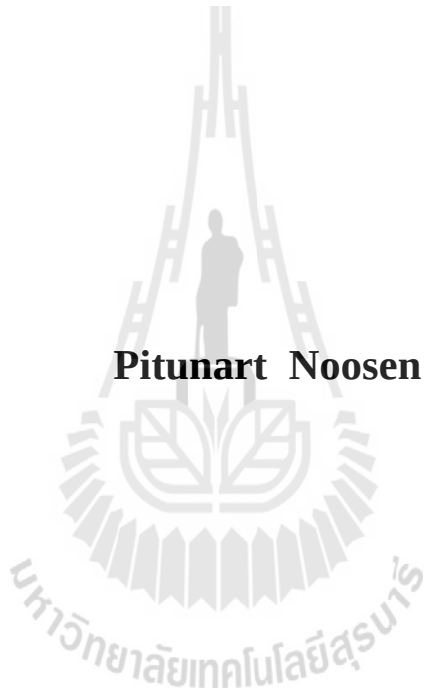
EFFECT OF LINSEED OIL SUPPLEMENTATION ON ACCUMULATION OF LINOLENIC ACID AND CHANGE IN n-6/n-3 RATIO IN BEEF AND COW'S MILK

Pitunart Noosen



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การเสริมน้ำมันลินสีด ต่อการสะสมกรดไขมันชนิดลิโนเลนิก และ การเปลี่ยนแปลงสัดส่วนของกรดไขมัน n-6/n-3 ในเนื้อ และ น้ำนม

นางสาวปิตุนาถ หนูเสน

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรดุษฎีบัณฑิต
สาขาวิชาเทคโนโลยีการผลิตสัตว์
มหาวิทยาลัยเทคโนโลยีสุรนารี
ปีการศึกษา 255

ปิตุนาถ หนูเสน : ผลของการเสริมน้ำมันลินสีดต่อการสะสมกรดไขมันลิโนเลนิก และการเปลี่ยนแปลงสัดส่วนของกรดไขมัน n-6/n-3 ในเนื้อ และน้ำนม (EFFECT OF LINSEED OIL SUPPLEMENTATION ON ACCUMULATION OF LINOLENIC ACID AND CHANGE IN n-6/n-3 RATIO IN BEEF AND COW'S MILK)
อาจารย์ที่ปรึกษา : รองศาสตราจารย์ ดร.วิศิษฐิพร สุขสมบัติ, 172 หน้า.

วัตถุประสงค์งานวิจัยนี้คือ เพื่อศึกษาผลของการใช้น้ำมันลินสีดเสริมในอาหารโคขุน และโครีดนมต่อการสะสมกรดไขมันและสัดส่วนของกรดไขมันชนิด n-6/n-3 ในเนื้อโค และ นมโค

การทดลองที่ 1 ทำการทดลองในโคเนื้อขุน (ระดับสายเลือดพันธุ์บรามันมากกว่า 87.5%) จำนวน 20 ตัว อายุเฉลี่ยประมาณ 2 ปี วางแผนการทดลองแบบสุ่มสมบูรณ์ โดยสุ่มสัตว์ทดลองแบบแบ่งชั้นจากน้ำหนักตัว จำนวน 4 กลุ่ม และ ทำการสุ่มอาหารทดลองให้แก่สัตว์ทดลอง แบ่งเป็น 4 กลุ่มตามอาหารทดลอง โดยที่โคทุกตัวได้รับอาหารข้นเป็นชนิดเม็ดมีโปรตีนไม่น้อยกว่า 14% มีน้ำให้กินตลอดเวลา และถูกเลี้ยงขังในคอกเดี่ยว กลุ่มการทดลองได้แก่ 1) กลุ่มควบคุมได้รับอาหารข้น 7 กิโลกรัม/ตัว/วัน และฟางข้าวเป็นแหล่งอาหารหยาบแบบไม่จำกัดปริมาณ 2) เสริมน้ำมันปาล์มปริมาณ 200 กรัม/ตัว/วันและอาหารข้น 4 กิโลกรัม/ตัว/วัน และหญ้าสดเป็นอาหารหยาบแบบไม่จำกัดปริมาณ 3) เสริมน้ำมันปาล์มปริมาณ 100 กรัม/ตัว/วัน ร่วมกับ น้ำมันลินสีดปริมาณ 100 กรัม/วันและได้รับอาหารข้น 4 กิโลกรัม/ตัว/วัน และหญ้าสดเป็นแหล่งของอาหารหยาบแบบไม่จำกัดปริมาณ 4) เสริมน้ำมันลินสีดปริมาณ 200 กรัม/ตัว/วัน และอาหารข้น 4 กิโลกรัม/ตัว/วัน และหญ้าสดเป็นอาหารหยาบแบบไม่จำกัดปริมาณ ผลการทดลองพบว่า อาหารทดลองไม่มีผลต่อการกินได้ของโภชนะในโคเนื้อขุน อย่างไรก็ตามการเสริมน้ำมันในอาหารโคเนื้อขุนมีผลในการลดปริมาณการกินได้ของวัตถุแห้ง ขณะที่ค่าความเป็นกรดด่าง แอมโมเนียไนโตรเจน โปรโตซัว และความเข้มข้นของกรดไขมันระเหยได้ ไม่แตกต่างกันอย่างมีนัยสำคัญทางสถิติ นอกจากนี้ในกลุ่มการทดลองที่ได้รับการเสริมน้ำมันลินสีดไม่ส่งผลกระทบต่อลักษณะซากและลักษณะทางประสาทสัมผัสต่อการยอมรับของผู้บริโภคแต่สามารถเพิ่มปริมาณกรดไขมันชนิด n-3 และลดสัดส่วนของกรดไขมัน n-6/n-3 ในเนื้อโคตามปริมาณการเพิ่มขึ้นของการเสริมน้ำมันลินสีดในอาหารโคเนื้อขุน

การทดลองที่ 2 ทำการทดลองในโคนมลูกผสมพันธุ์โฮลสไตน์ฟรีเชียน (ระดับสายเลือดพันธุ์โฮลสไตน์ฟรีเชียนมากกว่า 87.5%) จำนวน 24 ตัว วางแผนการทดลองแบบ 2 × 2 Factorial in Random Complete Block Design โดยโคทุกตัวได้รับอาหารข้นเป็นชนิดเม็ดประมาณ 6 กิโลกรัม/ตัว/วัน มีโปรตีนไม่น้อยกว่า 21% มีน้ำให้กินตลอดเวลา และถูกเลี้ยงขังในคอกเดี่ยว กลุ่มการทดลองได้แก่ 1) เสริมน้ำมันปาล์ม 300 กรัม/ตัว/วันร่วมกับข้าวโพดหมักเป็นแหล่งอาหารหยาบแบบไม่จำกัด

ปริมาณ 2) เสริมน้ำมันลินสีด 300 กรัม/ตัว/วัน ร่วมกับข้าวโพดหมักเป็นแหล่งอาหารหยาบแบบไม่จำกัดปริมาณ 3) เสริมน้ำมันปาล์ม 300 กรัม/ตัว/วัน ร่วมกับหญ้าสดเป็นแหล่งอาหารหยาบแบบไม่จำกัดปริมาณ 4) เสริมน้ำมันลินสีด 300 กรัม/ตัว/วัน ร่วมกับหญ้าสดเป็นแหล่งอาหารหยาบแบบไม่จำกัดปริมาณ ผลการทดลองพบว่าการเสริมน้ำมันลินสีดร่วมกับหญ้าสดเป็นแหล่งอาหารหยาบแบบไม่จำกัดปริมาณไม่ส่งผลต่อกระทบต่อปริมาณการกินได้ของวัตถุแห้ง, ปริมาณน้ำนม, และองค์ประ- กอบน้ำนม แต่อย่างไรก็ตามปริมาณการเสริมน้ำมันลินสีดในอาหารโคนมสามารถเพิ่มปริมาณกรดไขมันชนิด C18:3n3 และเมื่อเสริมร่วมกับหญ้าสดสามารถลดสัดส่วนของกรดไขมัน n-6/n-3 ในนมโคได้ต่ำกว่าในทุกกลุ่มการทดลอง

การทดลองที่ 3 โคเจาะกระเพาะลูกผสมพันธุ์โฮลสไตน์ฟรีเชียน จำนวน 4 ตัว วางแผนการทดลองแบบ 4 × 4 Latin square โดยโคทุกตัวได้รับอาหารข้นเป็นชนิดเม็ดประมาณ 3 กิโลกรัม/ตัว/วัน มีโปรตีนไม่น้อยกว่า21% มีน้ำให้กินตลอดเวลา และถูกเลี้ยงขังในคอกเดี่ยว ได้รับอาหารทดลองตามการทดลองที่ 2 ผลการทดลองพบว่า ในชั่วโมงที่ 2 หลังจากกินอาหารในกลุ่มที่ได้รับการเสริมน้ำมันลินสีด อาหารในกระเพาะหมักมีปริมาณของกรดไขมันชนิด C18:3n3 สูงกว่ากลุ่มการทดลองอื่น แต่ไม่ส่งผลกระทบต่อปริมาณของกรดไขมันชนิด C18:0, C18:2 และ CLA เนื่องด้วยการเสริมน้ำมันลินสีดมีผลในการยับยั้งการเกิดกระบวนการสังเคราะห์กรดไขมันอิ่มตัวชนิด C18:0 จาก C18:2 ในกระเพาะหมัก โดยอัตราการไหลผ่านของอาหารในกระเพาะหมักที่เพิ่มขึ้นจะมีผลต่อกระบวนการการสังเคราะห์กรดไขมันอิ่มตัวในกระเพาะหมักที่ลดลง นอกจากนี้เสริมน้ำมันลินสีดไม่มีผลต่อการเปลี่ยนแปลงกระบวนการหมักย่อยในกระเพาะหมัก, ประสิทธิภาพการย่อยได้ของวัตถุแห้งและเยื่อใยที่ไม่สามารถละลายได้ในสารละลายที่เป็นกลาง, ค่าความเป็นกรดด่าง, แอมโม-เนียไนโตรเจน, โปรโตซัว และความเข้มข้นของกรดไขมันระเหยได้

สาขาวิชา เทคโนโลยีการผลิตสัตว์ ลายมือชื่อนักศึกษา_____

ปีการศึกษา 2557 ลายมือชื่ออาจารย์ที่ปรึกษา_____

ลายมือชื่ออาจารย์ที่ปรึกษาร่วม_____

PITUNART NOOSEN : EFFECT OF LINSEED OIL SUPPLEMENTATION ON ACCUMULATION OF LINOLENIC ACIDS AND CHANGE IN n-6/n-3 RATIO IN BEEF AND COW'S MILK.
THESIS ADVISOR : ASSOC. PROF. WISITIPORN SUKSOMBAT, Ph.D., 172 PP.

