	22.0	20.4	1.43	3.37	-
	61.2 g CLA	21.4	20.9	1.38	3.53	-
	91.8 g CLA	20.2	18.3	1.23	3.46	-

[a,b,c] Means within row with different superscripts differ ($P<0.05$); [d,e,f] Means within row with different superscripts differ ($P<0.01$)
DMI = Dry matter intake, CLA = Conjugated linoleic acid, Ca-CLA = Calcium salts of conjugated linoleic acid, FP-CLA = Formaldehyde-protected of conjugated linoleic acid, AP-CLA = Amide-protected of conjugated linoleic acid, LE-CLA = Lipid encapsulated of conjugated linoleic acid

Table 2.9 Fatty acid composition of milk fat from cows fed rumen protected conjugated linoleic acid (RP-CLA)

References	Treatments	Items (% of fatty acid)				
		C18:1 *t*-11 Vaccenic acid	**C 18:2 Linoleic acid**	***c*-9, *t*-11 CLA**	***t*-10, *c*-12 CLA**	**Total CLA**
De Veth et al. (2005)	Control	1.70	3.30	0.78	<0.01[d]	-
	10 g *t*-10,*c*-12 CLA (Ca-CLA)	1.37	3.62	0.77	0.07[d]	-
	10 g *t*-10,*c*-12 CLA (FP-CLA)	1.67	3.85	1.02	0.18[e]	-
Castaneda- Cutierrez et al. (2005)	Control	1.53[d]	3.80[a]	0.04[d]	<0.01[d]	-
	31.6 g CLA (Ca-CLA)	1.73[e]	4.01[ab]	0.52[de]	0.02[e]	-
	63.2 g CLA (Ca-CLA)	2.07[f]	4.25[b]	0.61[f]	0.04[f]	-
Moore et al. (2004)	Control	1.31	3.66	0.34	<0.01[d]	0.51[d]
	62 g CLA (Ca-CLA)	1.11	4.05	0.31	0.08[de]	1.08[de]
	125 g CLA (Ca-CLA)	1.11	3.96	0.35	0.16[ef]	1.70[e]
	187 g CLA (Ca-CLA)	1.04	4.02	0.42	0.25[f]	2.69[f]

Table 2.9 Fatty acid composition of milk fat from cows fed rumen protected conjugated linoleic acid (RP-CLA) (Cont.)

References	**Treatments**	**Items (% of fatty acid)**				
		C18:1 *t*-11 Vaccenic acid	**C 18:2 Linoleic acid**	***c*-9, *t*-11 CLA**	***t*-10, *c*-12 CLA**	**Total CLA**
Perfield et al. (2004)	Control	1.75[d]	3.61[d]	0.57[d]	<0.01[d]	-
	54 g (AP-CLA)	2.06[e]	3.93[e]	0.83[e]	0.08[e]	-
	138 g (LE-CLA)	2.12[e]	4.41[f]	0.80[e]	0.09[e]	-
Piperova et al. (2004)	Control	-	-	76.82[e]	1.32[d]	0.52[a]
	13 g CLA (Ca-CLA)	-	-	67.94[d]	3.48[e]	0.83[b]
Bernal-Santos et al. (2003)	Control	1.15	3.38[d]	0.36	<0.01[d]	-
	30.4 g CLA (Ca-CLA)	1.12	3.75[e]	0.36	0.03[e]	-

Table 2.9 Fatty acid composition of milk fat from cows fed rumen protected conjugated linoleic acid (RP-CLA) (Cont.)

References	**Treatments**	**Items (% of fatty acid)**				
		C18:1 *t*-11 Vaccenic acid	**C 18:2 Linoleic acid**	***c*-9, *t*-11 CLA**	***t*-10, *c*-12 CLA**	**Total CLA**
Viswanadha et al.	Control	-	4.34	0.41	0.00	-
(2003)	2 g *t*-10,*c*-12 CLA	-	4.45	0.33	0.02	-
Linear, P<0.01	4 g *t*-10,*c*-12 CLA	-	4.75	0.47	0.06	-
	6 g *t*-10,*c*-12 CLA	-	5.06	0.47	0.10	-
Giesy et al. (2002)	Control	-	4.09	0.486	0.032	-
Linear	8.13 g CLA (Ca-CLA)	-	4.02	0.492	0.041	-
	16.25 g CLA (Ca-CLA)	-	4.33	0.508	0.043	-
	32.50 g CLA (Ca-CLA)	-	4.46	0.557	0.068	-
	65.00 g CLA (Ca-CLA)	-	4.57	0.646	0.128	-
Perfield et al. (2002)	Control	1.11	3.12	0.44[d]	<0.01[d]	-
	30.4 g CLA (Ca-CLA)	1.08	3.59	0.51[e]	0.04[e]	-

Table 2.9 Fatty acid composition of milk fat from cows fed rumen protected conjugated linoleic acid (RP-CLA) (Cont.)

References	Treatments	Items (% of fatty acid)				
		C18:1 *t*-11 Vaccenic acid	**C 18:2 Linoleic acid**	***c*-9, *t*-11 CLA**	***t*-10, *c*-12 CLA**	**Total CLA**
Chouinard et al.	Control	-	2.53	-	-	0.68
(1999)	30.6 g CLA	-	3.41	-	-	2.35
Linear, P<0.01	61.2 g CLA	-	3.64	-	-	4.66
	91.8 g CLA	-	3.74	-	-	6.36

[a,b,c] Means within row with different superscripts differ (P<0.05); [d,e,f] Means within row with different superscripts differ (P<0.01)
CLA = Conjugated linoleic acid, C 18:1 *t*-11 = vaccenic acid, LA = Linoleic acid C 18:2, CLA = conjugated linoleic acid, *c*-9, *t*-11 = *cis*-9, *trans*-11 CLA
t-10, *c*-12 = *trans*-10, *cis*-12 CLA
Ca-CLA = Calcium salts of conjugated linoleic acid, FP-CLA = Formaldehyde-protected of conjugated linoleic acid
AP-CLA = Amide-protected of conjugated linoleic acid, LE-CLA = Lipid encapsulated of conjugated linoleic acid

The objective of the present study is to determine the conjugated linoleic acid (CLA) accumulation in meat and milk. Thus, it is interesting that supplementation of sources of lipid rich in linoleic acid such as plant oils and oilseeds especially, soybean oil (SBO) (51.0% linoleic acid; NRC, 2001) and whole cottonseed (WCS) (55.72% linoleic acid; Bertrand et al., 2005) can increase CLA due to linoleic acid, a major precursor of CLA synthesis in rumen through intermediate (*trans*-11 C18:1 vaccenic acid) for intracellular CLA synthesis. Moreover, rumen protected conjugated linoleic acid (RP-CLA), which bypass rumen biohydrogenation can also increase CLA.

2.6 References

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http://www.raw-milk-facts.com/CLA_T3.html

CHAPTER III

THE STUDY OF SOYBEAN OIL AND WHOLE COTTONSEED SUPPLEMENTATION ON PERFORMANCES, CARCASS QUALITY AND CLA ACCUMULATION IN BEEF

3.1 Abstract

Effects of soybean oil (SBO) and whole cottonseed (WCS) supplementation fattening cattle's diets on performance and conjugated linoleic acids (CLA) accumulation in beef were investigated in this study. Eighteen fattening cattle, averaging 241±24 kg live weight (LW) and approximate 1 year old, were stratified by LW into three groups and each group was randomly assigned to three dietary treatments. The treatments were 1) control, 2) control plus 170 g SBO/day and 3) control plus 170 g of oil from WCS/day. At the end to feeding trial, the animals were weighed and slaughtered and carcass measurements were obtained. There were no significant differences in final body weight (BW), average daily gain (ADG) and dry matter intake (DMI) among treatments. Crude protein intake (CPI) was significantly decreased ($P<0.01$) when fed WCS compared with control and SBO treatments. Chemical composition of carcass (moisture percentage, protein percentage and lipid percentage) and carcass quality (meat color and shear force) were not significantly different in *longissimus dorsi* (LD) and *semimembranosus* (SM) muscle by feeding SBO

and WCS. Supplementing SBO increased (P<0.01) in C18:2 *cis*-9, *trans*-11 CLA by 116% in LD muscle (P<0.01) and 240% in SM muscle (P<0.01). However, feeding WSC did not increase C18:2 *cis*-9, *trans*-11 CLA in both muscle. Ruminal pH, ammonia N, total protozoa and volatile fatty acid (VFA) concentrations in rumen fluid were not significantly different when SBO and WCS were added. Addition of 170 g of SBO negligibly detected of C18:2 *cis*-9, *trans*-11 CLA content in rumen digesta than control and WCS supplemented groups. The proportion of C18:1 oleic acid was a high in the rumen digesta when SBO was fed compared with other treatments. This study has successfully increased the C18:2 *cis*-9, *trans*-11 CLA content of meat by source of SBO but not WCS.

3.2 Introduction

Conjugated linoleic acids (CLA) are fatty acids that are found naturally in foods derived from ruminant animals, mainly in meat and milk. Many research studies promote healthy benefits when these products are consumed. The benefits include anticarcinogenesis (Ip et al., 1999; Belury, 2002; Corl et al., 2003), antiobese effects (Park et al., 1997), modulation of the immune system (Cook et al., 1993), antiatherosclerosis (Nicolosi et al., 1997), antidiabetes (Houseknecht et al., 1998) and decrease of human body fat mass in humans (Blankson et al., 2000; Gaullier et al., 2005). CLA is a mixture of geometric and positional isomers of linoleic acid with conjugated double bonds. CLA is intermediate in the biohydrogenation of linoleic acid, which originates from the incomplete biohydrogenation of unsaturated fat by rumen function (Bauman et al., 1999). However, some research work found that cows can also synthesize CLA from *trans*-11 octadecadienoic acid, another intermediate in

the rumen biohydrogenation process by Δ^9 desaturase in tissue (Griinari et al., 1998; Corl et al., 2001).

Researchers have successfully increased the C18:2 *cis*-9, *trans*-11 CLA content of muscle lipids by feeding source of plant oils (Engle et al., 2000; Mir et al., 2002; Mir et al., 2003; Noci et al., 2005; Noci et al., 2007) and oilseeds (Bolte et al., 2002). Thus, supplementation with fat sources rich in linoleic acid such as plant oils and oilseeds may increase the proportion of CLA in meat. The objective of the present study is to investigate the effect of soybean oil (SBO) and oil from whole cottonseed (WCS) supplementation on CLA accumulation in beef.

3.3 Objective

The objective of this experiment was to investigate the effect of soybean oil and whole cottonseed supplementation on performances, carcass quality and CLA accumulation in beef.

3.4 Materials and Methods

Experiment 1

Animals and Feeding

Eighteen fattening cattle (9 dairy bulls and 9 beef bulls), averaging 241±24 kg live weigh (LW) and approximate 1 year old, were stratified by their LW into three groups and each group was randomly assigned to three dietary treatments. The treatments were control (T1), control and supplemented with 170 g SBO/day (T2) and control plus 170 g of oil from whole cottonseed (WCS)/day (T3). The animals were

individually housed and *ad libitum* access to water. All cattle were individually fed concentrate and received *ad libitum* rice straw. The experiment lasted for 109 days. At the end of feeding trial the animals were weighed, and 4 animals per treatment (2 dairy bulls and 2 beef bulls) were randomly sampled and transported to a commercial abattoir (505 Pokphan Co., Ltd, Thailand) and then slaughtered.

Sample collection and chemical analysis

Feeds offered and left after eating of individual cattle were weighed and collected on two consecutive days of each period (21 days). Samples were taken and dried at 60^{o}C for 48 hours. At the end of the experimental period, feed samples were mixed and sub samples were taken for further chemical analysis. Samples were ground through 1 mm screen and analyzed for chemical analysis. Dry matter (DM) was determined by hot air oven at 60^{o}C for 48 h. The crude protein (CP) was determined by Kjeldahl analysis (AOAC, 1995). Ether extract (EE) was determined using petroleum ether in a Soxtec System (AOAC, 1995). Fiber fraction, neutral detergent fiber (NDF) and acid detergent fiber (ADF) 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^{o}C for 3 h. The chemical analysis was expressed on the basis of the final DM. Fatty acids composition of concentrates and rice straw were determined by Gas chromatography.

Carcass collection and Analysis

Muscle samples were cut from outside *Longissimus dorsi* (LD) muscle and *Semimembranosus* (SM) muscle on the left side of each carcass. All samples were placed in plastic bags and placed in ice. At the laboratory, samples were chilled at 4^{o}C for 48 h. Meat sample were determined color and shear force. Meat samples were

removed from the plastic bags and cut. These samples were measured meat color by Chroma meter (Minolta CO., LTD) and then *L** (lightness), *a** (redness) and *b** (yellowness) value were measured in six locations. Shear force was done with a Warner-Bratzle shear attachment by Texture analyzer (TA-TX2 Texture Analyzer, Stable Micro Systems, UK). Marbling score measured by Thai Agricultural Commodity and Food Standard (TACFS 6001-2004 : level 5=Abundant, 4=Moderate, 3=Small, 2=Slight, 1=Devoid). Muscle samples (LD and SM) were ground using blender machine. Sub samples were analyzed in duplicate for CP using the Kjeldahl method and lipid by solvent extraction using petroleum ether extraction (AOAC, 1995).

Fatty acid analysis

Feed, rumen digesta and meat were extracted using a modified of the method used by Folch et al. (1957) and Metcalfe et al. (1966). Before the extraction, meat samples were thawed and each sample was chopped coarsely and blended in blender machine. 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 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 were added. The under layer of fatty acid methyl esters (FAME) was removed and placed in screw-cap test tube and stored at -20^{o}C 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 were added. The tubes were flushed with nitrogen, capped, heated at 100 C for 5 min with occasional shaking and then

cooled to room temperature. One ml of C17:0 internal standard (2.00 mg/mL in hexane) and 2 ml of boron trifluoride in methanol were added and heated at 100 C for 5 min with occasional shaking and 10 ml of deionized water were added. The solution was transferred to a 40-ml centrifuged tube and 5 ml of hexane were added for FAME extraction. The solution was centrifuged at 2,000 g, at 10 C for 20 min and then the hexane layer was dried over sodium sulfate and transferred into vial for analyzing by gas chromatography (GC) (Hewlett Packard GC system HP6890 A; Hewlett Packard, Avondale, PA) equipped with a 100 m x 0.25 mm x 0.2 μm film fused silica capillary column (SP2560, Supelco Inc, Bellefonte, PA, USA). Injector and detector temperatures were 240 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 31 min.

Experiment 2

Three fistulated non-lactating dairy cows were used in a 3x3 Latin Square Design. Concentrates included control, control plus 170 g SBO/d and control plus 170 g oil fromWCS/d. Experimental periods were 14 d with 12 d for adaptation and 2 d for sample collection. On d 13 and 14 of each period, rumen digesta was sampled via the rumen cannula from each fistulated non-lactating dairy cows at 0 (pre feeding), 2, 4 and 6 h post feeding. Rumen digesta were crushed through nylon cloth. Rumen fluid sample was analyzed for pH, VFA, ammonia N, protozoa count and rumen digesta was analyzed for fatty acids.

Rumen digesta was collected using a modified method described by AbuGhazaleh et al. (2002). Briefly, approximately 450 g of rumen digesta was removed by hand from different locations in the rumen and mixed. Additional rumen

digesta was taken and squeezed through nylon cloth and 100 ml of rumen fluid was added to each sample. Rumen digesta samples were then placed into plastic bags and stored on ice until processing in the laboratory. Each sample was mixed by hand, sub sampled (approximately 200 g) and then frozen. Frozen rumen digesta samples were dried and ground to pass 1 mm screen. Rumen digesta was extracted using a modified of the method used by Folch et al. (1957) and Metcalfe et al. (1966) and Fatty acid methyl esters (FAME) were prepared by procedure described by Ostrowska et al. (2000). The fatty acid in rumen digesta was determined by gas chromatography.

The pH of rumen fluid was determined at the time of sampling by pH meter. Ruminal volatile fatty acids (VFA) and ammonia N were determined in rumen fluid samples by taking 20 ml of rumen fluid, then combined with 5 ml 6N HCl to freezing at -20^{o}C until analysis of VFA and ammonia N. Samples were later thawed at 4^{o}C and centrifuged at 3,000 rpm for 15 min. The supernatant fluid was analyzed for ammonia N by Kjeldahl and VFAs (acetate, propionate and butyrate) concentrations by gas chromatography (Hewlett Packard GC system HP6890 A; Hewlett Packard, Avondale, PA) equipped with a 30 m x 0.25 mm x 0.25 μm film (DB-FFAP). Protozoa populations was determined in rumen fluid samples by taking 1 ml of rumen fluid, then diluted 9 ml with 10% formal saline solution (1:9 ml) and counted by Hematocytometer.

Statistical Analysis

All data in experiment 1 were statistically analyzed as a Randomized Completely Block Design and all data in experiment 2 were statistically analyzed as 3x3 Latin Square Design using ANOVA procedure of SAS (SAS, 1996).

Experimental location

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

Experimental period

The experiment was from February 2007 to November 2007.

3.5 Result and Discussion

Experiment 1

Feed composition and performances

The chemical compositions of concentrate, whole cotton seed and rice straw are reported in Table 3.1. Diets were control diet, control diet supplemented with 170 g SBO/d and 170 g of oil from WCS/d and rice straw. Concentrations of CP in diet were 15.21, 14.78 and 14.71 %, respectively. SBO supplemented diets had higher EE content than other diets. The fatty acid compositions of concentrates, SBO and WCS were summarized in Table 3.2. The concentration of C18:2 was increased with supplementing SBO and WCS in the diets.

The effects of dietary treatments on performance and nutrient intake of cattle are presented in Table 3.3. Intake of fatty acids is showed in Table 3.4. Final BW, average daily gain (ADG), Feed: Gain ratio and dry matter intakes (DMI) of experimental animals were not significantly different among treatments. The addition of WCS in diet, DMI was the lowest compared with other treatments (6.56 vs. 6.69, 6.78 kgDM/d), especially DMI in rice straw was also the lowest. The crude protein intake (CPI) in concentrate was significantly lower ($P<0.01$) in cattle which received WCS treatment than control and SBO treatments. The energy gain was significantly increased ($P<0.05$)

when receiving WCS treatments compared to the control treatment. Other researches, Luginbuhl et al. (2000) showed that the addition of 16 and 24% WCS in the diets of growing male goats decreased DMI ($P<0.04$) and CPI ($P<0.01$) DM and NDF digestibility. Decreases in DMI can be attributable to the fact that goats may refuse or be reluctant to consume WCS during the experiment. Moore et al. (1986) suggested that the additions of fat were higher than 4%, even in the form of whole cottonseed, and fiber digestion decreased.

The DMI decreased ($P>0.05$) when WCS was supplemented to cattle, probably because WCS is protected from ruminal digestion due to its encapsulation by the seed coat, which is high in CF, NDF and ADF (27.39, 47.76, and 38.49, respectively). Moreover, WCS had been associated with slow rates of ruminal passage digesta, thus increasing retention time of the digesta and limited intake. Palmquist (1995) suggested that the delay in the digestion of cotton fibers after colonization was caused by the highly crystalline structure of cotton fibers, which slow hydrolization and cellulolytic activity resulted in a limited rate of cotton linter digestion.

The results of this study are similar to other studies. For example, the addition of 3 and 6% SBO in beef heifer's diets had no effect on final BW and ADG (Whitney et al., 2000). Griswold et al. (2003) fed 4% SBO diet to Angus-Hereford finishing steers, DMI, ADG and Gain: Feed ratios were similar, and was is consistent with Beaulieu et al. (2002) when Angus-Wagyu heifers were fed finishing diets supplemented with 5% SBO. Mir et al. (2003) reported that the performance parameters such as LW gain, DMI and Feed: Gain ratio were similar when 3 and 6% sunflower oil (SFO) supplemented diets were fed. The addition of 5.5% SFO in concentrates did not affect the final LW and ADG (Noci et al., 2005) which is similar

to Noci et al. (2007), when 150 g/d SFO and 150 g/d linseed oil (LSO) were fed. Hristov et al. (2005) reported that DMI, ADG and Gain: Feed were unaffected by 5% safflower oil supplementation. Others have reported that the addition of oil to diet decreased DMI (Andrae et al., 2000; Engle et al., 2000). For example, steers fed 8.5% high oil diet caused a decrease in DMI and ADG compared with control treatment (Andrae et al., 2000). Engle et al. (2000) also reported that final BW, ADG and DMI were decreased when fed 4% SBO. The decreased DMI in SBO added steers may have been due to the high unsaturated fatty acid content of SBO affecting rumen fermentation, and inhibiting fiber digestion (Engle et al., 2000). Huerta-Leidenz et al. (1991), fed 15 and 30% WCS which showed no differences in the final BW, ADG and Gain: Feed ratios among treatments. Similar results were obtained when steers were fed 15%WCS compared with control treatments (Cranston et al., 2006). Moreover, increased DMI (P=0.02), ADG (P=0.01) and Gain: Feed (P=0.01) when feeding 9 and 14% whole sunflower seed (WSFS) were used. However, supplementing WSFS rich in linoleic acid did not affect DMI, ADG and Gain: Feed ratio (Gibb et al., 2004).

Table 3.1 Chemical composition of the experimental diets

Items	Treatments[1]			WCS	Rice straw
	T1	T2	T3		
	----------% of dry matter----------				
Dry matter	92.94	94.34	93.83	91.28	92.16
Ash	6.93	6.39	5.56	3.61	11.68
Crude protein	15.12	14.78	14.71	19.51	3.91
Ether extract	4.11	8.58	7.56	16.25	0.83
Crude fiber	16.66	16.46	17.02	27.39	40.89
Neutral detergent fiber	46.46	41.38	42.37	57.15	70.96
Acid detergent fiber	28.23	26.49	26.26	42.35	44.87
Acid detergent lignin	10.59	11.17	11.65	11.84	6.90
TDN_{1x} (%)[2]	62.96	71.02	69.11	78.20	45.85
DE_{1x} (Mcal/kg)[3]	2.84	3.17	3.07	3.52	1.95
MEp (Mcal/kgDM)[4]	2.33	2.60	2.52	2.89	1.60
NEm (Mcal/kgDM)[5]	1.46	1.69	1.62	1.94	0.76
NEg (Mcal/kgDM)[6]	0.87	1.08	1.02	1.29	0.22

[1]T1 = Control, T2 = 170 g SBO/d, T3 = 170 g oil from WCS/d
[2]Total digestible nutrients, TDN_{1x} (%) = tdNFC + tdCP + (tdFA x 2.25) + tdNDF – 7 (NRC, 2001)
[3]Digestible energy, DE_{1x} (Mcal/kg) = [(tdNFC/100)x4.2]+[(tdNDF/100) x 4.2]+[(tdCP/100) x 5.6]+[(FA/100) x 9.4] –0.3
[4]Metabolisable energy, ME = 0.82 x DE (NRC, 1996)
[5]Net energy for maintenance, $NEm = 1.37ME – 0.138ME^2 + 0.0105ME^3 – 1.12$ (NRC, 1996)
[6]Net energy for growth, $NEg = 1.42ME – 0.174ME^2 + 0.0122ME^3 – 1.65$ (NRC, 1996)
WCS = Whole cottonseed, RS = Rice straw

Table 3.2 Fatty acid compositions of concentrate, soybean oil (SBO) and whole cottonseed (WCS)

Items	Treatments[1]			SBO	WCS	RS
	T1	T2	T3			
	----------% of total fatty acid----------					
C 12:0	36.37	18.31	6.74	0.01	0.03	4.48
C 14:0	13.18	6.64	2.84	0.08	0.61	2.95
C 16:0	12.13	11.16	24.67	10.42	26.58	28.05
C 18:0	2.84	3.20	2.43	3.57	2.44	15.07
C 18:1	18.84	18.95	15.71	19.01	13.07	14.84
C 18:2	11.32	34.02	45.75	57.82	55.38	12.33
C 18:3	ND	1.14	0.02	7.67	0.19	2.26
Other[2]	5.32	6.57	1.84	1.43	1.71	20.00

[1]T1 = Control, T2 = 170 g SBO/d, T3 = 170 g oil from WCS/d
[2]Other = (Sum of C6:0, C8:0, C10:0, C16:1, C17:1, C20:1,C20:2, C22:0, C20:3n6, C22:1n9, C20:3n3, C23:0, C20:5n3, C24:1)
SBO = Soybean oil WCS = Whole cottonseed; RS = Rice straw
ND = Not detected. A value of 0 was used for statistical analyses

Table 3.3 Effect of soybean oil (SBO) and whole cottonseed (WCS) on performance and nutrient intake of cattle

Items	Treatments[1]			SEM	P-value
	T1	T2	T3		
Initial body weight, kg	242	239	241	9.44	0.971
Final body weight, kg	296	305	312	8.42	0.449
Average daily gain, kg/d	0.50	0.61	0.65	0.05	0.096
Energy gain[2]	1.64[b]	2.09[ab]	2.28[a]	0.17	0.046
Feed: Gain ratio	14.21	11.31	10.66	1.11	0.093
Dry matter intake (kg/d)					
Concentrate	3.04	3.04	3.02	0.01	0.396
Rice straw	3.65	3.74	3.54	0.29	0.882
Total	6.69	6.78	6.56	0.29	0.856
DMI, g/kg $BW^{0.75}$	93.77	93.03	88.43	1.90	0.140
Crude protein intake (g/d)					
Concentrate	460[d]	450[e]	444[e]	2.02	0.001
Rice straw	143	146	141	2.20	0.247
Total	603[d]	596[e]	585[f]	2.37	0.001
Ether extract intake (g/d)					
Concentrate	125[f]	261[d]	228[e]	0.96	0.001
Rice straw	30	31	29	0.54	0.131
Total	155[f]	292[d]	257[e]	0.95	0.001

[a,b,c] Means within row with different superscripts differ ($P<0.05$)
[d,e,f] Means within row with different superscripts differ ($P<0.01$)
[1]T1 = Control, T2 = 170 g SBO/d, T3 = 170 g oil from WCS/d
[2]Energy gain was calculated by the equation $EG = (0.0493BW^{0.75})*ADG^{1.097}$, where EG is the daily energy deposited (Mcal/d) and BW is the mean body weight (NRC, 1984 as reviewed by NRC, 1996)
SEM = Standard error of mean

Table 3.4 Intake of individual fatty acids

Items	**Treatments**[1]		
	T1	**T2**	**T3**
	---------- g/day ----------		
C12:0	46.88	46.90	31.36
C14:0	17.37	17.53	12.73
C16:0	23.66	41.59	63.38
C18:0	8.12	14.31	10.91
C18:1	28.06	60.48	42.06
C18:2	17.89	116.28	107.07

[1]T1 = Control, T2 = 170 g SBO/d, T3 = 170 g oil from WCS/d

Carcass quality

Chemical compositions of meat were not significantly different (P>0.05) among treatments (Table 3.5). The average percentages of protein, lipid and moisture in LD muscle were 22.06, 5.81 and 75.30, respectively and in SM muscle were 22.60, 4.28 and 75.38, respectively. Carcass quality including color, shear force and marbling score were not different when SBO and WCS were fed (P>0.05).

Researches had reported carcass composition and quality in response to supplemented sources of oil and oilseed. Addition of 5% SBO did not affect carcass weight and carcass quality parameters including fat thickness, LM area and marbling score (Beaulieu et al., 2002). In contrast to Engle et al. (2000), who reported that marbling scores of hot carcass weight were significantly decreased (P<0.01) when 4% SBO were fed. Griswold et al. (2003) showed increasing dietary SBO linearly decreased dressing percentage (P=0.04) and tended to linearly decrease marbling score (P=0.12). Moreover, Mir et al. (2003) reported that cattle fed 6% SFO, rib eye area was reduced (P<0.05) and back fat content was also reduced when received 3% SFO (P<0.05). Addition of 5.5 and 11 % SFO in concentrates did not affect carcass weight, moisture and fat contents of the LD muscle (Noci et al., 2005). Noci et al. (2007) reported that Charolais crossbred heifers fed 150 g/d SFO and 150 g/d linseed oil (LSO) showed no differences in carcass weight and dressing percentage. Andrae et al. (2001), with steers fed high-oil corn in diets did not affect carcass weight, dressing percentage, fat thickness and LM area, but increased marbling score (P<0.05).