LINSEED OIL/LINOLENIC ACID/FATTY ACID/BEEF/MILK

The objective of this research was to study supplementation of linseed oil in fattening cattle and crossbred Holstein Friesian cow diets and then to determine the fatty acid accumulation and n-6/n-3 ratio in beef and milk.

In Experiment I : twenty fattening steers (87.5% Brahman crossbred), approximately 2 years old, were assigned into a Completely Randomized Design (CRD) and stratified by their LW into 4 groups of which each group was randomly assigned to four dietary treatments. All steers were fed 14% CP control concentrate and free access to clean water and were individually housed in a free-stall unit. The treatments were 1) concentrate approximately 7 kg/d with *ad libitum* rice straw; 2) concentrate approximately 4 kg/d plus 200 g/d of palm oil with *ad libitum* fresh grass; 3) concentrate 4 kg/d plus 100 g/d of palm oil and 100 g/d of linseed oil with *ad libitum* fresh grass; 4) concentrate 4 kg/d plus 200 g/d of linseed oil with *ad libitum* fresh grass**.** The dietary treatment had no effect on nutrient intake. The oil supplement decreased DMI, while ruminal pH, NH_4-N VFA concentrations and protozoa count were not changed. LSO did not negatively affect carcass quality or sensory perceptions, but increased the n-3 FA and lowered the n-6/n-3 ratio in beef with increasing amounts of LSO supplement.

In Experiment II : twenty-four Holstein Friesian crossbred lactating dairy cows

(87.5% Holstein Friesian) were assigned into a 2 × 2 Factorial in Random Complete Block Design. All cows were fed approximately 6 kg/d of 21% CP concentrate and free access to clean water and were individually housed in a free-stall unit. Treatments were: 1) concentrate plus 300 g/d of palm oil (PO) together with *ad libitum* corn silage (CS); 2) concentrate plus 300 g/d of linseed oil (LSO) together with *ad libitum* CS; 3) concentrate plus 300 g/d of PO together with *ad libitum* fresh grass (FG); and 4) concentrate plus 300 g/d of LSO together with *ad libitum* FG. Supplementation with LSO had no effect on DMI, milk production and milk composition. However, the milk C18:3n3 percentage was increased by LSO supplementation. Finally, LSO supplemented with *ad libitum* fresh grass was able to decrease the n-6/n-3 FA ratio in the dairy cow milk.

In Experiment III : four ruminally fistulated crossbred Holstein Friesian cows were assigned to 4 dietary treatments in a 4 × 4 Latin square design. All cows were fed approximately 3 kg/d of 21% CP concentrate. Treatments were similar to those in Experiment II. The results demonstrated that post feeding LSO at 2 h provided higher C18:3n3, but it did not affect C18:0, C18:2 and CLA proportion in rumen digesta. Feeding LSO inhibited BH of C18:2 to C18:0, as indicated by the increased rumen flows. Furthermore, LSO did not negatively influence ruminal fermentation, DM or NDF digestibilities, and there was no change in ruminal pH, NH_3-N, protozoa or VFA concentration.

School of Animal Production Technology Student's Signature_____

Academic Year 2014 Advisor's Signature_____

Co-advisor's Signature_____

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ADF	=	Acid detergent fiber
ADICP	=	Acid detergent insoluble crude protein
ADIN	=	Acid detergent insoluble N
ADL	=	Acid detergent lignin
NDF	=	Neutral detergent fiber
NDICP	=	Neutral detergent insoluble crude protein
NDIN	=	Neutral detergent insoluble N
NE	=	Net energy
NFC	=	Non-fiber carbohydrate
NPN	=	Non protein nitrogen
NRC	=	National research council
RDP	=	Rumen degradable protein
RUP	=	Rumen undegradable protein
C4:0	=	Butyric acid
C6:0	=	Caproic acid
C8:0	=	Caprylic acid
C10:0	=	Capric acid
C11:0	=	Cis-10-Pentadecenoic acid
C12:0	=	Lauric acid
C13:0	=	Tridecanoic acid
C14:0	=	Myristic acid

LIST OF ABBREVIATIOS (Continued)

C14:1	=	Myristoleic acid
C15:0	=	Pentadecanoic acid
C16:0	=	Palmitic acid
C16:1	=	Palmitoleic acid
C17:1	=	Heptadecenoic Acid
C18:0	=	Stearic acid
C18:1n9t	=	Elaidic acid
C18:1n9c	=	Oleic acid
C18:2n6t	=	Linolelaidic acid
C18:2n6c	=	Linoleic acid
C18:3n3	=	α -Linoleic acid
C20:0	=	Arachidic acid
C20:1	=	Gondoic acid
C22:0	=	Behenic acid
C20:3n-6	=	dihomo-γ-linolenic acid
C20:4n-6	=	arachidonic acid
C22:6n-3	=	docosahexaenoic acid
FCM	=	Fat corrected milk
FCM	=	Fat corrected milk
NDF	=	Neutral detergent fiber
NDICP	=	Neutral detergent insoluble crude protein
NDIN	=	Neutral detergent insoluble N

LIST OF ABBREVIATIOS (Continued)

NE	=	Net energy
NFC	=	Non-fiber carbohydrate
NPN	=	Non protein nitrogen
NRC	=	National research council

CHAPTER I

INTRODUCTION

Products from ruminant contain high amount of saturated fatty acids (SFA), which are the result of biohydrogenation process occurring within the rumen (Scollan et al., 2001), particularly lauric acid (C12:0) and myristic acid (C14:0) found in milk. While palmitic acid (C16:0) and stearic acid (C18:0) are rich in beef (Moloney et. al., 2008; Suksombat et al., 2011). Consumption of foods rich in SFA can cause heart disease in human. While the amount of polyunsaturated fatty acid (PUFA) in ruminant products including linoleic acids (C18:2n-6) and linolenic acids (C18:3n-3) has a positive effect on consumer's health (Parodi, 2005). PUFAs, particularly n-3 fatty acids, can inhibit prostate cancer and breast cancer (Pandalai et al., 1996), reduce atherothrombotic vascular disease and the risk of heart disease (Kris-Etherton et al., 2002; Harper and Jacobson, 2005). In addition, n-3 FA helps to balance appropriate n-6/n-3 FA ratio for human's health. The Department of Health (1994) suggested that consumers should receive food with high amount of n-3 FA relative to the quantities of n-6 FA. At present, consumers receive an unbalanced n-6/n-3 ratio (10:1) while the appropriate ratio should be 1:1 (Eaton et al., 1996). Since the 2 groups of these FAs cannot be synthesized in human's body because of lacking Δ-12 and Δ-15 desaturase enzymes to insert double bonds. Thus, these 2 essential FAs must be directly supplied from the diets. Compositions and ratios of FAs in beef and milk are influenced by dietary FAs, an important factor controlling the proportion of fatty acids in the beef

and milk. Linseed oil is a natural source of alpha-linolenic fatty acid (ALA) and other PUFAs and it is important antithrombotic and anti-inflammatory roles (Palmquist, 2009). Petit et al. (2004) found that 9.7% of dietary DM flaxseed supplemented dairy cows produced milk containing higher n-3 FAs than those cows supplemented with palm oil and sunflower seeds, and the control group which did not add the oil. Bork et al., (2010) reported significant reduced milk n-6/n-3 FA ratio when 0.85% linseed of dietary DM was supplemented to lactating dairy cows. When comparison has been made among the fresh grass system, corn silage and corn silage plus linseed as roughage, the ratios of n-6/n-3 fatty acids in dairy cow's milk were 1.23, 2.93 and 1.88, respectively (Dutreuil, 2008). Supplementation of 3% linseed oil significantly increased the concentrations of n-3 fatty acids (alpha linolenic acid, C20: 5 n3 and C22:5 n3 and C22:6 n3) in beef (Herdmann et al., 2010). Thus, the present study aimed to determine the effect of LSO supplementation in beef and dairy cattle diets on n3 fatty acid profiles and n-6/n-3 ratio in beef and milk. The first part of this study was to supplement with linseed oil in fattening cattle's diets and the second part in Crossbred Holstein Friesian cow's diets.

1.1 Research hypothesis

1.1.1 Supplementation of linseed oil in fattening cattle's diets may increase n3 fatty acid accumulation and decrease n-6/n-3 ratio in beef.

1.1.2 Supplementation of linseed oil in Crossbred Holstein Friesian cow's diets may increase n3 fatty acid accumulation and decrease n-6/n-3 ratio in milk.

1.2 Research objectives

1.2.1 To study the effect of linseed oil supplementation in fattening cattle's diets on n3 fatty acid accumulation and n-6/n-3 ratio in beef.

1.2.2 To study the effect of linseed oil supplementation in Crossbred Holstein Friesian cow's diets on n3 fatty acid accumulation and n-6/n-3 ratio in milk.

1.3 Scope of the study

These researches intended to study the effect of linseed oil supplementation in fattening cattle's diets on change n-6/n-3 ratio and accumulation of n3 fatty acid in beef and the effect of linseed oil supplementation in Crossbred Holstein Friesian cow's diets on change n-6/n-3 ratio and accumulation of n3 fatty acid in milk.

1.4 Expected results

1.4.1 High level of n3 fatty acid accumulation and low n-6/n-3 ratio in beef may occur when linseed oil was supplemented in fattening cattle's diets.

1.4.2 High level of n3 fatty acid accumulation and low n-6/n-3 ratio in milk may occur when linseed oil was supplemented in lactating dairy's cow diets.

1.5 References

Bork, N. R., Schroeder J. W., Lardy G. P., Vonnahme K. A., Bauer M. L., Buchanan D. S., Shaver R. D., and Fricke, P. M. (2010). Effect of feeding rolled flaxseed on milk fatty acid profiles and reproductive performance of dairy cows. **J. Anim. Sci.** 88 : 3739-3748.

Department of Health. (1994). Nutritional aspects of cardiovascular disease. Report of

the Cardiovascular Review Group Committee on Medical Aspects of Food Policy. **Reports on Health and Social Subjects.** H.M. Stationery Office, London. No. 46.