Gibb et al. (2004) reported that dressing percentage and back fat thickness were not different, but carcass weight was increased (P=0.03) when whole sunflower seed was fed. Cranston et al. (2006) found that steers fed 15% WCS decreased

dressing percentage (63.02 vs. 61.81%) (P=0.02) and marbling scores (481 vs. 430) (P=0.02), however 12^{th} rib fat was not affected. Addition of high fat in diets did not affect percentages of DM, fat and protein of LD muscle (Garcia et al., 2003). Scollan et al. (2006) reviewed that the development of intramuscular fat concentration or marbling score is late maturing, it is due to maintained or increased fat synthesis in combination with declining muscle growth as animal. The intramuscular fat content at birth or at the beginning of the finishing period is explained by the number of preadipocytes which depends itself on genetic and nutrition factors, for example late-maturing beef breeds deposit more muscle and less fat compared to dairy breeds or early-maturing beef breeds.

Table 3.5 Effect of soybean oil (SBO) and whole cottonseed (WCS) on chemical composition and carcass quality

Items	Treatments[1]			SEM	P-value
	T1	T2	T3		
Chemical composition (%)					
Longissimus dorsi muscle					
Protein	22.07	22.22	21.89	0.24	0.650
Lipid	5.18	5.27	6.99	1.22	0.533
Moisture	72.43	72.11	72.35	0.28	0.723
Semimembranosus muscle					
Protein	22.20	22.53	23.08	0.22	0.073
Lipid	4.17	4.54	4.14	0.86	0.937
Moisture	72.26	72.60	72.28	0.38	0.787
Marbling score	1	1	1	-	-
Carcass quality					
Longissimus dorsi muscle					
Shear force, kg	5.76	7.12	6.14	0.52	0.246
Color L*	45.52	44.40	48.39	5.43	0.869
a*	14.34	14.58	13.50	1.11	0.779
b*	6.18	7.53	6.63	0.59	0.554
Semimembranosus muscle					
Shear force, kg	9.69	14.44	8.88	2.07	0.202
Color L*	44.87	43.12	47.09	6.08	0.900
a*	15.12	14.32	14.10	0.83	0.678
b*	7.66	9.54	8.14	0.57	0.126

[1]T1 = Control, T2 = 170 g SBO/d, T3 = 170 g oil from WCS/d
Color : *L** (lightness), *a** (redness) and *b** (yellowness)
SEM = Standard error of mean

Fatty acid composition in muscle

The fatty acid composition of fat extracted from LD and SM muscle are presented in Tables 5 and 6. There were significant decreases ($P<0.05$) in C12:0, C14:0 and C14:1 in LD and SM muscle when WCS was fed, in comparison with control and SBO treatments. Feeding SBO and WCS significantly decreased ($P<0.05$) C16:0 and C16:1 in LD muscle and C16:1 in SM muscle. The present report confirms the results of Engle et al. (2000), who found a decrease in C16:1 content in muscle and adipose tissue but not in C16:0 content in LD muscle when steers were fed diets containing 4% SBO. Supplementation with 5% SBO, the proportion of C16:0 and C16:1 tissue lipid from loin decreased (Beaulieu et al., 2002). Dhiman et al. (2005) reported that adipose tissue and muscle from steers fed 2 and 4% SBO were not affected the C12:0-C16:0 content, except for C14:0 content that increased and C16:1 content decreased. Moreover, Mir et al. (2002) reported the decreases in C16:0 and C16:1 when 6% SFO was supplemented because of negative feedback inhibition of fatty acid synthesis by the exogenous fatty acids. Mir et al. (2003) showed that C16:0 and C16:1 decreased ($P<0.05$) by 3 and 6% SFO supplementation. However, Noci et al. (2007) indicated that the proportion of C12:0, C14:0 and C16:0 increased in muscle tissue when cattle were supplemented with SFO and LSO (linseed oil) compared with the control treatment.

Stearic acid (C18:0) in LD and SM muscle of cattle fed WCS diet were higher ($P<0.01$) than those fed control and SBO diets. However, both LD and SM muscle showed no differences in C18:1, C18:2 and C18:3 contents among treatments. Other researchers have reported 10 and 12% increase in C18:0 of forequarter and loin, respectively while C18:2 concentration in muscle did not change by feeding 5% SBO

(Beaulieu et al., 2002) and 10% increase in C18:0 in adipose and muscle tissue when adding 4% SBO to steer diets compared with control and 2% SBO treatments (Dhiman et al., 2005). Griswold et al. (2003), reported linearly increase (P=0.04) in C18:2 content, while C18:3 was not affected when SBO (0, 4 and 8% SBO of diet DM) was added to steers diets. Mir et al. (2003) found that C18:2 in beef muscle was increased by 3 and 6% SFO addition because SFO contains high levels (68%) of C18:2. Noci et al. (2007), reported that C18:1 and C18:2 contents were increased (P<0.01), while C18:0 content was decreased (P<0.05) by SFO and LSO supplementation.

The main objective of this study was to increase the concentration of beneficial fatty acid, especially CLA by SBO supplemented in diets because SBO is major sources of C18:2 linoleic acid (57.82 %) which is a substrate in rumen biohydrogenation. CLA content (C18:2 *cis*-9, *trans*-11) significantly increased (P<0.01) by 116% in LD muscle when SBO were fed and compared with control. In SM muscle, the addition of SBO in diet significantly increased (P<0.01) CLA content by 240% when compared with control. However, the *trans*-10, *cis*-12 CLA content in LD and SM muscle were not detected in this study. Many reports demonstrated that C18:2 *cis*-9, *trans*-11 CLA is a major fatty acid in tissue and little to no *trans*-10, *cis*-12 CLA was detected (Beaulieu et al., 2002; Madron et al. 2002; Griswold et al., 2003). Increasing CLA content accumulation in muscle by SBO, can be explained by the fact that SBO is rich in C18:2 and is used to promote direct synthesis of CLA. The biohydrogenation is incomplete in the rumen, CLA isomer and C18:1 *trans*-11 vaccenic acid are an intermediate that escaped from the rumen and then converted to produce CLA (C18:2 *cis*-9, *trans*-11) in tissue by the action of Δ^9 desaturase (Griinari

et al., 1998; Bauman et al., 1999; Corl et al., 2001). In this study, the supplementation of WCS did not affect CLA content in muscle although it was rich in linoleic acid content. Page et al. (1997) expected that WCS depress stearoyl-coenzyme a desaturase activity in subcutaneous, adipose tissue and liver due to its cyclopropene fatty acid content in WCS if fed for a sufficiently long period of time. Madron et al. (2002) suggested that the possibility of the biohydrogenation is more complete in the rumen, resulting in the formation of more stearic acid and thus less C18:1 *trans*-11 vaccenic acid and CLA would escape the rumen.

Researchers have successfully increased the C18:2 *cis*-9, *trans*-11 CLA content of muscle lipids by sources of oils. For example, the addition of 4% SBO increased ($P<0.05$) C18-conjugated dienes by 45% (Engle et al., 2000). Mir et al. (2002) found the increase ($P<0.01$) in CLA contents in LD muscle by 339% but not muscle fat content when adding of 6% SFO in steer diets. Similarly, CLA content of muscle increased 30% and 75% ($P<0.05$) by 3% and 6% SFO supplementation, respectively (Mir et al., 2003). The addition of SFO and LSO in diets significantly increased ($P<0.01$) C18:2 *cis*-9, *trans*-11 CLA content in *longissimus dorsi* muscle by 144 and 73%, respectively while *trans*-10, *cis*-12 CLA content was similar (Noci et al., 2007). Moreover, the addition of oilseeds increased CLA content in muscle tissue. Bolte et al. (2002), reported that safflower seeds added to lamb diets increased ($P<0.01$) *cis*-9, *trans*-11 CLA content in adipose tissue and muscle.

However, many researchers had shown that CLA content in tissue did not affect when supplemented with oils. For example, Dhiman et al. (2005), supplementing with 4% SBO to diets did not affect the C18:2 *cis*-9, *trans*-11 CLA content of muscle lipids. Similar to Beaulieu et al. (2002), who reported that feeding 5%

SBO did not affect in C18:2 *cis*-9, *trans*-11 CLA content of muscle tissue, but increased *trans*-10, *cis*-12 CLA content in tissue lipid from forequarters ($P<0.03$) and hindquarters ($P<0.04$). Feeding 2 and 4% SBO did not affect C18:2 *cis*-9, *trans*-11 CLA content in adipose and muscle tissue, but increased *trans*-10, *cis*-12 CLA content in adipose tissue of the LD muscle (Dhiman et al., 2005). However, the levels of 0, 4 and 8% SBO of diet DM supplemented to Angus-Hereford steer diets decreased CLA content in lean tissues (Griswold et al., 2003). Dietary polyunsaturated fatty acid content is sufficient to limit ruminal of conjugated linoleic acid and vaccenic acid or decrease tissue stearoyl-CoA desaturase expression or activity.

The proportions of short- and medium-chain fatty acid (<16 carbons) significantly decreased ($P<0.01$) in LD muscle by WCS supplementation compared with control and SBO treatment. However, the proportion of long-chain fatty acids (>16 carbons) significantly increased ($P<0.01$) in the same treatments. In SM muscle, the supplementation of WCS significantly decreased ($P<0.01$) <16 carbons, while it increased >16 carbons. Saturated fatty acid and unsaturated fatty acid were not significantly affected by supplementation of either SBO or WCS.

Table 3.6 Effect of soybean oil (SBO) and whole cottonseed (WCS) on fatty acid composition in *Longissimus dorsi* muscle

Items	Treatments[1]			SEM	P-value
	T1	T2	T3		
	----- % of total fatty acid -----				
C 12:0	1.09[a]	0.91[a]	0.35[b]	0.14	0.019
C 14:0	7.13[a]	5.64[a]	3.88[b]	0.56	0.018
C 14:1	0.94[d]	0.69[de]	0.35[e]	0.12	0.002
C 15:0	0.66	0.53	0.55	0.06	0.343
C 15:1	0.26	0.14	0.19	0.07	0.478
C 16:0	29.37[a]	25.92[b]	27.63[ab]	0.62	0.022
C 16:1	3.64[a]	2.62[b]	2.60[b]	0.24	0.034
C 17:1	0.83	0.56	0.57	0.07	0.072
C 18:0	17.51[f]	21.06[ee]	25.21[d]	1.13	0.008
C 18:1	29.51	31.38	29.52	1.23	0.504
C 18:2	4.15	5.29	4.78	0.79	0.621
C 18:3	0.33	0.33	0.15	0.05	0.060
≥C 20:0	4.27	4.26	4.02	0.68	0.958
c-9,t-11CLA	0.32[e]	0.69[d]	0.22[e]	0.04	0.001
Summation by source[2]					
<C16:0	10.08[d]	7.90[d]	5.30[e]	0.73	0.010
C16:0 and C16:1	33.00[d]	28.54[e]	30.23[e]	0.66	0.008
>C16:0	56.92[e]	63.57[d]	64.47[d]	1.19	0.007
Saturated fatty acid	57.05	55.03	58.57	1.81	0.435
Unsaturated fatty acid	42.96	44.97	41.44	1.81	0.435

[a,b,c] Means within row with different superscripts differ (P<0.05)
[d,e,f] Means within row with different superscripts differ (P<0.01)
[1]T1 = Control, T2 = 170 g SBO/d, T3 = 170 g oil from WCS/d
[2]Fatty acids : <C16:0 represent de novo synthesized fatty acids, >C16:0 represent preformed fatty acids taken up from circulation, C16:0 fatty acids are derived from both sources
SEM = Standard error of mean

Table 3.7 Effect of soybean oil (SBO) and whole cottonseed (WCS) on fatty acid composition in *Semimembranosus* muscle

Items	Treatments[1]			SEM	P-value
	T1	T2	T3		
	----- % of total fatty acid -----				
C 12:0	0.69[e]	0.62[d]	0.32[e]	0.05	0.005
C 14:0	5.28[a]	4.53[ab]	3.42[b]	0.39	0.040
C 14:1	0.68[a]	0.63[a]	0.20[b]	0.13	0.041
C 15:0	0.50	0.43	0.48	0.03	0.307
C 15:1	0.34	ND	0.13	0.09	0.083
C 16:0	27.92	25.64	26.85	0.76	0.189
C 16:1	3.39[a]	2.65[b]	2.54[b]	0.21	0.059
C 17:1	1.03	0.51	0.51	0.17	0.113
C 18:0	16.25[e]	18.30[e]	24.00[d]	0.91	0.002
C 18:1	30.85	32.94	29.81	1.09	0.196
C 18:2	6.07	7.13	6.32	1.15	0.801
C 18:3	0.36	0.32	0.16	0.11	0.408
≥C 20:0	6.45	5.61	5.15	1.17	0.739
c-9,t-11CLA	0.20[e]	0.68[d]	0.14[e]	0.03	0.001
Summation by source[2]					
<C16:0	7.49[a]	6.21[a]	4.54[b]	0.48	0.014
C16:0 and C16:1	31.30	28.30	29.39	0.85	0.114
>C16:0	61.21[b]	65.50[a]	66.07[a]	1.26	0.052
Saturated fatty acid	52.31	50.42	55.71	1.55	0.125
Unsaturated fatty acid	47.69	49.58	44.29	1.55	0.125

[a,b,c] Means within row with different superscripts differ (P<0.05)
[d,e,f] Means within row with different superscripts differ (P<0.01)
[1]T1 = Control, T2 = 170 g SBO/d, T3 = 170 g oil from WCS/d
[2]Fatty acids : <C16:0 represent de novo synthesized fatty acids, >C16:0 represent preformed fatty acids taken up from circulation, C16:0 fatty acids are derived from both sources
ND = Not detected. A value of 0 was used for statistical analyses
SEM = Standard error of mean

Experiment 2

Ruminal fermentation

Ruminal pH, ammonia N and protozoa population in rumen fluid at 2, 4 and 6 h post-feeding are presented in Table 3.8. Ruminal pH ranged from 6.57 to 7.05 across treatments (P>0.05). Ammonia N and protozoa population were not different (P>0.05) when SBO and WCS were supplemented. The concentration of VFA in rumen is showed in Table 3.9. Addition of SBO and WCS in diets did not affect acetate, propionate, butyrate and acetate : propionate ratio. Dayani et al. (2007) suggested that VFA concentration was not changed by WCS supplement due to 1) low oil supplement in diets containing WCS, 2) WCS fat may be released slowly in the rumen, 3) WCS leaves the rumen still partially enclosed within the seed. Krysl et al. (1991) reported that the addition of 3% SBO did not effect ruminal ammonia N, total VFA and acetate while ruminal pH was significantly decreased (P<0.01), however, propionate was significantly increased (P<0.05). As reported by Brokaw et al. (2001), supplementation of 12.5% SBO did not affect ruminal pH, ammonia N and total VFA concentration. Dayani et al. (2007) showed that feeding 20% cottonseed significantly decreased (P<0.01) ammonia N while ruminal pH and total VFA concentration were similar. In this study, supplementation of 170 g SBO/d and 170 g oil from WCS/d would not be expected to influence ruminal fermentation because amount of oil has a few, and the seeds oil were not readily fermented.

Table 3.8 Effect of soybean oil (SBO) and whole cottonseed (WCS) on pH, NH_3-N and protozoa population

Items	**Treatmens**[1]			**SEM**	**P-value**
	T1	**T2**	**T3**		
pH					
0 h	7.02	7.05	6.87	0.07	0.317
2 h	6.71	6.90	6.81	0.08	0.403
4 h	6.57	6.81	6.80	0.06	0.179
6 h	6.57	6.86	6.78	0.08	0.217
NH_3-N (mg/dl)					
0 h	5.33	4.88	6.07	0.56	0.465
2 h	6.49	5.03	5.34	0.40	0.215
4 h	3.93	4.74	3.71	1.05	0.787
6 h	3.31	3.03	3.30	0.25	0.714
Protozoa (x 10^5 cells/ml)					
0 h	1.21	2.67	4.33	1.45	0.463
2 h	2.17	2.96	3.13	0.37	0.343
4 h	3.33	4.38	1.50	0.52	0.114
6 h	2.54	3.54	2.38	0.54	0.424

[1]T1 = Control, T2 = 170 g SBO/d, T3 = 170 g oil from WCS/d
NH_3-N = Ammonia nitrogen
SEM = Standard error of mean

Table 3.9 Effect of soybean oil (SBO) and whole cottonseed (WCS) on volatile fatty acids

Items	Treatments[1]			SEM	P-value
	T1	T2	T3		
VFA, mol/100 mol					
Acetate, C_2					
0 h	77.78	73.40	71.34	1.36	0.145
2 h	75.83	70.90	70.90	2.12	0.356
4 h	74.94	71.43	71.29	2.02	0.488
6 h	73.80	71.08	71.41	2.20	0.687
Propionate, C_3					
0 h	14.24	16.02	18.67	0.72	0.094
2 h	16.04	17.69	19.06	0.74	0.193
4 h	15.29	16.87	18.48	0.70	0.159
6 h	16.02	16.57	18.10	0.62	0.249
Butyrate, C_4					
0 h	7.98	10.57	10.00	0.87	0.291
2 h	8.13	11.41	10.04	1.44	0.431
4 h	9.77	11.70	10.23	1.34	0.637
6 h	10.18	12.35	10.48	1.58	0.644
Acetate : Propionate					
0 h	5.51	4.59	3.82	0.32	0.123
2 h	4.80	4.01	3.74	0.32	0.249
4 h	4.97	4.24	3.87	0.33	0.254
6 h	4.64	4.29	3.95	0.30	0.436

[1]T1 = Control, T2 = 170 g SBO/d, T3 = 170 g oil from WCS/d
VFA = volatile fatty acids
SEM = Standard error of mean

Fatty acid composition of rumen digesta

The rumen is the site of an intense microbial lipid metabolism. Lipolysis of dietary glycolipids, phospholipids and triglycerides leads to free fatty acids that are hydrogenated by microbes to more saturated fatty acid end products. Conjugated linoleic acids and *trans* vaccenic acid are intermediates formed during biohydrogenation of dietary linoleic acid. AbuGhazaleh et al. (2002) reported that the extent of biohydrogenation of unsaturated fatty acids reflected by : 1) accumulation of *trans* fatty acids in the rumen, mainly C18:1 *trans* vaccenic acid; 2) change in the percentages of saturated and unsaturated fatty acid in the rumen digesta compared with dietary fatty acid; and 3) greater concentration of C18:0 in the rumen compared with dietary fatty acid.

The objective of this study is to determine the effect of SBO and WCS supplementation on ruminal fatty acid profiles and CLA content. The proportions of fatty acids in rumen digesta are presented in Table 3.10, 3.11, 3.12 and 3.13. Addition of 170 g of SBO in diet negligibly detected of C18:2 *cis*-9, *trans*-11 CLA content in rumen digesta while those in control and WCS supplemented were not detected. The proportion of C18:1 oleic acid in the rumen digesta was higher than WCS and control treatments when SBO was fed. Rumen digesta of WCS supplemented cattle contained lower ($P<0.05$) C18:0 stearic acid and higher ($P<0.05$) C18:2 linoleic acid than control and SBO treatments. The present study suggested that the biohydrogenation in the rumen of WCS supplemented cattle occurred only little, presumably because of mastication of the seed coat of WCS, thus linoleic acid remained at a high level, resulting in a low level of stearic acid. In contrast, rumen digesta of SBO supplemented cattle contained lower C18:2 linoleic acid and higher C18:0 stearic acid than those WCS supplemented and control group.

Table 3.10 Effect of soybean oil (SBO) and whole cottonseed (WCS) on fatty acid composition in rumen digesta at 0 h

Items	Treatments[1]			SEM	P-value
	T1	T2	T3		
	----- % of total fatty acid -----				
C 12:0	6.84	1.84	ND	2.70	0.367
C 14:0	11.17	4.52	1.18	1.18	0.050
C 16:0	34.08	24.16	34.05	2.15	0.124
C 18:0	48.51	28.06	44.28	5.70	0.218
C 18:1	3.63	16.78	10.39	3.30	0.201
C 18:2	<0.01[e]	0.90[e]	25.41[d]	1.00	0.004
cis-9, *trasns*-11CLA	ND	3.28	0.92	1.69	0.500

[d,e,f] Means within row with different superscripts differ (P<0.01)
[1]T1 = Control, T2 = 170 g SBO/d, T3 = 170 g oil from WCS/d
ND = Not detected. A value of 0 was used for statistical analyses
SEM = Standard error of mean

Table 3.11 Effect of soybean oil (SBO) and whole cottonseed (WCS) on fatty acid composition in rumen digesta at 2 h post feeding

Items	Treatments[1]			SEM	P-value
	T1	T2	T3		
	----- % of total fatty acid -----				
C 12:0	18.64	7.38	2.09	4.79	0.243
C 14:0	11.52	5.28	2.59	1.15	0.059
C 16:0	23.74	19.92	30.61	3.12	0.249
C 18:0	33.28	33.91	25.62	5.20	0.559
C 18:1	9.06[b]	23.83[a]	12.94[b]	1.35	0.030
C 18:2	1.18	8.07	26.67	4.92	0.127
C 20:0	0.68	0.23	ND	0.28	0.388
cis-9, *trasns*-11CLA	ND	0.48	ND	0.28	0.500
trans-10,*cis*-12 CLA	ND	0.22	ND	0.13	0.500
Other	1.89	0.66	ND	0.98	0.511

[a,b,c] Means within row with different superscripts differ (P<0.05)
[1]T1 = Control, T2 = 170 g SBO/d, T3 = 170 g oil from WCS/d
ND = Not detected. A value of 0 was used for statistical analyses
SEM = Standard error of mean

Table 3.12 Effect of soybean oil (SBO) and whole cottonseed (WCS) on fatty acid composition in rumen digesta at 4 h post feeding

Items	Treatments[1]			SEM	P-value
	T1	T2	T3		
	----- % of total fatty acid -----				
C 12:0	21.32	7.61	2.93	3.94	0.145
C 14:0	19.95	6.06	2.96	1.08	0.053
C 16:0	21.07	18.52	27.89	1.96	0.143
C 18:0	33.02[a]	36.92[a]	26.65[b]	0.79	0.022
C 18:1	8.12[b]	27.44[a]	11.11[b]	1.31	0.015
C 18:2	0.63[b]	2.48[b]	27.78[a]	3.03	0.038
C 20:0	1.36	0.24	0.25	0.21	0.093
cis-9, *trasns*-11CLA	ND	0.36	ND	0.24	0.500
Other	2.52	0.37	0.42	0.41	0.102

[a,b,c] Means within row with different superscripts differ (P<0.05)
[1]T1 = Control, T2 = 170 g SBO/d, T3 = 170 g oil from WCS/d
ND = Not detected. A value of 0 was used for statistical analyses
SEM = Standard error of mean

Table 3.13 Effect of soybean oil (SBO) and whole cottonseed (WCS) on fatty acid composition in rumen digesta at 6 h post feeding

Items	Treatments[1]			SEM	P-value
	T1	T2	T3		
	----- % of total fatty acid -----				
C 12:0	18.96[a]	8.55[ab]	0.41[b]	1.82	0.036
C 14:0	11.66[a]	6.91[b]	1.57[c]	0.32	0.004
C 16:0	22.78	21.25	30.40	1.76	0.114
C 18:0	36.28	38.63	21.84	5.39	0.259
C 18:1	7.02	23.65	11.39	2.85	0.082
C 18:2	1.17[b]	0.62[b]	34.39[a]	1.86	0.009
C 20:0	0.77	ND	ND	0.22	0.200
cis-9, *trasns*-11CLA	ND	0.39	ND	0.22	0.500
Other	1.35	ND	ND	0.40	0.500

[d,e,f] Means within row with different superscripts differ (P<0.01)
[1]T1 = Control, T2 = 170 g SBO/d, T3 = 170 g oil from WCS/d
ND = Not detected. A value of 0 was used for statistical analyses
SEM = Standard error of mean

3.6 Conclusion

This present study demonstrated that supplementation of SBO which rich in C18:2 linoleic acid increased in C18:2 *cis*-9, *trans*-11 CLA content of muscle tissue. Although WCS is also rich in C18:2 linoleic acid, However, its supplementation did not affect accumulation of CLA in muscle lipid. Supplementation of SBO did not influence on performances (final BW, ADG, and DMI) and carcass quality (meat color and shear force) of the fattening cattle. Ruminal fermentation parameters such as pH, ammonia N, protozoa population and VFA concentration were not affected among treatments. Moreover, C18:2 *cis*-9, *trans*-11 CLA content in rumen digesta was negligibly detected which SBO supplemented while those in control and WCS supplemented were not detected. It can be clearly concluded that SBO addition to fattening cattle's diets was superior to WCS in accumulation of CLA in beef.

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CHAPTER IV

THE STUDY OF SOYBEAN OIL AND RUMENPROTECTED CONJUGATED LINOLEIC ACID SUPPLEMENTATION ON PERFORMANCES, MILK PRODUCTION, MILK COMPOSITION AND CLA ACCUMULATION IN MILK

4.1 Abstract

The effects of feeding soybean oil (SBO) and rumen protected conjugated linoleic acid (RP-CLA) on CLA accumulation in milk, performances, milk production and milk composition of dairy cows were studied. Twenty four Holstein Friesian crossbred (>87.5% Holstein Friesian) lactating dairy cows (averaged 126±45 days in milk) were stratified randomly assigned in a randomized complete block design (RCBD) experiment. The treatments were control diet, 150 g of SBO and 150 g of RP-CLA supplementation in the diet. Performance parameters showed that dry matter intake (DMI), net energy for lactation (NE_{LP}) intake and body weight change were similar across treatments ($P<0.05$), while crude protein intake (CPI) was decreased by SBO and RP-CLA supplementation. Milk yield and milk composition were not significantly different among treatments ($P<0.05$), However, milk fat percentage and fat yield that were significantly decreased by 27% ($P<0.05$) from SBO and by 28%

(P<0.01) by RP-CLA supplements compared with control diet. Feeding RP-CLA reduced 3.5%FCM compared with the other treatments (P<0.01). Both SBO and RP-CLA supplementation reduced <C16:0 fatty acids but increased > C16:0 and CLA concentration in milk fat. Addition of SBO and RP-CLA did not significantly affect ruminal pH, ammonia N, protozoa population and volatile fatty acid (VFA) production. Fatty acids in rumen digesta were not altered by treatments. However, CLA isomers particularly *cis*-9, *trans*-11 CLA in rumen digesta were increased by RP-CLA compared with other treatments.

4.2 Introduction

Conjugated linoleic acid (CLA), a mixture of positional and geometric isomers of octadecadienoic acid with conjugated double bonds, have been demonstrated to have a range of potent health effects, including suppression of carcinogenesis (Ip et al., 1999; Belury, 2002; Corl et al., 2003), antibioses effect (Park et al., 1997), modulation of the immune system (Cook et al., 1993), reductions in atherosclerosis (Nicolosi et al., 1997), diabetes (Houseknecht et al., 1998) and decreased body fat mass in human (Blankson et al., 2000; Gaullier et al., 2005). Animal products from ruminants, particularly dairy products are the main dietary source of CLA. It is accepted that CLA are intermediates in the biohydrogenation of linoleic acid, which originated from the incomplete biohydrogenation of unsaturated fat by rumen function (Bauman et al., 1999). However, research work found that cows can also synthesis CLA from *trans*-11 octadecadienoic acid, another intermediate in the rumen biohydrogenation process by Δ^9 desaturase in tissue (Griinari et al., 1998; Corl et al., 2001).