Dutreuil, M., Hurtaud, C., Martin, B., Agabriel, C., and Cabrera, V. E. (2008). How feeding systems influence milk quality on French dairy farm? Online: http://dairymgt.info/publications/FeedMilkQuality.pdf

Eaton S. B., Konner M. J., and Shosta, M. (1996). An evolutionary perspective enhances understanding of human nutritional requirements. **J. Nutr.** 126 : 1732-1740.

Harper, C. R., and Jacobson T. A. (2005). Usefulness of omega-3 fatty acids and the prevention of coronary heart disease**. Am. J. Cardiol.** 96 : 1521-1529.

Herdmann, A., Martin, J., Nuernberg, G., Dannenberger, D., and Nuernberg, K. (2010). Effect of dietary n-3 and n-6 PUFA on lipid composition of different tissues of German Holstein bulls and the fate of bioactive fatty acids during processing. **J. Agric. Food Chem.** 58 : 8314-8321.

Kris-Etherton P. M, Harris, W. S., and Appel L. J. (2002). Fish consumption, fish oil, omega-3 fatty acids, and cardiovascular disease. **Circulation**. 106 : 2747-2757.

Moloney, A, P., Keane M.G., Dunne P.G., Mooney, M.T., and Troy, D.J. (2008). Effect of concentrate feeding pattern in a grass silage/concentrate beef finishing system on performance, selected carcass and meat quality characteristics. **Meat Sci.** 79 : 355-364.

Palmquist, D. L. (2009). Omega-3 fatty acids in metabolism, health, and nutrition and for modified animal product foods. **Prof. Anim. Sci.** 25(3) : 207-249.

Pandalai, P. K., Pilat, M. J., Yamazaki K., Naik, H., and Pienta, K. J. (1996). The effects of omega-3 and omega-6 fatty acid on in vitro prostate cancer growth.

Anticancer Res. 16 : 815-820.

Parodi, P.W. (2005). Dairy product consumption and the risk of breast cancer. **J. Am. Coll. Nutr.** 24 : 556-568.

Petit, H. V., Germiquet, C., and Lebel, D. (2004). Effect of feeding whole, unprocessed sunflower seeds and flaxseed on milk production, milk composition, and prostaglandin secretion in dairy cows. **J. Dairy. Sci.** 87 : 3889-3898.

Scollan, N. D., Choi, N.J., Kurt, E., Fisher, A.V., Enser, M., and Wood, J.D. (2001). Manipulating the fatty acid composition of muscle and adipose tissue in beef cattle. **Br. J. Nutr.** 85 : 115-124.

Suksombat, W., Lounglawan P., and Paengsai, P. (2011). Effects of biotin supplementation on milk production, milk composition, milk fatty acids, ruminal pH, ammonia nitrogen and volatile fatty acids in lactating dairy cows**. J. Anim. Vet. Adv.** 10(16) : 2186-2192.

CHAPTER II

LITERATURE REVIEW

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

2.1 n-3 Fatty acids

n-3 Fatty acids (omega-3 fatty acids or ω-3 fatty acids) are family of unsaturated fatty acids that the first carbon-carbon double bond exits as the third carbon-carbon bond from the terminal methyl end (ω) of the carbon chain of fatty acid. n-3 Fatty acids are considered essential fatty acids. The human body cannot synthesize n-3 fatty acids *de novo* but it can form 20-carbon unsaturated n-3 fatty acids (eicosapentanoic acid, EPA) and 22-carbon unsaturated n-3 fatty acids (docosahexaenoic acid, DHA) from the eighteen-carbon n-3 fatty acid α-linolenic acid, witch the chemical structures are shown in Figure 2.1 These three polyunsaturated fatty acids have 3, 5 and 6 double bonds in the carbon chain of 18, 20 and 22 carbon atoms, respectively (Kapoor and Patil, 2011). The research indicates

and prevention risk factors associated with chronic and prevention risk factors for brain (brian memory and performance), the central nervous, retinal and eye development of infants (Uauy and Valenzuala, 2000). Infants who do not get enough n-3 fatty acids from their mothers during pregnancy are at risk for developing vision and nerve problems (Mirajkar et al, 2011). Plants can synthesize n-3 fatty acid in a reaction catalyzed by the enzyme Δ-15 desaturase; mammals do not possess this enzyme and thus they have to obtain this fatty acid from the diet. n-3 Fatty acid can be found in green leafy vegetables, seeds such as flaxseed and linseed oil, nuts, and legumes, and a small percentage of n-3 fatty acid is also found in corn oil, sunflower oil, or safflower oil (Kromhout et al., 2012).

Figure 2.1 Chemical structure of (a) α-linolenic acid, ALA; (b) eicosapentaenoic acid, EPA; (c) docosahexaenoic acid, DHA (Kashiwagi and Huang, 2012)

2.2 n-6 Fatty acids

n-6 Fatty acids (omega-6 fatty acids or ω-6 fatty acids) are a family of unsaturated fatty acids that have in common a final carbon-carbon double bond in the n-6 position, that is the sixth bond, counting from the methyl end. n-6 Fatty acids are considered as essential fatty acids. The biological effects of the n-6 fatty acids are

largely mediated by their conversion to 20-carbon unsaturated n-6 fatty acids (arashidonic acid, AA) and 22-carbon unsaturated n-6 fatty acids (docosapentanoic acid, DPA) from the 18-carbon n-6 fatty acid (linoleic acid, LA) (Kuang, 2001). The chemical structures of n-6 fatty acid are shown in Figure 2.2. The conversion of tissue arachidonic acid (C20:4n-6) to n-6 prostaglandin and n-6 leukotriene hormones provides many targets for pharmaceutical drug development and treatment to diminish excessive n-6 actions in atherosclerosis, asthma, arthritis, vascular disease, thrombosis, immune-inflammatory processes, and tumor proliferation (Simopoulos, 2002). n-6 Fatty acid is a major fatty acid in plant lipids. In animals it is derived mainly from dietary plant oils such as palm oil, sunflower oil, corn oil and soybean oil (Hibbeln, 2006).

Figure 2.2 Chemical structure of (a) linoleic acid, LA; (b) arachidonic acid, AA; (c) docosapentanoic acid, DPA (Kashiwagi and Huang, 2012)

2.3 Metabolism of n-3 and n-6 fatty acids

The enzymes responsible for desaturation and chain elongation in both n-3 and n-6 families are identical (Figure 2.3). Imbalance in the intake of n-6 versus n-3 fatty acids sometimes leads to over-production of eicosanoids with less preferred activities. Eicosanoids made from n-3 fats are often referred to as anti-inflammatory, but in fact

they are just less pro-inflammatory than those made from n-6 fats. If both n-3 and n-6 are present, they will “compete” to be transformed, so the ratio of n-6/n-3 directly affects the type of eicosanoids that are produced. This as to greater interest in finding ways to control the synthesis of n-6 eicosanoids. The simplest way would be by consuming more n-3 and fewer n-6 fatty acids. Guebre et al. (2008) studied the effect of diet modification to decrease the n-6/n-3 ratio on cardiovascular risk factors and resting energy expenditure. Decreased n-6/n-3 ratio can be achieved with simple dietary counseling, resulting in multiple, potentially favorable effects on the metabolic and inflammatory profiles. Serhan et al. (2002) discovered that the EPA is responsible for the formation of potent antiinflamatory nanomolecules, called Resolvins in the human body and discovered that n-3 fatty acids are converted into other anti-inflammatory molecules called Maresins and n-3-oxylipins, which partly explain the versatile health effects of n-3 fatty acid enriched foods. It has been reported that conversion of ALA to EPA and further to DHA in humans is limited, but varies with individuals. Generally, women have higher ALA conversion efficiency than men, probably due to the lower rate of utilization of dietary ALA for β-oxidation. This suggests that biological engineering of ALA conversion efficiency is possible. However, Goyens et al. (2006) reported the absolute amount of ALA, rather than the ratio of n-6 and n-3 fatty acids, which affects the conversion.

2.4 Rumen biohydrogenation of n-3 and n-6 fatty acids

Lipids in ruminant feed are derived from forages, grains and oil supplements. The lipid content in ruminant diets is approximately 3-7% DM intake. The pathways of biohydrogenation of the major dietary PUFA, n-6 and n-3 fatty acids, were

established in classical studies conducted during the 1960s through to 1980s (Palmquist et al., 2005; Bauman and Lock, 2006; Jenkins et al., 2008). Dietary lipids entering the rumen are usually triglycerides, phospholipids and galactolipids. The first transformation is lipolysis, the hydrolysis of the ester linkages releasing free fatty acid. The establishment of a free carboxyl group is a critical first step before hydrogenation may proceed. The first stage in the saturation of both n-6 and n-3 involves an isomerisation reaction that converts the *cis*-12 double bond to a *trans*-11 isomer, resulting in conjugate linoleic acid (CLA). Hydrogenation of the *cis*-9 bond can then proceed by a microbial reductase, with the formation of vaccenic acid. The final step in the ruminal biohydrogenation pathway involves a further hydrogenation of the *trans*-11 double bond producing C18:0 (C18:2n-6 pathway; Figure 2.4) or *trans*-15 C18:1 (C18:3n-3 pathway; Figure 2.4). Partially n-6 and n-3 fatty acid bypassing microbial hydrogenation which escapes from the rumen and are absorbed by intestine and incorporated into milk fat and beef (Fuentes et al., 2011).