Plant oils and oil seeds rich in linoleic acid supplementation in the diet showed an increase of CLA in milk fat of cows (Kelly et al., 1998; Leonardi et al., 2005; Loor et al., 2005; Zheng et al., 2005; Shingfield et al., 2006; Bu et al., 2007) and RP-CLA also showed similar trend (Perfield et al., 2002; Perfield et al., 2004; Piperova et al., 2004; Castaneda-Gutierrez et al., 2005). Recently, comparison between oils and RP-CLA supplementation in dairy cows is very limited. The aim of the present study is to compare soybean oil (SBO) and rumen protected conjugated linoleic acid (RP-CLA) supplementation in dairy cow on CLA accumulation in milk fat and performance of lactating dairy cows.

4.3 Objective

The objective of this experiment was to determine the effect of soybean oil and rumen protected conjugated linoleic acid (RP-CLA) supplementation on performance, pH, volatile fatty acid, ammonia N, protozoa and CLA accumulation of cow's milk and rumen digesta.

4.4 Materials and methods

Experiment 1

Animals and treatments

The experiment was conducted at The Suranaree University of Technology dairy farm. Twenty four Holstein Friesian lactating dairy cows (n=24) that averaged 126±45 days in milk (DIM) were allotted in a Randomized Complete Block Design (RCBD) experiment. The average milk production and body weight of cows were

15.6±2.43 kg and 452±51 kg, respectively. Cows were randomly divided into two block base on DIM. Cows within each block were randomly assigned to three treatments of 8 cows. Each treatment group received SBO and RP-CLA (BASF Thailand Co., Ltd) supplement that was top-dressed once daily on their concentrate. After the adjustment period, cows were assigned to three treatments. The first treatment was control diet (T1), control diet plus 150 g of SBO (T2) and control diet plus 150 g of RP-CLA (T3) per cow per day, respectively.

All cows were individually fed concentrate and received *ad libitum* grass silage. All cows were housed in a free-stall unit and had free-choice access to water. The experiment lasted for 40 days (8 periods of 5 d), with the first 2 periods (10 days) was the adjustment period, and followed by 30 days (6 periods) of measurement period.

Measurements, Sample Collection, and Chemical Analysis

Feeds offered and left after eating of individual cow was weighed on two consecutive days of each period. Feed samples were taken and dried at 60^{o}C for 48 hours. At the end of the experimental period, feed samples were mixed and sub samples were taken for further chemical analysis. Feed samples were ground through 1 mm screen and subjected to proximate analysis. The crude protein (CP) was determined by Kjeldahl analysis (AOAC, 1995). Ether extract (EE) was determined using petroleum ether in a Soxtec System (AOAC, 1995). Neutral detergent fiber (NDF) and Acid detergent fiber (ADF) was determined using the method described by Van Soest et al. (1991), adapted for Fiber Analyzer. Chemical analysis was expressed on the basis of this final DM. Fatty acids composition of concentrates and grass silages were determined by Gas chromatography (Hewlett Packard GC system HP 6890).

Cows were weighed at the start and at the end of the experiment. Cows were milked twice daily at 05.00 and 15.00 h and milk yields were recorded for each cow. Sample of milks (evening+morning) were collected at each milking for two consecutive days in each period and stored at 4^{o}C with a preservative until analyzed for fat, protein, lactose and solid not fat content using a Milko-Scan S50 analyzer (Tecator, Denmark). All cows were weighed at the start and at the end of the experiment. In addition, milk samples were collected on day 0, 10, 20 and 30 of the experiment and stored at -20^{o}C until analyzed free fatty acids and CLA analyses (Gas chromatography; Hewlett Packard GC system HP 6890).

Analysis of fatty acids by Gas chromatography (GC)

Feeds were extracted using a modified of the method used by Folch et al. (1957) and Metcalfe et al. (1966) (See in chapter III). Milk samples were collected from individual cow on day 0, 10, 20 and 30 of the experiment. Milk samples of each period were centrifuged to fat cake and extraction. Lipid extraction was that of the procedures described by Hara and Radin (1978), using a volume of 18 ml of hexane and isopropanol (3:2, vol/vol)/g of fat cake. After vortexing, a sodium sulfate solution (6.7% $NaSO_4$ in distilled H_2O) was added at a volume of 12 ml/g of fat cake. The hexane layer was transferred to a tube containing 1 g of $NaSO_4$ and after 30 min, the hexane layer was removed and stored at –20°C until methylation. Fatty acid methyl esters (FAME) were prepared by procedure described by Ostrowska et al. (2000) (See in chapter III) and analyzed by gas chromatography.

Experiment 2

Three fistulated non-lactating dairy cows were used in 3x3 Latin Square Design. Concentrate mixes included control diet, control diet with 150 g/d SBO and

control diet with 150 g/d RP-CLA. Experimental periods were 14 d duration with 12 d for diet adaptation and 2 d for sample collection. At the end of each period, rumen digesta was sampled during the experiments from each fistulated non-lactating dairy cows at 0 (prefeeding), 2, 4 and 6 h post feeding on d 13 and 14 of each period. Rumen digesta were crushed through nylon cloth. Rumen fluid samples were analyzed for pH, VFA, ammonia N, protozoa count and rumen digesta fatty acid.

The pH of rumen fluid was determined at the time of sampling by pH meter. Ruminal volatile fatty acids (VFA) and ammonia N were determined in rumen fluid samples taken on 20 ml of rumen fluid was combined with 5 ml 6N HCl to freezing for analysis of VFA and ammonia N. Later the samples were thawed at 4^{o}C and centrifuged at 3,000 rpm for 15 min. The supernatant fluid was analyzed ammonia N by Kjeldahl and concentrations of VFA were determined by GC. Protozoa populations were counted by Hematocytometer in rumen fluid samples which preserved with 10% formal saline solution.

Statistical Analysis

Measurements of DMI, milk production and milk fatty acid composition were analyzed by repeated measures ANOVA for a Randomized Completely Block Design, all data in experiment 2 were analyzed as 3x3 Latin Square Design using Statistical Analysis System (SAS, 1996).

Experimental location

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

Experimental period

The experiment was from August 2006 to February 2007.

4.5 Result and Discussion

Experiment 1

Feed composition and performances

Chemical and fatty acid compositions of feed used in the experiment are presented in Table 4.1 and Table 4.2, respectively. This diets were control diet, the diet supplemented with 150 g/day SBO and 150 g of RP-CLA/day and grass silage. The EE content and energy values of control diet were lower than the SBO and RP-CLA supplementation diets. Control diet had higher C12:0 and C14:0 than other diets, while SBO supplemental diet was rich in C18:2 and C18:3. RP-CLA diet contained 2.59 % total CLA of total fatty acids. The average values for production parameters are presented in Table 4.3. Intake of fatty acids are showed in Table 4.4. Dry matter (DM) and net energy for lactation (NE_{LP}) intakes of the experimental cows were similar among treatments and averaged 13.62 kg/d and 17.86 Mcal/d, respectively. However, CP intake was significantly higher ($P<0.01$) in cows received control diet than SBO and RP-CLA diets (Table 4.3).

Similar results were previously reported when oils were supplemented (Dhiman et al., 2000; Loor et al., 2005; Zheng et al., 2005) and with RP-CLA (Chouinard et al., 1999; Giesy et al., 2002; Perfield et al., 2002; Bernal-Santos et al., 2003; Moore et al., 2004; Piperova et al., 2004; Perfield et al., 2004; Castaneda-Gutierrez et al., 2005; De Veth et al., 2005). The present study showed no significant difference in DMI, although there was a trend towards a reduction in grass silage and total DMI due to SBO and RP-CLA addition. High oil addition in the diet limited

DMI in previous studies (Gagliostro and Chilliard, 1991; Litherland et al., 2005; Shingfield et al., 2006). For example, Shingfield et al. (2006) reported a decrease in DMI by 20.5% according to 45 g FSO (fish oil and soybean oil mix, 1:2; wt/wt) supplementation. Supplementation of diets with oils rich in polyunsaturated fatty acids (PUFA) often results in reduction in nutrient intake or might be related in part to possible negative effects of unsaturated oils in the diet on rumen function (Jenkins, 1993).

Table 4.1 Chemical composition of the experimental diets

Items	Treatments[1]			Grass silage
	T1	T2	T3	
	---------- % of DM ----------			
Dry matter	88.03	90.18	89.19	31.11
Ash	7.58	7.56	8.21	8.17
Crude protein	23.57	22.16	22.00	3.95
Ether extract	2.86	4.59	4.04	1.65
Crude fiber	12.53	12.52	12.38	39.88
Neutral detergent fiber	46.98	48.54	42.17	81.41
Acid detergent fiber	22.77	21.42	21.89	52.61
Acid detergent lignin	7.27	5.57	6.56	7.95
TDN_{1x} (%)[2]	59.23	63.48	63.12	45.56
DE_{1x} (Mcal/kgDM)[3]	2.77	2.93	2.92	1.93
DEp (Mcal/kgDM)[4]	2.74	2.83	2.82	2.11
MEp (Mcal/kgDM)[5]	2.31	2.41	2.40	1.68
NE_{lp} (Mcal/kgDM)[6]	1.44	1.51	1.50	0.99

[1]T1 =Control, T2 = 150 g SBO/d, T3 = 150 g RP-CLA/d
[2]Total digestible nutrients, TDN_{1X} (%) = tdNFC + tdCP + (tdFA x 2.25) + tdNDF – 7 (NRC, 2001)
[3]Digestible energy, DE_{1X} (Mcal/kg) = [(tdNFC/100)x4.2]+[(tdNDF/100) x 4.2]+[(tdCP/100) x 5.6]+[(FA/100) x 9.4] –0.3
[4]DE_P (Mcal/kgDM) = DE_{1X} x Discount (NRC, 2001)
[5]Metabolisable energy, ME_p = [1.01 x (DE_p) – 0.45] + [0.0046 x (EE – 3)] (NRC, 2001)
[6]Net energy for lactation, NE_{lp} = ([0.703 x ME_p (Mcal/kg)] – 0.19) + ([(0.097 x ME_p + 0.19)/97] x [EE – 3]) (NRC, 2001)

Table 4.2 Fatty acid compositions of concentrate, soybean oil (SBO) and rumen protected conjugated linoleic acid (RP-CLA)

ems	Treatments			Grass
	Concentrate	**SBO**	**RP-CLA**	**silage**
	----------% of total fatty acid----------			
C 12:0	25.86	0.01	0.10	5.29
C 14:0	9.42	0.08	0.33	3.52
C 16:0	13.03	10.42	21.17	32.26
C 18:0	2.87	3.57	44.63	10.74
C 18:1	21.48	19.01	24.40	6.40
C 18:2	22.81	57.82	0.58	23.21
C 18:3	-	7.67	-	-
C 20:1	-	-	0.18	18.60
Other[1]	4.53	1.43	6.02	-
cis-9, *trans*-11CLA	-	-	1.15	-
trans-10, *cis*-12 CLA	-	-	1.04	-
trans-9, *trans* -11 CLA	-	-	0.40	-
Total CLA[2]	-	-	2.59	-

[1]Other = (Sum of C6:0, C8:0, C10:0,C16:1, C17:1, C20:1,C20:2, C22:0, C20:3n6, C22:1n9, C20:3n3, C23:0, C20:5n3, C24:1)
[2]Total CLA = (Sum of *cis*-9,*trans*-11 CLA; *trans* -10, *cis* -12 CLA; *trans* -9, *trans* -11 CLA)
CLA = Conjugated linoleic acid (*cis-9, trans-11 octadecadienoic acid*)
SBO = Soybean oil, RP-CLA = rumen protected conjugated linoleic acid

Table 4.3 Effect of soybean oil (SBO) and rumen protected conjugated linoleic acid (RP-CLA) on nutrient intake of lactating cows

Items	Treatments[1]			SEM	P-value
	T1	T2	T3		
Dry matter intake (kg/d)					
Concentrate	8.91	8.91	8.91	-	-
Grass silage	5.07	4.49	4.52	0.30	0.448
Total	13.98	13.40	13.44	0.30	0.451
DMI, g/kg $BW^{0.75}$	144.27	144.93	138.83	2.91	0.339
Crude protein intake (g/d)					
Concentrate	2,100	1,975	1,961	-	-
Grass silage	211	195	196	9.29	0.443
Total	2,311[d]	2,170[e]	2,157[f]	9.58	0.001
Ether extract intake (g/d)					
Concentrate	255	409	360	-	-
Grass silage	84	78	75	4.51	0.436
Total	338[f]	487[d]	435[e]	4.52	0.001
NE_{LP} intake (Mcal/d)					
Concentrate	12.83	13.45	13.37	-	-
Grass silage	5.02	4.44	4.48	0.38	0.274
Total	17.85	17.89	17.84	0.27	0.988

[d,e,f] Means within row with different superscripts differ (P<0.01)
[1]T1 =Control, T2 = 150 g SBO/d, T3 = 150 g RP-CLA/d
SEM = Standard error of mean

Table 4.4 Intake of individual fatty acids

Items	**Treatments**[1]		
	T1	**T2**	**T3**
	---------- g/day ----------		
C12:0	70.32	70.12	69.94
C14:0	26.93	26.89	26.95
C16:0	60.17	70.63	78.43
C18:0	16.30	19.89	59.95
C18:1	60.09	81.09	83.91
C18:2	77.53	141.28	76.02
C18:3	ND	8.57	ND
cis-9, *trans*-11CLA	ND	ND	1.15
trans-10, *cis*-12 CLA	ND	ND	1.04
trans-9, *trans* -11 CLA	ND	ND	0.40
Total CLA	ND	ND	2.59

[1]T1 =Control, T2 = 150 g SBO/d, T3 = 150 g RP-CLA/d
ND = Not detected. A value of 0 was used for statistical analyses

Milk yield and milk composition

Milk yield and composition data are presented in Table 4.5. There were no significant differences in milk, protein, lactose and SNF yields (P>0.05). However, 3.5% FCM, fat yield and total solid yield were significantly reduced (P<0.01) when RP-CLA was supplemented. Milk compositions were unaffected by SBO and RP-CLA addition except for fat percentage, which was significantly decreased (P<0.05) when RP-CLA was added. The reduction in 3.5% FCM yield and total solid yield reflects the depression in milk fat percentage and yield. Many previous studies showed no difference in milk yield (Chouinard et al., 1999; Dhiman et al., 2000; Giesy et al., 2002; Perfield et al., 2002; Bernal-Santos et al., 2003; Moore et al., 2004; Piperova et al., 2004; Perfield et al., 2004; Castaneda-Gutierrez et al., 2005, De Veth et al., 2005). Moreover, Zheng et al. (2005), also reported that milk yield was unaffected when cows received oils from cottonseed, soybean and corn. However, Leonardi et al. (2005) found that milk yield was significantly increased (P<0.005) by 5.8% with 1.5% corn oil supplementation.