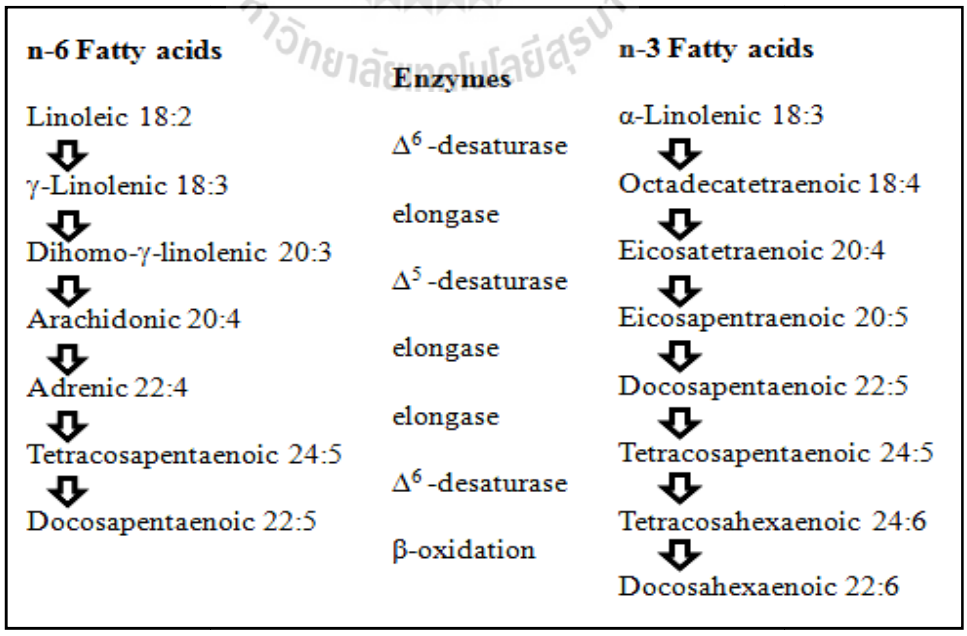

Figure 2.3 Essential fatty acids metabolism (Kapoor and Patil, 2011)

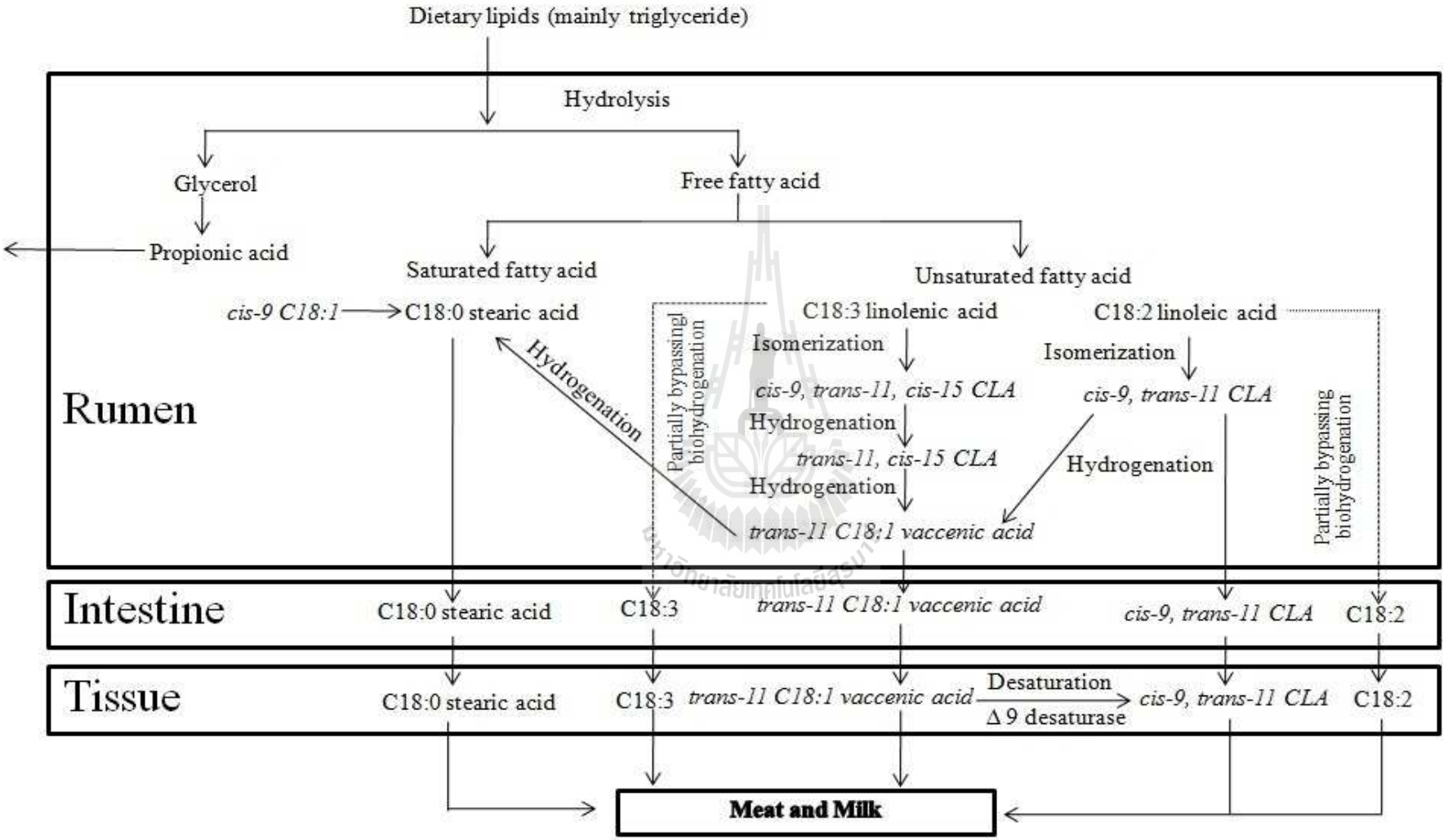

Figure 2.4 Lipid metabolisms in the rumen (Tanaka, 2005)

2.5 Lipid transport in ruminants

Fatty acids in the small intestine predominantly originate from the diet or microbial *de novo* synthesis; however a significant proportion (roughly 10 to 20%) of the fatty acid arises from microbial phospholipids. Once lipids reach the duodenum, fatty acid blends with bile salts to form micelles which are then absorbed across the mucosal cells. The capacity to absorb fatty acid diminishes with increasing lipid supplementation in the diet, assumably due to limitations in the production of pancreatic lipases and bile salts (Bauchart, 1993). Once absorbed, medium-chain fatty acid (C6 to C12) are transported as non esterified fatty acid (NEFA) via the portal vein and can be either be absorbed in the liver or peripheral tissues. In the liver, the NEFA can undergo oxidation to form acetate, or be formed into triacylglycerols and re-excreted as very low density lipoproteins (VLDL) (Hocquette and Bauchart, 1999). Fatty acids greater than C12 are transported as triacylglycerols by lipoproteins called chylomicrons through the lymphatic system to the peripheral tissues. Lipoprotein lipases hydrolyse the triacylglycerols, releasing NEFA, which are then absorbed and incorporated into membrane or adipose tissue (Hocquette and Bauchart, 1999). Further elongation and desaturation of the absorbed lipids occurs via lipogenic enzymes (Hocquette et al., 2010).

2.6 Dietary ratio of n-3 and n-6 fatty acids

The n-6/n3 ratio may be as high as 17 : 1 in some Western diets (Simopoulos, 2006). It is roughly 10 : 1 in the U.S. diet (Etherton et al., 2002). In the Women's Health Study, participants had an average dietary ratio of about 8 : 1. Although some women ate diets with a low ratio of about 1 : 1 while others ate diets with a high ratio

of 33 : 1 (Miljanovic et al., 2005). The n-6/n-3 ratio recommended by international agencies and some European countries ranges from 4 : 1 to 10 : 1 (Gebauer et al., 2006). The U.S. Institute of Medicine (IOM) supports a ratio of 5 : 1 for the U.S. and Canadian populations (Institute of Medicine, 2002).

2.7 Linseed oil

Linseed oil is derived from the seeds of flaxseed (*Linum usitatissimum L.*), a plant widely cultivated in Europe for fiber or oil for industrial use (Bayrak et al., 2010). The most important linseed producing countries are Canada, Argentina, USA, China, India and Europe (Lidefelt, 2007). Generally linseed contains 40% oil, 30% diet fiber, 20% protein, 4% ash and 6% moisture (Wang et al., 2008). Linseed oil richs in polyunsaturated fatty acids, particularly n-3 about 53% of the total fatty acids (Table 2.1), whereas the n-6 fatty acids comprise about 13%, the essential for humans, the body needs them. They must be obtained from the fats and oils in foods because our bodies cannot make them. Thus, linseed oil contains more than three times as much n-3 as n-6 fatty acids, giving an n-6/n-3 ratio of 0.3 : 1 (Katare, 2012). By comparison, the n-6/n-3 ratio for corn oil is 46 : 1; for soybean oil, 7 : 1; and for canola oil, 2 : 1. The high level of n-3 in linseed oil makes it a good source of n-3 fat in the North American diet. Consuming linseed oil or foods rich in n-3, such as n-3 enriched milk and beef derived from ruminant, increases n-3 fat intake and improves the dietary n-6/n-3 ratio (Chilliard et al., 2007; Herdmann et al., 2010).

Table 2.1 Fatty acid profile of oils (Diane, 2007)

	Fatty acids (g /100 g fatty acids)					
Feeds	**C16:0**	**C18:0**	**C18:1**	**C18:2n-6**	**C18:3n-3**	**n-6/n-3**
Linseed	5.00	4.10	20.20	12.70	53.30	0.24
Corn	22.70	2.30	27.30	53.50	1.16	46.10
Palm	43.50	4.30	36.60	9.10	0.20	45.50
Rice bran	16.90	1.60	39.10	33.40	1.60	20.90
Sunflower	5.90	4.50	19.50	65.70	-	-
Soybean	10.30	3.80	22.80	51.00	6.80	7.50
Canola	4.00	1.80	56.10	20.30	9.30	2.20

2.8 Effect of plant oil and oilseeds on performance, carcass and n-3 fatty acid accumulation in beef

Animal performance data are summarized in Table 2.5 Daily total DM intakes (DMI) were not significantly affected by dietary treatment (Dawson et al., 2007). Scholljegerdes and Kronberg (2010) reported that compared with nonsupplemented controls, steers grazing native rangeland gained on average 0.27 kg more BW per day and had greater G : F when fed linseed. Although ADG did not differ between steers fed a corn-based supplement or linseed, G : F was greater for the steers supplemented with linseed (Scholljegerdes and Kronberg, 2010). In other studies, Holstein calves fed a palm oil supplement attained a growth rate of 1.3 kg/d (Partida et al., 2007), while Italian Holstein calves fed a concentrate that included 5% whole linseed during the growth period and 8% whole linseed during the finishing period achieved an average daily gain of 1.21 kg/d (Corazzin, et al, 2012). Furthermore, no significant differences in carcass weight or carcass quality were observed due to dietary supplement (Table 2.5). Greater carcass fatness has been recorded in some studies

carried out with steers or cull cows fed concentrate that included linseed (Kim et al., 2009; Hernandez-Calva et al., 2011), but no such differences were found in some researches. This could be because the diets used were isoenergetic, while in some papers cited the concentrates supplemented with linseed contained a higher percentage of lipids than the control diets. Works comparing concentrates supplemented with linseed and concentrates supplemented with another lipid ingredient having a similar energy content published by Mach et al. (2006), Kronberg et al. (2011) and Corazzin, et al. (2012) also found no variation in carcass fatness. Comparing the effect of dietary linseed, no significant differences in marbling were found (Mach et al., 2006; Kronberg et al., 2011; and Corazzin et al.,2012)