In the present study, addition of 1.5% SBO was not affected in milk fat percentage and yield (P>0.05). Similar to Dhiman et al. (2000) who reported that, supplemented with SBO at 1 to 2 % in the diet DM, while milk fat percentage was decreased when supplemented with 3 to 4 % SBO. Moreover, milk fat percentage and yield were significantly decreased (P<0.05) by 27% and 28% (P<0.01), respectively, due to RP-CLA supplementation. Similar results were reported by Chouinard et al. (1999), Giesy et al. (2002), Perfield et al. (2002), Bernal-Santos et al. (2003), Moore et al. (2004), Piperova et al. (2004), Perfield et al. (2004), Castaneda-Gutierrez et al. (2005) and De Veth et al. (2005). Chouinard et al. (1999) who reported that infusion of the

CLA (contained 61.2% CLA, the major CLA isomers were *cis*-8, *trans*-10, *cis*-9, *trans*-11, *cis*-10, *trans*-12 and *cis*-11, *trans*-13) reduced milk fat content and yield by 52 and 55%, respectively. Similarly, Giesy et al. (2002) showed that supplementation of 100 g CLA-60 in calcium salt form reduced milk fat percentage by 34%, because milk fat depression (MFD) was induced by supplementing cows with a source of CLA isomers in the form that reduce biohydrogenation by ruminal microorganisms. Bernal-Santos et al. (2003) summarized that feeding fat supplements (90 g/d of fatty acids) consisting of Ca salt of either palm fatty acid dilsitillated (control) or mixture of control and mixed isomer of CLA (CLA 30.4 g/d), resulted in reducing milk fat percentage by 12.5% during early lactation ($P<0.001$) and milk fat yield was reduced by only 7.5% ($P<0.11$) because of the increased milk yield. De Veth et al. (2005) reported that RP-CLA supplementation reduced milk fat yield and fat content compared with control ($P<0.01$).

The reduction in milk fat due to CLA isomer supplementation demonstrated that the *trans*-10, *cis*-12 CLA isomer inhibited milk fat synthesis in dairy cow (Baumgard et al., 2000; 2001), whereas the *cis*-9, *trans*-11 CLA had no effect. The mechanism by which *trans*-10, *cis*-12 CLA alters lipid mechanism involving many aspects of milk fat synthesis. Specifically, this CLA isomer dramatically reduced the mammary gland's lipogenesis (rates of acetate incorporation into fatty acids) and decreased the expression of genes encoding enzyme (mRNA abundance of acetyl CoA carboxylase) involved in the uptake and transport of circulating fatty acids *de novo* fatty acid synthesis, desaturation of fatty acids and formation of triglycerides, as found in mice (Baumgard et al., 2000; 2001; Piperova et al., 2000). Lin et al. (2004) also indicated that reduced lipogenesis in the mammary gland of lactating mice was caused

by reducing acetyl CoA carboxylase activity and mRNA abundance of acetyl CoA carboxylase, the critical enzyme in *de novo* fatty acid synthesis, and also inhibited mammary desaturation by reducing mammary stearoyl-CoA desaturase activity and mRNA abundance. In the present study reduction in milk fat was similar to that reported by Peterson et al. (2003), who indicated that *trans*-10, *cis*-12 CLA decreased mRNA abundance for key enzyme involved in the production of milk fat.

Table 4.5 Effect of soybean oil (SBO) and rumen protected conjugated linoleic acid (RP-CLA) on milk yield and milk composition of lactating cows

Items	**Treatments[1]**			**SEM**	**P-value**
	T1	**T2**	**T3**		
Initial body weight, kg	452	454	450	19.76	0.993
Final bogy weight, kg	448	428	449	16.41	0.627
Body weight change, kg/d	-0.11	-0.86	-0.02	0.52	0.524
Milk yield, kg/d	15.16	16.05	14.46	0.98	0.518
3.5%FCM yield, kg/d	14.87[d]	16.07[d]	12.02[e]	0.58	0.001
Milk composition, kg/d					
Fat yield	0.51[d]	0.57[d]	0.37[e]	0.02	0.001
Protein yield	0.39	0.44	0.43	0.02	0.086
Lactose yield	0.62	0.67	0.65	0.03	0.611
Solid not fat (SNF)	1.20	1.30	1.18	0.07	0.383
Total solid	1.70[ab]	1.86[a]	1.56[b]	0.07	0.028
Milk composition, %					
Fat	3.49[a]	3.62[a]	2.55[b]	0.25	0.015
Protein	2.63	2.75	2.89	0.11	0.267
Lactose	4.14	4.2	4.36	0.09	0.208
Solid not fat (SNF)	7.94	8.14	8.16	0.14	0.457
Total solid	11.43	11.74	10.8	0.38	0.224

[a,b,c] Means within row with different superscripts differ (P<0.05)
[d,e,f] Means within row with different superscripts differ (P<0.01)
[1]T1 =Control, T2 = 150 g SBO/d, T3 = 150 g RP-CLA/d
3.5 % FCM (Fat Corrected Milk) = (0.432 x kg of Milk) + (16.2 x kg of Fat)
SEM = Standard error of mean

Fatty acids composition in milk

Fatty acids composition of milk fat are presented in Table 4.6. The proportions of fatty acid which had carbon less than 14 (≤C14:0) (C4:0, C6:0, C8:0, C10:0, C12:0 and C14:0) in milk fat were significantly decreased (P<0.01) by supplementation with SBO and RP-CLA. However, C16:0 was not altered across treatments. Fatty acids, C18:0, C18:1 and C18:2 in milk fat were significantly increased (P<0.01) by SBO supplementation in diet. Short- and medium-chain fatty acids were reduced when cows received both SBO and RP-CLA supplementation. This resulted in decreasing (P<0.01) the proportions of *de novo* (≤C16:0) fatty acids and increasing (P<0.01) in preformed (>C16:1) fatty acids in milk fat. Similar patterns to shift these fatty acids were also observed with oils (Dhiman et al., 2000; Kay et al., 2004; Zheng et al., 2005; Shingfield et al., 2006; Bu et al., 2007).

The addition of dietary SBO significantly increased (P < 0.01) *cis*-9, *trans*-11 CLA concentration by 65% and 38% in milk fat when compared with control and RP-CLA treatments, respectively. RP-CLA significantly increased (P<0.01) *trans*-10, *cis*-12 CLA concentration compared with control and SBO treatments. The increase in *trans*-10, *cis*-12 CLA concentration in milk fat caused milk fat depression (Table 4.4). However, total CLA concentration was significantly increased by SBO and RP-CLA addition. In the present study, increase in *cis*-9, *trans*-11 CLA was due to a high linoleic acid and linolenic acid in SBO (Table 4.1). The *cis*-9, *trans*-11 CLA in milk fat was probably formed by incomplete biohydrogenation of dietary linoleic acid in rumen and by *trans*-11 C18:1 vaccenic acids (the intermediate in biohydrogenation of linoleic acid, linolenic acid and oleic acid) which can endogenous synthesize *cis*-9, *trans*-11 CLA via Δ^9 desaturase in mammary gland (Corl et al., 2001).

As confirmed by Dhiman et al. (2000), who discussed that feeding lipid sources rich in linoleic acid and linolenic acid increased the CLA concentration in milk fat by 77% and 188% according to 2.0% and 4.0% SBO addition, respectively. Leonardi et al. (2005) showed that cows fed fish oil and sunflower oil increased total CLA concentration by 42 and 594%, respectively. Shingfield et al. (2006) summarized that concentrations of *cis*-9, *trans*-11 CLA in milk fat was increased by dietary supplementation with fish and sunflower oil in the diet. As reviewed by Zheng et al. (2005), they reported that cows fed with 5% SBO increased the *cis*-9, *trans*-11 CLA by 97%, while increased by 318% also found when fed with 4% SBO (Bu et al., 2007)

Previous studies with RP-CLA supplementation, Chouinard et al. (1999) reported that infusion of the CLA at 50, 100 and 150 g/d increased ($P<0.01$) the CLA content of milk fat by 246, 585 and 835%, respectively. Similarly, Perfield et al. (2004) found that both *cis*-9, *trans*-11 CLA and *trans*-10, *cis*-12 CLA were increased by the amide protected CLA (AP-CLA) and lipid encapsulated CLA (LE-CLA) supplements. Calcium salts of CLA (Ca-CLA) addition also increased *cis*-9, *trans*-11 CLA and *trans*-10, *cis*-12 CLA concentration in milk fat (Giesy et al., 2002). As reviewed by Moore et al. (2004), concentration of total CLA in milk fat were linearly increased by dietary RP-CLA supplementation, while Viswanadha et al. (2003) showed that the proportion of *trans*-10, *cis*-12 CLA was linearly increased ($P<0.05$) by dose of CLA, but *cis*-9, *trans*-11 CLA concentration was not different, which is similar to Bernal-Santos et al. (2003).

Moreover, Piperova et al. (2004) reported that the proportion of total CLA and *trans*-10, *cis*-12 CLA were increased ($P<0.01$) by 60 and 164% with Ca-CLA treatment,

respectively, while *cis*-9, *trans*-11 CLA concentration in milk fat was decreased ($P<0.01$) by 12%. De Veth et al. (2005) showed that the Ca-CLA and formaldehyde-protected CLA (FP-CLA) supplementation in the diet increased *trans*-10, *cis*-12 CLA concentration in milk fat, similar to Castaneda-Gutierrez et al. (2005), who reported that the proportion of *cis*-9, *trans*-11 CLA and *trans*-10, *cis*-12 CLA were increased 12% and 1,425% by Ca-CLA treatment, respectively.

Table 4.6 Effect of soybean oil (SBO) and rumen protected conjugated linoleic acid (RP-CLA) on fatty acid composition in milk fat of lactating cows

Items	**Treatments**			**SEM**	**P-value**
	T1	**T2**	**T3**		
	------% of total fatty acid------				
C4:0	1.85[a]	1.56[b]	1.63[b]	0.07	0.012
C6:0	1.39[d]	1.13[e]	0.76[f]	0.06	0.001
C8:0	0.89[d]	0.68[e]	0.44[f]	0.05	0.001
C10:0	1.95[a]	1.48[b]	1.06[c]	0.13	0.001
C11:0	0.27[a]	0.18[b]	0.10[c]	0.02	0.001
C12:0	6.80[d]	5.42[e]	5.43[e]	0.24	0.001
C13:0	0.23[d]	0.16[e]	0.15[e]	0.01	0.002
C14:0	11.51[a]	10.00[b]	10.11[b]	0.38	0.018
C14:1	1.61[a]	1.17[b]	1.29[b]	0.10	0.012
C15:0	0.74	0.65	0.69	0.04	0.370
C16:0	28.13	25.86	26.49	0.96	0.249
C16: 1	2.90[a]	2.41[b]	2.65[ab]	0.19	0.052
C17: 1	0.25	0.25	0.22	0.02	0.367
C18:0	7.35[e]	10.16[d]	9.80[d]	0.52	0.002
C18:1	30.29[b]	33.69[a]	35.09[a]	1.19	0.029
C18:2	2.19[b]	2.89[a]	2.26[b]	0.19	0.037
C18:3	0.17[ab]	0.20[a]	0.13[b]	0.02	0.044
C20:0	0.12[e]	0.15[d]	0.15[d]	0.01	0.004

Table 4.6 Effect of soybean oil (SBO) and rumen protected conjugated linoleic acid (RP-CLA) on fatty acid composition in milk fat of lactating cows (Cont.)

Items	**Treatments**[1]			**SEM**	**P-value**
	T1	**T2**	**T3**		
	------% of total fatty acid------				
Others[2]	0.54[d]	0.57[d]	0.37[e]	0.03	0.001
cis-9,*trans*-11 CLA	0.80[e]	1.32[d]	0.96[e]	0.08	0.001
trans -10, *cis* -12 CLA	0.0025[e]	0.0075[e]	0.1386[d]	0.01	0.001
trans -9, *trans* -11 CLA	0.0300[e]	0.0863[d]	0.0925[d]	0.01	0.005
Total CLA[3]	0.83[e]	1.41[d]	1.19[d]	0.08	0.001
Summation by source[4]					
<C16:0	27.24[d]	22.43[e]	21.66[e]	0.80	0.001
C16:0 and C16:1	31.04	28.27	29.14	0.95	0.137
>C16:0	41.73[e]	49.30[d]	49.20[d]	1.27	0.005
Saturated fatty acid	61.11	57.28	56.66	1.47	0.095
Unsaturated fatty acid	38.89	42.72	43.34	1.47	0.095

[a,b,c] Means within row with different superscripts differ (P<0.05)
[d,e,f] Means within row with different superscripts differ (P<0.01)
[1]T1 =Control, T2 = 150 g SBO/d, T3 = 150 g RP-CLA/d
[2]Other = (Sum of C20:1,C20:2, C22:0, C20:3n6,C22:1n9+C20:3n3, C20:4n6, C20:5n3)
[3]Total CLA = (Sum of *cis*-9,*trans*-11 CLA; *trans* -10, *cis* -12 CLA; *trans* -9, *trans* -11 CLA)
[4]Fatty acids : <C16:0 represent de novo synthesized fatty acids, >C16:0 represent preformed fatty acids taken up from circulation, C16:0 fatty acids are derived from both sources
SEM = Standard error of mean

Experiment 2

Ruminal fermentation

Supplementation of SBO and RP-CLA had no significant effect (P>0.05) on ruminal pH, NH_3-N and protozoa population at 2, 4 and 6 hours post-feeding (Table 4.7). The concentrations of ruminal acetate and propionate and acetate to propionate ratio (A:P ratio) were not significantly different (P>0.05) among treatments (Table 4.8). However, A:P ratio at 2 h post-feeding, showed that RP-CLA supplementation had higher A:P ratio than other treatments. Kim et al. (1993), fed control diet and extruded soybeans and Ca soaps of fatty acid (Ca-LCFA) diets had lower concentrations of total VFAs (P<0.05) than when fat sources were fed. Supplementation of high fat diet had no significantly effect (P>0.05) on pH and NH_3-N, but increased (P<0.05) butyrate content compared with low fat diet (Chan et al., 1997). Ueda et al. (2003) noted that ruminal fluid pH and total VFA concentration did not affect (P>0.05) by 3% linseed oil (LSO) supplementation. Harvatine and Allen (2006) reported that fatty acid supplements decreased (P<0.05) ruminal VFA concentration and changed VFA by decreasing acetate and increasing propionate concentrations (P<0.01).