Fatty acid compositions of beef are summarized in Table 2.5. Linseed oil, as a major source of C18:3n-3, was used to augment the supply of C18:3n-3 from grass and so promote indirect synthesis of CLA via tissue desaturation of ruminally derived C18:1trans-11. The management strategy imposed was successful in ensuring similar mean carcass weights and muscle fatty acid concentrations across the treatments as intended. Interpretation of the effects of diet on fatty acid composition is therefore not confounded by differences in carcass fatness (Leat, 1978). Despite the high degree of biohydrogenation of dietary PUFA reported by Scollan et al. (2001) and by Doreau and Ferlay (1994), supplementation with PUFA-rich rations decreased the SFA and increased in the PUFA proportion in the muscle and in subcutaneous adipose tissue. This decrease in SFA suggests an increase in the incorporation of PUFA in muscle and subcutaneous adipose tissue at the expense of SFA, due to the different proportions of fatty acids in the unsupplemented and supplemented diets. A diet rich in C18:3n-3 (crushed or extruded linseed) was

shown by Raes et al. (2004) to decrease the n-6/n-3 PUFA ratio from 6 : 1 to less than 4 : 1 in Belgian Blue bulls, and similar results were obtained by Scollan et al. (2001) by feeding a 60 : 40 silage to concentrate ratio with a whole linseed-based ration. The n-6/n-3 PUFA ratio of beef is of relevance in its contribution to the whole diet of humans.

Table 2.2 Effect of dietary source of n-3 fatty acids on steer growth characteristics

Reference	Diet[1/]	DMI (kg/d)	BW gain (kg)	ADG (kg/d)	Gain/kg feed (g)
Noci et al. (2007)	Grass Only	8.8	162.0	1.0	-
	Safflower oil	8.8	186.0	1.1	-
	Linseed oil	8.8	174.0	1.1	-
Razminowicz et al. (2008)	Grass only	-	Same Live Weight	0.9	96.0
	Cereal based type	-	(560 kg, 17realized :	1.0	97.0
	125 g/kg Flaxseed	-	557±9 kg)	1.1	100.0
He and Armentano (2011)	Control	8.6	114.0	1.3	147.0
	FW × 1	9.4	107.0	1.4	149.0
	FW × 4	9.4	123.0	1.4	151.0
Kim et al. (2009)	Control	-	86.3	0.7	70.0[a]
	10% Whole linseed	-	96.1	0.8	81.0[b]
	15% Whole linseed	-	94.4	0.8	82.0[b]

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

[1/] No FW = no feed withdrawal of equal mixture of flax oil and sunflower oil at 5% of diet; FW × 1 = single feed withdrawal; FW × 4 = feed withdrawal every 8 weeks for 48 h treatments

Table 2.2 Effect of dietary source of n-3 fatty acids on steer growth characteristics (Continue)

Reference	Diet	DMI (kg/d)	BW gain (kg)	ADG (kg/d)	Gain/kg feed (g)
Doreau et al. (2009)	Pastures	10.5	-	-	-
	Roll linseed	10.0	-	-	-
	Extrude linseed	9.9	-	-	-
	Linseed oil	9.9	-	-	-
Kronberg et al. (2011)	Grass grazing only	-		1.04[b]	-
	0.20% of BW/d Flaxseed	-	Before slaughter 499±26 kg	1.09[a]	-
	0.28% of BW/d CSBM	-		0.83[a]	-

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

[1/] CSBM = grass grazing and daily supplement mixture of corn and soybean meal (0.28% of BW/d)

Table 2.3 Effect of dietary source of n-3 fatty acids on steers carcass characteristics.

Reference	Diet[1/]	CCW (kg)[2/]	HCW (kg)[2/]	Fat weight (kg)	Marbling score[3/]	Rib eye area (cm^2)
Noci et al. (2007)	Grass Only	258	-	5.15	-	
	Safflower oil	277	-	5.41	-	
	Linseed oil	267	-	5.33	-	
Kronberg et al. (2011)	Grass grazing only	-	263.3[a]	-	366	-
	0.20% of BW/d Flaxseed	-	281.6[b]	-	367	-
	0.28% of BW/d CSBM	-	275.4[ab]	-	358	-
Kim et al. (2009)	Control	-	344.8	-	2.55 [a]	75.0
	10% Flaxseed	-	351.3	-	3.33[b]	79.0
	15% Flaxseed	-	350.3	-	3.14 [b]	77.0

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

[1/] CSBM = grass grazing and daily supplement mixture of corn and soybean meal (0.28% of BW/d)

[2/]CCW = Cold Carcass Weight

[3/] 300 = slight, 400 = small

Table 2.4 Effect of dietary source of n-3 fatty acids on beef fatty acid composition.

Reference	Diet	Fatty acids (g /100 g fatty acids)						
		C18:2n-6	C18:3n-3	SFA[1/]	PUFA[2/]	n-6 FA	n-3 FA	n-6/n-3 ratio
Noci et al. (2007)	Grass Only	2.53[a]	1.37[a]	43.38[c]	6.65[a]	3.13[a]	2.37	1.46[a]
	Safflower oil	3.36[b]	0.87[b]	39.06[a]	8.02[b]	4.26[b]	1.91	2.24[c]
	Linseed oil	2.93[a]	1.35[a]	41.01[b]	7.49[b]	3.82[b]	2.34	1.72[b]
Razminowicz et al. (2008)	Grass only	3.37	1.74	47.46	10.44	4.54	3.96	1.25[a]
	Cereal based type	2.71	1.13	48.62	8.15	3.64	2.76	1.45[b]
	125 g/kg Flaxseed	2.91	1.37	47.61	9.08	3.84	3.13	1.33[a]
Kim et al. (2009)	Control	3.33	0.09 [a]	50.78 [b]	4.13 [a]	3.66	0.47 [a]	7.78 [b]
	10% Flaxseed	4.98	0.16 [b]	45.21 [a]	6.01 [b]	3.55	0.46 [a]	7.71 [b]
	15% Flaxseed	5.00	0.20 [b]	42.92 [a]	6.10 [b]	3.59	0.51 [b]	7.03 [a]

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

[1/]SFA = Saturated fatty acid

[2/]PUFA = Poly unsaturated fatty acid

Table 2.4 Effect of dietary source of n-3 fatty acids on beef fatty acid composition (continue)

Reference	Diet[1/]	Fatty acids (g /100 g fatty acids)						
		C18:2n-6	C18:3n-3	SFA[2/]	PUFA[3/]	n-6 FA	n-3 FA	n-6/n-3 ratio
Baird et al. (2010)	Control	2.03	0.24 [a]	19.54	3.09 [a]	2.58 [a]	0.51 [a]	5.06 [b]
	400 g/d Flaxseed	2.86	0.32 [b]	19.24	4.93 [b]	3.96 [b]	0.98 [c]	4.04 [a]
	800 g/d Flaxseed	2.36	0.29 [b]	20.05	4.17 [b]	3.28 [b]	0.89 [b]	3.68 [a]
	1,200 g/d Flaxseed	2.07	0.33 [b]	18.84	3.52 [b]	2.79 [a]	0.73 [b]	3.82 [a]
Corazzin et al. (2012)	Control	3.70	0.35 [a]	61.95	4.11	3.76	0.35 [a]	10.74 [b]
	Whole linseed	3.20	0.64 [b]	53.41	8.95	3.31	0.64 [b]	5.17 [a]
Nassu et al. (2011)	Hay							
	No Flaxseed	2.60	0.51 [b]	43.13	5.35	3.97	1.19 [b]	3.32 [b]
	Flaxseed	2.40	1.22 [d]	40.39	5.58	3.36	1.89 [d]	1.78 [a]
	Silage							
	No Flaxseed	2.10	0.31 [a]	42.52	4.16	3.24	0.74 [a]	4.39 [c]
	Flaxseed	2.12	1.06 [c]	40.70	4.89	2.99	1.64 [c]	1.83 [a]

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

2.9 Effect of plant oil and oilseeds on performance, carcass and n-3 fatty acid accumulation in dairy cow's milk

Milk compositions were not affected by oil supplement (Loor et al., 2005; Flowers et al., 2008; Filleau et al., 2011), however report by Rego et al. (2009), supplementation with rapeseed and sunflower oils decreased milk fat content and milk production, with linseed oil having no effect. In general, supplementation of grazing dairy cows with unsaturated fat (0.2 to 1.0 kg/d) significantly decreases milk fat concentration (-8%) and production, as reviewed by Schroeder et al. (2004). However, the responses can be quite variable with the lipid source used. Lawless et al. (1998), who supplemented grazing dairy cows with full-fat soybeans and full-fat rapeseed (1.65 kg/d; approximately 0.65 kg of oil), observed a decrease in milk fat (only for full-fat rapeseed) and protein content without effects on milk and milk solid production, when compared with a control diet. Flowers et al. (2008), who supplemented diets with increasing levels of linseed oil (0.17, 0.34, and 0.51 kg/d), showed no effect on milk fat content and production. Another study using grazing dairy cows supplemented with a mixture of fish and sunflower oils (0.1 kg of fish oil + 0.3 kg of SO/d) reported no significant effect on milk production and composition (AbuGhazaleh and Holmes, 2007). Overall, the available literatures on this topic suggest that supplementation with linseed oil exerts no negative effects on milk fat content, when diets are mainly based on forage. It has been well established that the inclusion of unsaturated fat in dairy cow diets inhibits the *de novo* synthesis of short- and medium-chain fatty acids and increases the concentration of C18 fatty acids, resulting in a more unsaturated milk fat (Chilliard et al., 2007). Odd- and branched-chain fatty acids mainly originate from microbial matter in the rumen, and feeding

unprotected lipid supplements to ruminants induces changes in the rumen microbial population that can result in a lower outflow of this fatty acids. However, it was demonstrated that milk odd- and branched chain fatty acids are highly correlated with milk short- and med- chain fatty acid, and not with their duodenal concentrations (Glasser et al., 2007), suggesting that its concentration in milk would be mainly regulated at the mammary level (Glasser et al., 2008). The proportion of C18:0 was higher when feeding oil sources, which it is in line with previous results (Rego et al., 2005). Increases in oleic acid can be attributed to differences in C18:1 intake (and duodenal flow of C18:1 *cis*-9) and to availability of C18:0 for Δ9-desaturation in the mammary gland, which is linked to a less complete biohydrogenation of C18:3n-3 than of C18:2n-6 (Glasser et al., 2008). Linoleic acid was the highest, with sunflower oil reflecting the higher intake of C18:2n-6. The report of Rego et al., (2009) did not detect any positive additive or synergistic effect between pasture and linseed oil because both are good sources of C18:3n-3. Surprisingly, linseed oil lowered this fatty acid in milk fat, yet this strategy failed to promote milk fat enrichment in n-3 fatty acid. Conversely, Flowers et al. (2008), who supplemented grazing dairy cows with increasing levels of linseed oil (0.17, 0.34, and 0.51 kg/d), observed an increase in C18:3n-3. A possible explanation for the reduction in C18:3n-3 in the milk fat of cows fed fat-supplemented diets might be a reduction of pasture intake. A reduction of pasture DMI (-3.9 kg of DM) after lipid supplementation (0.5 and 1.0 kg of partially hydrogenated oil) has been reported by Schroeder et al. (2002). FA yields reflect the total fat yield and milk fatty acid pattern. In contrast to supplementation with rapeseed oil and sunflower oil, linseed oil supplementation did not depress total milk fat yield, and this was reflected in yields of individual milk fatty acid and partial sums of FA.

Table 2.5 Effect of oil source on dry matter intake (DMI), body weight (BW), milk yield and milk composition

Reference	Diet[1/]	Roughage	Milk yield (kg/d)	Milk composition (%)			DMI (kg/d)	BW(kg)
				Fat	Protein	Lactose		
Loor et al. (2005)	Fish oil	Hay	27.2	2.56	2.97	4.77	17.1	-
	Linseed oil		24.4	2.75	3.18	4.71	17.2	-
	Sunflower oil		26.5	2.62	3.50	4.68	19.3	-
Bu et al. (2007)	Control	TMR (tie-stall barn)	21.7 [a]	3.49	3.15	4.99	16.2	-
	4% Soybean oil		25.8 [b]	3.21	3.20	5.00	16.2	-
	4% Linseed oil		25.0 [b]	3.26	3.17	5.00	15.9	-
	2% Soybean oil + 2 % Linseed oil		25.2 [b]	3.30	3.15	5.04	16.2	-
Flowers et al. (2008)	Control	Alfalfa-fescue-clover-weed mixed pasture	18.93	3.23	3.03	4.56	-	631
	170 g/d Linseed oil		18.50	3.44	3.19	4.40	-	599
	340 g/d Linseed oil		19.60	3.35	3.12	4.59	-	600
	510 g/d Linseed oil		19.10	3.27	3.08	4.66	-	604

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

Table 2.5 Effect of oil source on dry matter intake (DMI), body weight (BW), milk yield and milk composition (Continue)

Reference	Diet	Roughage	Milk yield (kg/d)	Milk composition (%)			DMI (kg/d)	BW (kg)
				Fat	Protein	Lactose		
Rego et al. (2009)	Control	Pasture (ryegrass)	22.2	3.75[a]	3.51	-	-	555[a]
	Sunflower oil		22.0	3.27[b]	3.45	-	-	564[ab]
	Linseed oil		22.2	3.59[a]	3.43	-	-	566[b]
Chilliard et al. (2009)	Control	TMR (corn silage, grass hay)	23.0[b]	4.11[b]	3.40	4.83	19.8[b]	717
	Crude linseed		21.5[b]	4.54[b]	3.46	4.82	19.5[b]	714
	Extrude linseed		20.8[ab]	3.53[a]	3.33	4.80	16.7[ab]	708
	Linseed oil		18.9[a]	3.23[a]	3.47	4.86	14.7[a]	708
Filleau et al. (2011)	Control	Red cover silage	31.1	3.96	3.30	4.60	23.3	-
	Linseed oil		32.3	3.86	3.20	4.60	23.4	-
	Sunflower oil		32.3	3.64	3.26	4.63	23.0	-
	Camellia oil		31.2	3.93	3.23	4.61	23.3	-

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

Table 2.6 Effect of oil source on fatty acid (mg/g) milk fat

Reference	Diet	Milk fatty acid(g/kg fatty acid)							
		SFA[1/]	C18:2n-6	C18:3n-3	n-6 FA	n-3 FA	MUFA[2/]	PUFA[3/]	n-6/n-3
Bu et al. (2007)	Control	72.06[b]	2.35 [a]	0.37[a]	2.42	0.60[a]	24.24[c]	3.75[c]	4.25[b]
	4% Soybean oil	66.45[a]	2.84 [b]	0.37[a]	2.89	0.69[a]	27.45[a]	6.18[b]	4.35[b]
	4% Linseed oil	63.61[a]	2.13 [a]	1.00[b]	2.30	1.13[b]	31.37[b]	5.08[a]	2.13[c]
	2% Soybean oil + 2 % Linseed oil	66.25 [a]	2.40[ab]	0.44[a]	2.50	0.80 [a]	28.73[ab]	5.20 [a]	3.44[a]
Rego et al., (2009)	Control	48.40	11.20 [b]	6.00 [c]	1.35[a]	0.96 [b]	26.10[a]	1.97[c]	1.41[b]
	Sunflower oil	33.90	12.50 [c]	4.20 [a]	1.82[b]	0.59 [a]	34.10[c]	1.83[b]	3.08[c]
	Linseed oil	38.50	9.90 [a]	5.30 [b]	1.92[b]	1.83 [b]	31.10[b]	1.85[b]	1.04[a]

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

[1/]SFA = Saturated fatty acid

[2/]MUFA = Monounsaturated fatty acid

[3/]PUFA = Polyunsaturated fatty acid

Table 2.6 Effect of oil source on fatty acid (mg/g) milk fat (Continue)

Reference	Diet	Milk fatty acid(g/kg fatty acid)							
		SFA	**C18:2n-6**	**C18:3n-3**	**n-6 FA**	**n-3 FA**	**MUFA**	**PUFA**	**n-6/n-3**
Chilliard et al. (2009)	Control	68.95^{d}	2.59^{b}	0.67^{a}	2.59^{a}	0.67^{a}	26.14^{a}	4.42^{b}	3.87
	Crude linseed	66.27^{c}	2.05^{a}	0.65^{a}	2.05^{a}	0.65^{a}	29.89^{b}	3.45^{a}	3.15
	Extrude linseed	53.74^{b}	4.21^{b}	1.20^{b}	4.21^{b}	1.20^{b}	38.61^{c}	6.94^{c}	3.50
	Linseed oil	42.38^{a}	7.17^{c}	0.54^{a}	7.17^{c}	0.54^{a}	48.48^{d}	8.48^{d}	3.27
Filleau et al. (2011)	Control	71.00^{b}	2.08	1.15	2.96^{a}	1.45	23.20^{a}	5.33^{a}	2.04^{a}
	Linseed oil	66.10^{a}	1.99	1.07	2.97^{a}	1.32	28.10^{b}	5.40^{b}	2.25^{a}
	Sunflower oil	65.90^{a}	2.55	1.05	3.50^{b}	1.31	27.70^{b}	5.99^{b}	2.67^{b}
	Camellia oil	65.40^{a}	2.10	1.13	3.23^{a}	1.49	28.20^{b}	5.93^{b}	2.17^{a}

$^{a, b, c}$ Means within a column with different superscripts differ ($P < 0.05$)

$^{1/}$SFA = Saturated fatty acid

$^{2/}$MUFA = Monounsaturated fatty acid

$^{3/}$PUFA = Polyunsaturated fatty acid

2.10 Energy and protein requirement of beef cattle

2.10.1 Energy requirement

2.10.1.1 Energy unit

Energy is defined as the potential to do work and can be measured only in reference to define, standard conditions; thus, all defined units are equally absolute.

Nutritionists now standardize their combustion calorimeters using specifically purified benzoic acid, the energy content of which has been determined in electrical units and computed in terms of joules/g mole. The calorie has been standardized to equal 4.184 joules and is approximately equal to the heat required to raise the temperature of 1 g of water from 16.5° to 17.5° C.

In practice the calorie is a small amount of energy; thus, the kilocalorie (1 kcal = 1,000 calories) and megacalorie (1 Mcal = 1,000 kcal) are more convenient for use in conjunction with animal feeding standards.

2.10.1.2 Expressing energy values of feeds

1. Gross energy (GE) or heat of combustion is the energy released as heat when an organic substance is completely oxidized to carbon dioxide and water. The main source of GE (the primary gas being methane) is microbial fermentation, which also results in heat production. GE is related to chemical composition, but it does not provide any information regarding availability of that energy to the animal. Thus, GE is of limited use for assessing the value of a particular diet or dietary ingredient as an energy source for the animal.

2. Digestible energy (DE) is termed GE of the food minus the energy lost in the feces (FE).

$$DE = GE - FE$$

DE as a proportion of GE may vary from 0.3 for a very mature, weathered forage to nearly 0.9 for processed, high quality cereal grains. DE has some value for feed evaluation because it reflects diet digestibility and can be measured with relative ease; however, DE fails to consider several major losses of energy associated with digestion and metabolism of food. As a result, DE overestimates the value of high-fiber feedstuffs such as hays or straws relative to low-fiber, highly digestible feedstuffs such as grains.

3. Total digestible nutrients (TDN) is similar to DE but includes a correction for digestible protein. TDN has no particular advantages or disadvantages over DE as the unit to describe feed values or to express the energy requirements of the animal. TDN can be converted to DE by the equation

$$1 \text{ kg TDN} = 4.4 \text{ Mcal DE}$$

4. Metabolizable energy (ME) is defined as DE minus fecal energy (FE), urinary energy (UE), and gaseous energy (GE) losses, or

$$ME = DE - (UE + GE).$$

ME is an estimate of the energy available to the animal and represents an accounting progression to assess food energy values and animal requirements. ME, however, has many of the same weaknesses as DE; and because UE and GE are highly predictable from DE, ME and DE are strongly correlated. The ME values were estimated as

$$ME = DE * 0.82$$

5. Net energy (NE) are that animal requirements stated as net energy are independent of the diet, and the energy value of feeds for different

physiological functions are estimated separately for example, NE requirement for maintenance (NE_m), NE requirement for growth (NE_g), NE requirement for lactation (NE_l). This requires, however, that each feed must be assigned multiple NE values because the value varies with the function for which energy is used by the animal. Alternatively, the animal's energy requirement for various physiological functions may be expressed in terms of a single NE value, provided the relationships among efficiencies of utilization of ME for different functions are known.