.

Table 4.7 Effect of soybean oil (SBO) and rumen protected conjugated linoleic acid (RP-CLA) on pH, NH_3-N and protozoa population

Items	**Treatments**[1]			**SEM**	**P-value**
	T1	**T2**	**T3**		
pH					
0 h	7.00	7.04	6.96	0.08	0.412
2 h	6.84	6.69	6.62	0.17	0.281
4 h	6.45	6.47	6.30	0.30	0.634
6 h	6.57	6.45	6.40	0.22	0.505
NH_3-N (mg/dl)					
0 h	9.65	9.15	10.50	1.88	0.561
2 h	12.04	12.88	13.57	0.74	0.133
4 h	10.48	9.55	10.50	0.74	0.238
6 h	8.18	8.48	8.50	1.22	0.884
Protozoa (x 10^5 cells/ml)					
0 h	1.21	3.00	1.58	1.10	0.352
2 h	1.58	2.58	1.63	0.65	0.395
4 h	1.83	3.04	1.58	0.59	0.089
6 h	1.58	1.88	2.38	0.59	0.271

[1]T1 =Control, T2 = 150 g SBO/d, T3 = 150 g RP-CLA/d
SEM = Standard error of mean

Table 4.8 Effect of soybean oil (SBO) and rumen protected conjugated linoleic acid (RP-CLA) on volatile fatty acids

Items	Treatments[1]			SEM	P-value
	T1	T2	T3		
VFA, mol/100 mol					
Acetate, C2					
0 h	63.42	55.37	62.15	2.21	0.206
2 h	65.89[b]	65.11[b]	70.20[a]	0.68	0.057
4 h	65.68	66.04	68.76	0.47	0.072
6 h	66.85	65.97	69.07	0.98	0.274
Propionate, C3					
0 h	18.37	18.10	18.07	0.16	0.502
2 h	18.82[a]	18.71[a]	17.70[b]	0.14	0.049
4 h	18.09	18.02	17.42	0.41	0.550
6 h	17.61	17.98	17.24	0.17	0.164
Butyrate, C4					
0 h	14.01[ab]	15.57[a]	12.67[b]	0.35	0.056
2 h	15.30	16.18	12.10	0.59	0.071
4 h	16.23	15.94	13.83	0.76	0.248
6 h	15.54	16.04	13.69	0.92	0.353
Acetate : Propionate					
0 h	3.46	3.06	3.44	0.14	0.277
2 h	3.51[b]	3.49[b]	3.97[a]	0.06	0.047
4 h	3.64	3.67	3.95	0.06	0.122
6 h	3.80	3.68	4.01	0.08	0.167

[a,b,c] Means within row with different superscripts differ ($P<0.05$)
[1]T1 =Control, T2 = 150 g SBO/d, T3 = 150 g RP-CLA/d
VFA = volatile fatty acid
SEM = Standard error of mean

Fatty acid composition of rumen digesta

The objective of this study was to determine the effect of SBO and RP-CLA on ruminal fatty acid in rumen digesta. Biohydrogenation of C18:2 linoleic acid involves an initial isomerization of cis-12 double bond to a *trans*-11 bond, forming *cis*-9, *trans*-11 CLA. Microbial reductase hydrogenates the *cis*-9 bond, resulting in formation of *trans* C18:1 vaccenic acid, which in turn is reduced to C18:0 stearic acid.

The proportions of fatty acids in rumen digesta are presented in Table 4.9, 4.10, 4.11 and 4.12. Most fatty acids were not altered ($P>0.05$) by treatments. However, CLA isomers in rumen digesta particularly *cis*-9, *trans*-11 CLA were increased by RP-CLA compared with other treatments. Increase in CLA contents in rumen digesta was probably due to the fact that RP-CLA already contains CLA isomers and it was protected from rumen biohydrogenation. The proportion of C12:0, C14:0, C16:0 and C18:1 were decreased or tended to decrease in RP-CLA group. Researches determining ruminal fatty acid profiles were very limited. Abughazaleh et al. (2002), reported that concentration of CLA and *trans* vaccenic acid in ruminal digesta were increased ($P<0.01$) by 2% FO and 2% SBO from extruded soybean. Beam et al. (2000) suggested that the rate of biohydrogenation of the C18:2 linoleic acid in SBO was not affected by the amount of grain or fat fed to the cow, or the time after feeding that ruminal inoculum was collected. The primary factor that affected the rate of lipolysis of SBO and biohydrogenation of C18:2 linoleic acid was the concentration of SBO in the culture substrate. As SBO percentage in the substrate increased, the rates of lipolysis and biohydrogenation of C18:2 linoleic acid both declined (Beam et al., 2000).

Table 4.9 Effect of soybean oil (SBO) and rumen protected conjugated linoleic acid (RP-CLA) on fatty acid composition in rumen digesta at 0 h

Items	**Treatments**[1]			**SEM**	**P-value**
	T1	**T2**	**T3**		
	------% of total fatty acid------				
C 12:0	8.03	4.55	2.98	1.36	0.216
C 14:0	7.62[a]	5.68[b]	2.94[c]	0.29	0.014
C 16:0	24.98	23.03	16.64	1.17	0.066
C 18:0	44.88	44.18	57.80	2.01	0.064
C 18:1	12.26	20.45	11.04	1.38	0.067
C 18:2	1.73	2.11	1.06	0.94	0.756
≥C 20:0	0.51	ND	0.45	0.28	0.500
cis-9, *trasns*-11CLA	ND	ND	0.3067	0.18	0.500
trans-10, *cis*-12 CLA	ND	ND	1.60	0.47	0.500
trans-9, *trans*-11 CLA	<0.01[b]	<0.01[b]	5.18[a]	0.59	0.036
Total CLA	ND	ND	7.09	1.12	0.070

[a,b,c] Means within row with different superscripts differ (P<0.05)
[1]T1 =Control, T2 = 150 g SBO/d, T3 = 150 g RP-CLA/d
ND = Not detected. A value of 0 was used for statistical analyses
SEM = Standard error of mean

Table 4.10 Effect of soybean oil (SBO) and rumen protected conjugated linoleic acid (RP-CLA) on fatty acid composition in rumen digesta at 2 h post feeding

Items	**Treatments**[1]			**SEM**	**P-value**
	T1	**T2**	**T3**		
	------% of total fatty acid------				
C 12:0	10.27	5.40	5.65	1.82	0.305
C 14:0	7.37	5.26	3.69	1.08	0.255
C 16:0	20.54	20.04	16.18	1.55	0.296
C 18:0	33.42	33.84	43.32	5.07	0.450
C 18:1	17.58	20.80	12.66	3.12	0.366
C 18:2	6.50	9.92	2.60	1.90	0.213
≥C 20:0	2.79	1.18	1.23	0.25	0.067
cis-9, *trasns*-11CLA	1.19	2.50	3.26	1.67	0.716
trans-10, *cis*-12 CLA	ND	0.70	4.75	0.90	0.109
trans-9, *trans*-11 CLA	0.36[b]	0.37[b]	6.76[a]	0.65	0.030
Total CLA	1.55	3.57	14.77	3.21	0.168

[a,b,c] Means within row with different superscripts differ (P<0.05)
[1]T1 =Control, T2 = 150 g SBO/d, T3 = 150 g RP-CLA/d
ND = Not detected. A value of 0 was used for statistical analyses
SEM = Standard error of mean

Table 4.11 Effect of soybean oil (SBO) and rumen protected conjugated linoleic acid (RP-CLA) on fatty acid composition in rumen digesta at 4 h post feeding

Items	**Treatments**[1]			**SEM**	**P-value**
	T1	**T2**	**T3**		
	------% of total fatty acid------				
C 12:0	14.63	5.29	10.32	1.84	0.137
C 14:0	9.17[a]	5.90[b]	5.10[b]	0.44	0.040
C 16:0	20.60	20.64	16.29	0.65	0.063
C 18:0	34.86[b]	32.40[b]	40.75[a]	0.75	0.029
C 18:1	14.28	29.35	10.76	2.53	0.061
C 18:2	3.94	3.63	2.93	1.19	0.838
C 18:3	0.69	ND	ND	0.20	0.200
≥C 20:0	0.56	1.59	0.69	0.93	0.736
cis-9, *trasns*-11CLA	ND	1.19	2.82	0.54	0.126
trans-10, *cis*-12 CLA	0.67	ND	3.85	0.54	0.063
trans-9, *trans*-11 CLA	0.58[e]	<0.001[e]	6.51[d]	0.20	0.003
Total CLA	1.25[b]	1.19[b]	13.18[a]	1.10	0.024

[a,b,c] Means within row with different superscripts differ (P<0.05)
[d,e,f] Means within row with different superscripts differ (P<0.01)
[1]T1 =Control, T2 = 150 g SBO/d, T3 = 150 g RP-CLA/d
ND = Not detected. A value of 0 was used for statistical analyses
SEM = Standard error of mean

Table 4.12 Effect of soybean oil (SBO) and rumen protected conjugated linoleic acid (RP-CLA) on fatty acid composition in rumen digesta at 6 h post feeding

Items	**Treatments**[1]			**SEM**	**P-value**
	T1	**T2**	**T3**		
	------% of total fatty acid------				
C 12:0	9.70	3.75	8.53	1.48	0.330
C 14:0	8.11	5.03	4.15	0.29	0.104
C 16:0	23.98[a]	21.54[a]	14.74[b]	0.26	0.050
C 18:0	41.28	31.67	37.39	1.00	0.129
C 18:1	12.90	32.40	19.76	0.04	0.068
C 18:2	4.02[b]	5.60[a]	1.57[c]	1.48	0.016
cis-9, *trasns*-11CLA	ND	ND	2.45	0.11	0.062
trans-10, *cis*-12 CLA	ND	ND	4.14	0.46	0.154
trans-9, *trans*-11 CLA	<0.01[e]	<0.01[e]	7.27[d]	0.04	0.008
Total CLA	<0.01[b]	<0.01[b]	13.86[a]	0.53	0.053

[a,b,c] Means within row with different superscripts differ (P<0.05)
[d,e,f] Means within row with different superscripts differ (P<0.01)
[1]T1 =Control, T2 = 150 g SBO/d, T3 = 150 g RP-CLA/d
ND = Not detected. A value of 0 was used for statistical analyses
SEM = Standard error of mean

4.6 Conclusion

In the present study, both SBO and RP-CLA supplementation in the diet had no affect on DMI and milk production of lactating dairy cows. A reduction of fatty acid with carbon less than 16 (<C16:0) and increased fatty acid with carbon more than 16 (>C16:0) and CLA in milk fat. In addition, RP-CLA reduced 3.5% FCM, milk fat yield, milk fat percentage and total solid yield. Addition of SBO and RP-CLA did not significantly affect ruminal pH, ammonia N, protozoa population and VFA production in rumen ($P>0.05$). Most of fatty acids were not altered by treatments. However, CLA isomers particularly *cis*-9, *trans*-11 CLA were increased by RP-CLA compared with other treatments. Increase in CLA contents in rumen digesta was probably due to the fact that RP-CLA already contains CLA isomers and it was protected from rumen biohydrogenation. This study suggested that SBO supplementation in the diet was better than RP-CLA in accumulation of CLA in dairy cows' milk.

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CHAPTER V

OVERALL CONCLUSION, DISCUSSION AND IMPLICATION

The purposes of the present study were to investigate the effects of sources of lipid rich in linoleic acid, particularly soybean oil (SBO), oil from whole cottonseed (WCS) and rumen-protected conjugated linoleic acid (RP-CLA) on conjugated linoleic acid (CLA) content in beef and milk of cattle. The present studies had successfully.

The first experiment was conducted to determine whether SBO or WCS can increase CLA accumulation in beef. The results showed that accumulations of CLA in both *Longissimus dorsi* (LD) and *Semimembranosus* (SM) muscles were increased by the addition of SBO but not WCS. Many researchers also found higher in CLA content in muscle lipid by supplementing with SBO (Eagle et al., 2000) or with sunflower oil and linseed oil (Mir et al., 2002; Mir et al., 2003; Noci et al., 2007). However, some researches reported no significant differences in CLA content due to SBO supplementation (Beaulieu et al., 2002; Dhiman et al., 2005). The differences in responses to SBO or plant oils were probably due to variations in stage of growth of cattle, levels of oil supplementation, levels of oil in total ration and amount of linoleic acid in oils. The lack of increasing CLA due to WCS supplementation depressed stearoyl-coenzyme A desaturase activity in subcutaneous adipose tissue and liver due to

its cyclopropene fatty acid content in WCS if fed for a sufficiently long period of time (Page et al., 1997). In addition, performance parameters (final BW, DMI, Feed: Gain) and carcass quality (meat color and shear force) were unaffected by SBO and WCS supplementations.

The second experiment was carried out to investigate the effects of SBO or RP-CLA supplementation on CLA content in dairy milk. The results revealed that CLA content of milk was increased by both SBO and RP-CLA. The CLA content was increased by SBO in a greater extent than by RP-CLA. Moreover, RP-CLA reduced 3.5% fat-corrected-milk, milk fat yield and milk fat percentage compared with SBO and control treatments. Other researchers also found increases in CLA content in milk and reductions in milk fat percentage and milk fat yield due to plant oils or RP-CLA supplementation (Chouinard et al., 1999; Dhiman et al., 2000; Giesy et al., 2002; Viswanadha et al., 2003; Bernal-Santos et al., 2003; Moore et al., 2004; Perfield et al., 2004; Piperova et al., 2004; De Veth et al., 2005; Castaneda-Gutierrez et al., 2005; Leonardi et al., 2005; Shingfield et al., 2006; Zheng et al., 2005; Bu et al., 2007). The reduction in milk fat percentage and fat yield reflected the *trans*-10, *cis*-12 CLA isomer in RP-CLA which reduced the mammary gland's lipogenesis (rates of acetate incorporation into fatty acids) and decreased the expression of genes encoding enzyme (mRNA abundance of acetyl CoA carboxylase) involved in the uptake and transport of circulating fatty acids de novo fatty acid synthesis, desaturation of fatty acids and formation of triglycerides, as found in mice (Baumgard et al., 2000; 2001; Piperova et al., 2000). Lin et al. (2004) also indicated that reduced lipogenesis in the mammary gland of lactating mice caused by reducing acetyl CoA carboxylase activity and mRNA abundance of acetyl CoA carboxylase, the critical enzyme in de novo fatty acid

synthesis, and also inhibited mammary desaturation by reducing mammary stearoyl-CoA desaturase activity and mRNA abundance. Peterson et al. (2003) also indicated that *trans*-10, *cis*-12 CLA decreased mRNA abundance for key enzyme involved in the production of milk fat.

Beside the two experiments, rumen digesta were collected from fistulated cows receiving the same treatment feeds as above and CLA isomers in the digesta were detected. From the first experiment, CLA content in rumen digesta was negligibly detected in SBO supplemented cows while those in WCS supplemented and control cows were not detected. At 2, 4 and 6 h post feeding, rumen digesta of WCS supplemented cows contained higher C18:2 linoleic acid and lower C18:0 stearic acid than those SBO supplemented and control group. This suggested that the biohydrogenation in the rumen of WCS supplemented group occurred only little, presumably because of mastication of the seed coat of WCS, thus linoleic acid remained at a high level, resulting in a low level of stearic acid. Since little biohydrogenation occurred in the rumen, level of the *trans*-11 vaccenic acid, intermediate from this process, was also low. Thus, small amount of vaccenic acid was transported to the tissue resulting in little CLA was accumulated in beef. In contrast, at 2, 4 and 6 h post feeding, rumen digesta of SBO supplemented cattles contained lower C18:2 linoleic acid and higher C18:0 stearic acid than those WCS supplemented and control group. This suggested that a large extent of biohydrogenation process occurred in the rumen resulting in a high proportion of vaccenic acid reached the tissue, and thus accumulated CLA in beef. In the second experiment, SBO supplementation showed similar response in CLA concentration in digesta as in the first experiment resulting in accumulation of

CLA in milk fat. However, RP-CLA also increased CLA content in milk fat but in a lesser extent than SBO.

Increases in CLA content in meat and milk can be achieved by supplementing SBO in the present study. WCS did not increase CLA content in beef cattle while RP-CLA increased CLA content in milk with out any effect on milk fat percentage and yield. Milk price in Thailand relies partly on milk fat percentage, thus reduction in milk fat caused by RP-CLA supplementation results in lowering milk income for the farmers. If this is the case, Thai farmers who would like to increase CLA content in milk or meat should supplement with SBO rather than WCS or even RP-CLA.

In the present study, the proportion of CLA concentration accumulated in beef cattle lipid was lower than in of milk fat. It can be explained by the fact that milk fat composition designed by diet lipid composition while only part of the beef lipid fractions were accumulated during the experimental period. Moreover, the rates of passage from the rumen of lipid fractions differ between milking cows with a very high DMI and growing cattle with a lower DMI. Furthermore, the efficiency with which CLA was synthesized in the mammary glands may differ from that in the adipose tissue or the muscle. The applicability of the first mechanism was obvious, and expected that longer experimental period, beginning earlier in the life of the growing cattle, would result in a larger response.

Further studies should be emphasized on other plant oil and oilseed supplementation, on shorter term period and on other stage of growth and stage of lactation in order to compare cost of supplementation with benefit from increased CLA content in beef or milk. The present study suggests that fattening cattle should be supplemented with high linoleic acid fat sources during late-mature period. Since, it

was clear that the development of intramuscular fat deposition or marbling score was in late maturing. At this stage, cattle maintain or increase fat synthesis in combination with declining in muscle growth as animals get older (Scollan et al., 2006). The present study used cattle at an average initial weight of 241 kg and an average final body weight of 304 kg. Thus, intramuscular fat deposition was negligible. The initial weight should be 350-400 kg and the final weight of cattle should reach 450-500 kg which is in the period of fat deposition. In lactating dairy cows, they should be supplemented with 1.5-2.0% of SBO in total ration since the level of addition beyond 2% may depress milk fat percentage. Dhiman et al. (2000) suggested that milk fat percentage was decreased when cows were fed 2-4% SBO in the diet, while at 0.5-2.0% SBO supplementation did not affect milk fat percentage. Lounglawan (2006) also showed that supplementation of 200 g/d SBO or SFO did not alter milk fat percentage. Zheng et al. (2005) reported that milk fat percentage was decreased ($P<0.05$) when cow received 500 g/d/head SBO. Bu et al. (2007) showed that milk fat content was numerically lower in milk from cow fed 4% SBO. Huang et al. (2008) reported that 5% SBO supplementation resulted in 30% reduction ($P<0.05$) in milk fat percentage. Moreover, intermediate composed i.e. *trans*-11 C18:1 vaccenic acid in rumen digesta, meat and milk should be determined in future research. In addition cellulolytic bacteria particularly *Butyrivibrio fibrisovens* should also be investigated.

In conclusion, the addition of SBO in fattening cattle or lactating dairy cow diets increased CLA content in beef or milk which improved the nutritional value of beef and milk with compromising milk composition, carcass characteristic or other performances. However, more beneficial was found to supplement SBO than RP-CLA and WCS.

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APPENDIX A

Table 1 A Effect of soybean oil (SBO) and whole cottonseed (WCS) on fatty acid composition in *Longissimus dorsi* muscle (Chapter III)

Items	Treatments[1]			SEM	P-value
	T1	T2	T3		
	----- mg/g fat -----				
C 12:0	4.22[d]	3.13[d]	0.89[d]	0.34	0.001
C 14:0	29.58[e]	19.65[e]	10.04[f]	1.29	0.001
C 14:1	4.37[d]	2.41[e]	0.90[e]	0.46	0.005
C 15:0	2.71[d]	1.83[e]	1.42[e]	0.15	0.002
C 15:1	0.95	0.50	0.46	0.20	0.230
C 16:0	121.29[d]	90.27[e]	72.08[e]	5.53	0.002
C 16:1	15.28[a]	9.00[b]	6.82[b]	1.44	0.014
C 17:1	3.27[a]	1.92[ab]	1.51[b]	0.40	0.045
C 18:0	68.93	73.45	65.28	5.53	0.584
C 18:1	121.90[a]	108.74[ab]	77.01[b]	9.59	0.039
C 18:2	15.36	18.56	12.67	3.30	0.492
C 18:3	1.31[a]	1.16[a]	0.40[b]	0.20	0.034
≥C 20:0	15.88	14.93	10.68	2.69	0.402
c-9,t-11CLA	1.43[e]	2.40[d]	0.58[e]	0.16	0.001
Summation by source[2]					
<C16:0	41.83[d]	27.49[e]	13.76[f]	1.72	0.001
C16:0 and C16:1	136.57[d]	99.27[e]	78.89[e]	6.58	0.002
>C16:0	228.09	221.14	168.11	17.67	0.100
Saturated fatty acid	231.73[d]	191.68[e]	152.23[f]	10.43	0.005
Unsaturated fatty acid	174.73[a]	156.20[ab]	108.48[b]	15.35	0.053

[a,b,c] Means within row with different superscripts differ (P<0.05)
[d,e,f] Means within row with different superscripts differ (P<0.01)
[1]T1 = Control, T2 = 170 g SBO/d, T3 = 170 g oil from WCS/d
[2]Fatty acids : <C16:0 represent de novo synthesized fatty acids, >C16:0 represent preformed fatty acids taken up from circulation, C16:0 fatty acids are derived from both sources
SEM = Standard error of mean

Table 2 A Effect of soybean oil (SBO) and whole cottonseed (WCS) on fatty acid composition in *Semimembranosus* muscle (Chapter III)

Items	Treatments[1]			SEM	P-value
	T1	T2	T3		
	----- mg/g fat -----				
C 12:0	2.30[a]	1.55[ab]	0.52[b]	0.31	0.019
C 14:0	18.40[a]	12.18[ab]	6.07[b]	2.82	0.057
C 14:1	2.59[a]	1.74[ab]	0.40[b]	0.52	0.065
C 15:0	1.74	1.60	0.82	0.28	0.145
C 15:1	1.06[a]	<0.01[b]	0.21[b]	0.24	0.044
C 16:0	97.12[a]	67.03[ab]	47.06[b]	13.40	0.096
C 16:1	11.66[a]	6.89[b]	4.52[b]	1.37	0.026
C 17:1	3.43[a]	1.37[b]	0.97[b]	0.40	0.010
C 18:0	54.80	46.24	41.23	9.23	0.602
C 18:1	106.70[a]	83.30[ab]	52.51[b]	14.08	0.088
C 18:2	20.07[a]	15.82[ab]	10.30[b]	2.50	0.084
C 18:3	1.27	0.88	0.28	0.28	0.112
≥C 20:0	21.60[a]	12.16[b]	8.40[b]	2.46	0.022
c-9,t-11CLA	0.84[ab]	1.80[a]	0.26[b]	0.34	0.046
Summation by source[2]					
<C16:0	26.08[a]	16.62[ab]	8.01[b]	3.77	0.040
C16:0 and C16:1	108.78	73.92	51.57	14.64	0.082
>C16:0	208.70	161.55	113.94	25.24	0.097
Saturated fatty acid	180.15	130.36	96.79	25.85	0.151
Unsaturated fatty acid	163.41[a]	121.73[ab]	76.73[b]	17.64	0.036

[a,b,c] Means within row with different superscripts differ (P<0.05)
[1]T1 = Control, T2 = 170 g SBO/d, T3 = 170 g oil from WCS/d
[2]Fatty acids : <C16:0 represent de novo synthesized fatty acids, >C16:0 represent preformed fatty acids taken up from circulation, C16:0 fatty acids are derived from both sources
SEM = Standard error of mean

Table 3 A Effect of soybean oil (SBO) and rumen protected conjugated linoleic acid (RP-CLA) on fatty acid composition in milk fat of lactating cows (Chapter III)

Items	**Treatments**			**SEM**	**P-value**
	T1	**T2**	**T3**		
	-----mg/g fat------				
C4:0	12.95^{d}	10.81^{e}	9.09^{f}	0.52	0.002
C6:0	9.72^{d}	7.86^{e}	4.28^{f}	0.54	0.001
C8:0	6.26^{d}	4.70^{e}	2.46^{f}	0.41	0.001
C10:0	13.69^{d}	10.23^{e}	5.98^{f}	1.03	0.002
C11:0	1.93^{d}	1.24^{e}	0.59^{f}	0.14	0.001
C12:0	47.74^{d}	37.48^{e}	29.63^{f}	2.13	0.001
C13:0	1.63^{d}	1.11^{e}	0.84^{f}	0.11	0.003
C14:0	80.66^{d}	68.68^{e}	56.64^{f}	3.46	0.004
C14:1	11.41^{d}	8.10^{e}	7.02^{e}	0.68	0.005
C15:0	5.22^{e}	4.45^{de}	3.84^{e}	0.29	0.010
C16:0	197.67^{d}	177.14^{e}	149.51^{e}	8.80	0.003
C16: 1	20.45^{d}	16.62^{e}	14.23^{e}	0.98	0.009
C17: 1	1.73^{d}	1.70^{e}	1.22^{e}	0.12	0.010
C18:0	51.21^{d}	69.40^{e}	5.76^{e}	3.79	0.007
C18:1	212.88^{de}	231.48^{d}	189.97^{e}	8.46	0.008
C18:2	15.58^{e}	19.99^{d}	12.12^{e}	1.30	0.001
C18:3	1.21^{d}	1.38^{d}	0.64^{e}	0.12	0.009
C20:0	0.82	0.98	0.83	0.05	0.060

Table 3 A Effect of soybean oil (SBO) and rumen protected conjugated linoleic acid (RP-CLA) on fatty acid composition in milk fat of lactating cows. (Cont.) (Chapter III)

Items	**Treatments**			**SEM**	**P-value**
	T1	**T2**	**T3**		
	-----mg/g------				
Others[2]	3.76[d]	3.92[d]	2.03[e]	0.18	0.001
cis-9,*trans*-11 CLA	5.57[e]	9.15[d]	5.22[e]	0.53	0.001
trans -10, *cis* -12 CLA	0.016[d]	0.044[e]	0.753[d]	0.03	0.001
trans -9, *trans* -11 CLA	0.22[e]	0.58[d]	0.54[d]	0.06	0.001
Total CLA[3]	5.81[e]	9.77[d]	6.51[e]	0.53	0.001

[d,e,f] Means within row with different superscripts differ (P<0.01)
[1]T1 =Control, T2 = 150 g SBO/d, T3 = 150 g RP-CLA/d
[2]Other = (Sum of C20:1,C20:2, C22:0, C20:3n6,C22:1n9+C20:3n3, C20:4n6, C20:5n3)
[3]Total CLA = (Sum of *cis*-9,*trans*-11 CLA; *trans* -10, *cis* -12 CLA; *trans* -9, *trans* -11 CLA)
SEM = Standard error of mean

BIOGRAPHY

Khukhuan Chullanandana was born on May 26th, 1976 in Chaiyaphom Province, Thailand. She graduated Bachelor of Science in Animal Production Technology, Institute of Agricultural Technology, Suranaree University of Technology in 1998. She received Master of Science in Animal Production Technology, Institute of Agricultural Technology, Suranaree University of Technology in 2000. She worked at King Mongkut's Institute of Technology Ladkrabang, Chumphon Campus, Chumphon Province, Thailand for 3 years. In 2004, she continued to study Doctor of Philosophy in Animal Production Technology, Institute of Agricultural Technology, Suranaree University of Technology.