Relationships for converting ME values to NE_m and NE_g (Mcal/kg DM) have been reported by Garrett (1980) and are

$$NE_m = 1.37\ ME - 0.138\ ME^2 + 0.0105\ ME^3 - 1.12$$

$$NE_g = 1.42\ ME - 0.174\ ME^2 + 0.0122\ ME^3 - 1.65$$

Caution should be exercised in use of these equations for predicting NE_m or NE_g values for individual feed ingredients or for feeds outside the ranges indicated above. The relationship between DE and ME can vary considerably among feed ingredients or diets as a result of differences in intake, rate of digestion and passage, and composition (for example, fiber vs starch vs fat). In addition, conversion of ME to NE_m or NE_g may vary beyond that associated with variation in dietary ME in part because of differences in composition of absorbed nutrients.

2.10.1.3 Measurement of maintenance requirement

The maintenance requirement for energy has been defined as the amount of feed energy intake that will result in no net loss or gain of energy from the tissues of the animal body. Processes or functions comprising maintenance energy requirements include body temperature regulation, essential metabolic processes, and physical activity. Energy maintenance does

not necessarily equate to maintenance of body fat, body protein, or body weight.

Changes in body composition and composition of weight change in growing, pregnant, or lactating cattle are problematic with this approach. Expression of the results in terms of ME or NE requirements depends on use of information from other approaches.

$$NE_R = NE_m + NE_g + NE_p + NE_l$$

The California Net Energy System (CNES), proposed by Lofgreen and Garrett (1968) and adopted in NRC (2000), assigned 2 NE values to each feed NE_m for maintenance and NE_g for energy gain. Animal requirements were stated using the same terms. The terms, NE_m and NE_g are related to k_m and k_p as follows :

$$NE_m = k_m \times ME$$

$$NE_g = k_g \times ME$$

where

k_m is the efficiency of utilization of ME for maintenance.

$$k_m = NE_m/ME_m$$

k_g is the efficiency of utilization of ME for RE in growing animals.

Dietary ME was calculated from TDN. Subsequently, NE_m and NE_g were calculated from ME by use of the NRC (1984) equations. The NEm requirements of beef cattle have been estimated as :

$$NE_m = 0.077 \text{ Mcal/EBW}^{0.75}$$

EBW is the average empty body weight in kilograms (Lofgreen and Garrett, 1968; Garrett, 1980).

This expression was derived using data from, primarily, growing steers and heifers of British ancestry that were penned in generally non stressful environments. Effects of activity and environment are implicitly incorporated into NE_m in this system. Similarly, influences of increased feed during the feeding period, altered activity, or environmental effects differing from those at maintenance are implicitly incorporated into estimates of NE_g. Application to differing situations requires appropriate adjustments.

2.10.1.4 Energy requirements for growing cattle

Net energy for gain (NE_g) is defined herein as the energy content of the tissue deposited, which is a function of the proportion of fat and protein in the empty body tissue gain (Garrett et al., 1959; fat contains 9.367 kcal/g and nonfat organic matter contains an average of 5.686 kcal/g).

The energy content of weight gain across a wide range of ME intakes and rates of gain was described in equation formed by Garrett (1980), equations that were adapted by the Subcommittee on Beef Nutrition for use in the preceding edition of NRC (2000). The equation developed with British-breed steers describes the relationship between retained energy (RE) and empty body weight gain (EBG) for a given empty body weight (EBW);

$$RE = 0.0635 \times EBW^{0.75} \times EBW^{1.097}$$

To predict NE_g required for SBW and SWG, EBW and EBG were converted to 4 percent shrunk live weight gain with the following equations developed for use in the 1984 edition of NRC (2000) from the Garrett (1980) body composition data base :

$$EBW = 0.88 \times SBW + 14.6 \times NE_m - 22.9 \ (r = 0.98)$$

$$EBG = 0.93 \times SWG + 0.174 \times NE_m - 0.28 \ (r = 0.96)$$

or with constants of 0.891 * SBW and 0.956 * SWG. These equations were rearranged to predict EBG and SWG;

$$EBG = 12.341 \times (RE/EBW^{0.75})^{0.9116}$$

$$= 12.341 \times EBW^{-0.6837} \times RE^{0.9116}$$

$$SWG = 13.91 \times RE^{0.9116} \times SBW^{-0.6837}$$

In the rearranged equations, RE is equivalent to NE available for gain. Thus, if intake is known, the net energy required for gain (NEFG) may be calculated as :

$$NEFG = DMI \times NE_g$$

NEFG can then be substituted into equations for RE to predict ADG.

2.10.2 Protein requirement of beef cattle

The Nutrient Requirements of Dairy Cattle (NRC, 1989) and by the Agricultural and Food Research Council (1992) was adopted change from the crude protein (CP) system to the metabolizable protein (MP) system. MP is defined as the true protein absorbed by the intestine, supplied by microbial protein and undegraded intake protein (UIP). Crude protein can be calculated from the sum of UIP and degraded intake protein (DIP).

$$CP = UIP + DIP \ (NRC, 2000)$$

MP requirements estimates of daily crude protein requirements can be obtained by dividing MP amounts by a value between 0.64 and 0.80, depending on degradability of protein in the feed. The coefficients of 0.64 and 0.80 apply when all of the protein is degradable and undegradable, respectively.

MP = 0.64 DIP (NRC, 2000)

MP = 0.80 UIP (NRC, 2000)

2.10.2.1 Microbial protein synthesis

Bacterial crude protein (BCP) can supply from 50 percent (NRC, 1985; Spicer et al., 1986) to essentially all the MP required by beef cattle, depending on the UIP content of the diet. Clearly, efficiency of synthesis of BCP is critical to meeting the protein requirements of beef cattle economically; therefore, prediction of BCP synthesis is an important component of the MP system. Burroughs et al. (1974) proposed that BCP synthesis averaged 13.05 percent of total digestible nutrients (TDN). In Ruminant Nitrogen Usage (NRC, 1985), two equations were developed to predict BCP synthesis. Both forage and concentrate intakes (percent of body weight) are needed to calculate the less than 40 percent forage equation :

BCP (g/d) = 6.25 TDN (kg intake/day) × (8.63 + (14.6 × forage intake) – (5.18 × forage intake)^2 + (0.59 × concentrate intake))

The more than 40 percent forage equation was developed primarily for dairy cattle :

BCP (g/d) = 6.25 × (-31.86 + 26.12 TDN (kg intake/day))

or BCP = 12.8 TDN intake (NRC, 2000)

The value 13 g BCP/100 g TDN for BCP synthesis is a good generalization but it does not fit all situations. At both high- and low-ration digestibilities, efficiency may be lower but for different reasons. Logically, the higher digestibility diets are based primarily on grain. High grain finishing diets have lower rumen pH values and slower microbial turnover, which leads to lower efficiency for converting fermented protein and energy to BCP.

The requirement for rumen degradable protein (RDP) (including nonprotein nitrogen; NPN) is considered equal to BCP synthesis. This assumes that the loss of ammonia from the rumen as a result of flushing to the duodenum and absorption through the rumen wall is equal to the amount of recycled nitrogen.

RDP = BCP (NRC, 2000)

2.10.2.2 Metabolizable protein requirement

The Institute National de la Recherche Agronomique (INRA) (1988), using nitrogen balance studies that included scurf, urinary, and metabolic fecal losses, determined that the maintenance requirement was 3.25 g MP/kg $SBW^{0.75}$. This system simplifies calculations and is based on metabolic body weight ($BW^{0.75}$), as are maintenance energy requirements. Their diets were high in roughage and were based on the assumption that

0.13 TDN = BCP

If actual BCP synthesis efficiency was less than 0.13, the estimate of the maintenance would be less than 3.8 g MP/kg $BW^{0.75}$. In NRC (2000) used

MP_M = 3.8 g MP/kg $BW^{0.75}$

Because the maintenance requirement estimated was based on animal growth rather than on nitrogen balance.

3. Conversion of metabolizable protein to net protein

A constant conversion of MP to net protein (NP) for gain of 0.5 and to NP for milk of 0.65 was assumed (NRC, 1985). These efficiency values are based on two components the biological value of the protein and the efficiency of use of an “ideal mixture of amino acids” (Oldham, 1987). Oldham (1987) suggests that

the efficiency value is 0.85 for all physiological functions. Efficiency of use for gain is not likely to be constant across body weights (maturity) and rates of gain. The INRA (1988) system assumes a decreasing efficiency as body weight increases. This was confirmed by Ainslie et al. (1993) and Wilkerson et al. (1993). Based on these data, the following equation is used :

If EQEBW $\leq$ 300 kg,

Percent efficiency of MP to NP = 83.4 – (0.114 × EQEBW), otherwise 49.2,

$$EQSBW = SBW \times (SRW/FSBW)$$

where;

EQSBW is equivalent shrunk body weight in kilograms.

SBW is shrunk body weight being evaluated,

SRW is standard reference weight for the expected final body fat

FSBW is final shrunk body weight at the expected final body fat

The equation predicts a conversion efficiency of MP to NP of 66.3 percent for a 150 kg calf. A 300 kg steer has an efficiency of only 49.2 percent. For cattle weighing more than 300 kg, this maintains similar protein requirements to previous NRC publications (NRC, 1984, 1985) and recognizes the low CP requirements of cattle weighing more than 400 kg (Preston, 1982).

Given the relationship between energy retained and protein content of gain, protein content of SWG is given as (NRC, 1984) :

$$\text{Protein retained} = SWG \times (268 - (29.4 \times (RE/SWG))); r^2 = 0.96$$

2.11 Energy and protein requirement of dairy cattle

2.11.1 Energy requirement of dairy cattle

2.11.1.1 Energy value of feed

In NRC (2001) the concentrations (percent of dry matter) of truly digestible non fiber carbohydrate (NFC), CP, ether extract (EE), and NDF for each feed areestimated (Weiss et al, 1992) using

Truly digestible NFC (tdNFC)

$$= 0.98\ (100 - [(NDF - NDICP) + CP + EE + Ash]) \times PAF$$

Truly digestible CP for forages (tdCPf)

$$= CP \times \exp[-1.2 \times (ADICP/CP)]$$

Truly digestible CP for concentrates (tdCPc)

$$= [1 - (0.4 \times (ADICP/CP))] \times CP$$

Truly digestible FA (tdFA)

$$= FA$$ Note : If EE < 1, then FA = 0

Truly digestible NDF (tdNDF)

$$= 0.75 \times (NDFn - L) \times [1 - (L/NDFn)^{0.667}]$$

when; all values are expressed as a percent of dry matter (DM).

NDICP = neutral detergent insoluble N × 6.25,

PAF = processing adjustment factor (Table 2.7)

ADICP = acid detergent insoluble N × 6.25

FA = fatty acids (i.e., EE - 1),

L = acid detergent lignin

NDFn = NDF - NDICP.

Ether extract does not represent a nutritionally uniform fract-

ion and there fore does not have a constant digestibility across feedstuffs. Fatty acids (FA) are a uniform fraction with a true digestibility of 95 to 100 percent when diets contain 3percent or less EE (Palmquist, 1991). A value of 100 percent digestibility was chosen. FA content of feed can be estimated as FA = EE - 1 (Allen, 2000). A more accurate approach would be to measure FA directly. In all equations listed below, measured FA or EE - 1 can be used to represent the FA fraction.

The equations of true digestible NFC, CPf, CPc, FA, and NDF are based on true digestibility, but TDN is based on apparent digestibility; therefore, metabolic fecal TDN must be subtracted from the sum of the digestible fractions. Weiss et al. (1992) determined that, on average, metabolic fecal TDN equaled 7. The TDN_{1X} is then calculated using equation

$$TDN_{1X}\,(\%) = tdNFC + tdCP + (tdFA \times 2.25) + tdNDF - 7$$

Table 2.7 Processing adjustment factors (PAF) for NFC

Feedstuff	PAF
Bakery waste	1.04
Barley grain, rolled	1.04
Bread	1.04
Cereal meal	1.04
Chocolate meal	1.04
Cookie meal	1.04
Corn grain, cracked dry	0.95
Corn grain, ground	1.00
Corn grain, ground high moisture	1.04
Corn and cob meal, ground high moisture	1.04

For feeds not shown PAF = 1.0

Table 2.7 Processing adjustment factors (PAF) for NFC (Continue)

Feedstuff	PAF
Corn grain, steam flaked	1.04
Corn silage, normal	0.94
Corn silage, mature	0.87
Molasses	1.04
Wheat grain, rolled	1.00
All other feeds	1.04

For feeds not shown PAF = 1.0

Different equations are used to estimate TDN for animal protein meals and fat supplements.

Animal protein meals

Animal products contain no structural carbohydrates; however, certain animal products contain substantial amounts of neutral detergent insoluble residue. Because this material is not cellulose, hemicellulose, or lignin, the above equations can not be used. For those feeds, TDN_{1X} was estimated using :

$$TDN_{1X} = (CP_{digest} \times CP) + (FA \times 2.25) + 0.98(100 - CP - Ash - EE) - 7$$

Where CP_{digest} = estimated true digestibility of CP (Table 2.8) and FA = EE -

Fat supplements

The TDN_{1X} values of different fat supplements were calculated from measured fatty acid digestibility. Partial digestion coefficients (Table 2.9) of fatty acids from supplemental fat sources were determined indirectly by difference ((additional fatty acid intake during fat supplementation minus additional fecal fatty acid output during fat supplementation)/(additional fatty acid intake during fat supplementation); (Grummer, 1988). Assumptions associated with this method are that endogenous lipid

Table 2.8 True digestibility coefficients of CP used to estimate TDN_{1X} values of animal-based feedstuffs.

Feedstuff	True digestibility
Blood meal, batch dried	0.75
Blood meal, ring dried	0.86
Hydrolyzed feather meal	0.78
Hydrolyzed feather meal with viscera	0.81
Fish meal (Menhaden)	0.94
Fish meal (Anchovy)	0.95
Meat and bone meal	0.80
Meat meal	0.92
Whey	1.00

remains constant, and digestibility of fatty acids in the basal diet does not change when supplemental fat is fed. For fat sources containing triglycerides (tallow, partially hydrogenated tallow, and vegetable oil), ether extract was assumed to contain 90% fatty acids and 10% glycerol, and the glycerol was assumed to be 100% digestible at 1X. In the experiments used to determine fat digestibility, cows were fed at approximately 3X maintenance. Therefore, the original values were divided by 0.92 to adjust values to TDN_{1X}. After adjusting digestibility for intake (Table 2.9), digestible fat was multiplied by 2.25 to convert to TDN_{1X}.

For fat sources that contain glycerol :

$$TDN_{1X}\ (\%) = (EE \times 0.1) + [FAdigest \times (EE \times 0.9) \times 2.25]$$

For fat sources that do not contain glycerol :

$$TDN_{1X}\ (\%) = (EE \times FAdigest\ ) \times 2.25$$

where FAdigest = digestibility coefficients for fatty acids (Table 2.9).

Table 2.9 True digestibilities at maintenance (assumed 8% increase in digestibility compared with 3X maintenance) of fatty acids from various fat sources.

Fat	Fat type	True digestibility
Calcium salts of fatty acids	Fatty acids	0.86
Hydrolyzed tallow fatty acids	Fatty acids	0.79
Partially hydrogenated tallow	Fat plus glycerol	0.43
Tallow	Fat plus glycerol	0.68
Vegetable oil	Fat plus glycerol	0.86

2.11.1.2 Estimating DE of feeds

Crampton et al. (1957) and Swift (1957) computed that the gross energy of TDN is 4.409 Mcal/kg. Because nutrients have different heats of combustion (e.g.,4.2 Mcal/kg for carbohydrates, 5.6 Mcal/kg for protein, 9.4 Mcal/kg for long chain fatty acids, and 4.3 Mcal/kg for glycerol; Maynard et al., 1979), the gross energy value of TDN is not constant among feeds. The gross energy of TDN of a feed that has a high proportion of its TDN provided by protein will be greater than 4.409. Conversely the gross energy of TDN of a feed with a high proportion of its TDN provided by carbohydrate or fat will be less than 4.409. Therefore, the calculation of DE as 0.04409 × TDN (%) as in the previous edition (NRC, 1989) was abandoned. Digestible energy was calculated by multiplying the estimated digestible nutrient concentrations by their heats of combustion. Since DE is based on apparent digestibility are based on true digestibility, a correction for metabolic fecal energy is needed. The heat of combustion of metabolic fecal TDN was

assumed to be 4.4 Mcal/kg; metabolic fecal DE = 7 × 0.044 = 0.3 Mcal/kg.

For most feeds :

DE_{1X} (Mcal/kg) = [(tdNFC/100) × 4.2] + [(tdNDF/100) × 4.2] + [(tdCP/100) × 5.6]+[(FA/100) × 9.4] –0.3

For animal protein meals :

DE_{1X} (Mcal/kg) = [(tdNFC/100) × 4.2] + [(tdCP/100) × 5.6] +[(FA/100) × 9.4] –0.3

For fat supplements with glycerol :

DE_{1X} (Mcal/kg) = [9.4 × (FAdigest × 0.9 × (EE/100))] + [4.3 × 0.1 × (EE/100)]

For fat supplements without glycerol :

DE_{1X} (Mcal/kg) = [9.4 × (FAdigest × 0.9 × (EE/100))]

In the above equations, tdNFC, tdNDF, tdCP, and FA are expressed as %DM.

2.11.1.3 Estimating DE at actual intake

The digestibility of diets fed to dairy cows is reduce with increasing feed intake (Tyrrell and Moe, 1975). This reduces the energy value of any given diet as feed intake increases. This is particularly important in today's high producing dairy cows where it is not uncommon for feed intake to exceed 4 times maintenance level of intake. The rate of decline in digestibility with level of feeding has been shown to be related to digestibility of the diet at maintenance (Wagner and Loosli, 1967). Diets with high digestibility at maintenance exhibit a greater rate of depression in digestibility with level of feeding than diets with low digestibility fed at maintenance. Previous National Research Council reports (NRC, 1978, 1989) used a constant depression of 4% per multiple of maintenance to adjust maintenance energy values to 3X maintenance energy values. Using this method of discounting, the percentage unit decline in TDN for a diet containing 75% TDN_{1X} would be 3% units per multiple of maintenance, while the depression for a diet

containing 60% TDN_{1X} would be 2.4 units. The differences in rate of depression in digestibility are generally negligible for diet shaving maintenance TDN values of 60% or less. It was apparent that the rate of decline in digestibility with level of feeding was a function of the maintenance digestibility of the diets fed :

$$\text{TDN percentage unit decline} = 0.18TDN_{1X} - 10.3 \ (r^2 = 0.85)$$

Because DE, not TDN, is used to calculate ME and NEL, this equation was converted so that a percent discount, not a TDN percent age unit discount, was calculated :

$$\text{Discount} = [(TDN_{1X} - [(0.18 \times TDN_{1X}) - 10.3]) \times \text{Intake})]/TDN_{1X}$$

where TDN_{1X} is as a percent of dry matter and is for the entire diet, not the individual feed ,and intake is expressed as incremental intake above maintenance (e.g., for a cow consuming 3X maintenance, intake above maintenance = 2).

For example, for a cow consuming a diet that contains 74% TDN_{1X} at 3X intake, digestibility would be expected to be 0.918 times the value obtained at maintenance.

2.11.1.4 Estimating ME at actual intake

ME at production levels of intake (ME_p) should be calculated from DE_p. Equation was developed with diets containing about 3% ether extract, but because the efficiency of converting DE from fat into ME is approximately 100% (Andrews et al.,1991; Romo et al., 1996), Equation under estimates ME of high fat diets. A theoretical approach was used to adjust ME values of feeds with more than 3% EE. Assuming a feed with 100% EE has ME = DE and subtracting that equation from ME (Mcal/kg) = (1.01 × DE) - 0.45 and dividing by the change in EE concentration (100 -3) yields the expression : 0.000103 × DE + 0.00464 change in ME per increase in EE content (percentage unit). The DE term was assumed to be negligible; therefore, MEp values of feeds with more than 3% EE were increased

by 0.0046 per percentage unit increase in EE content above 3%. For feeds with less than 3% EE, Equation is used to calculate MEp.

$$ME_p = [1.01 \times (DE_p) - 0.45] + [0.0046 \times (EE - 3)]$$

where DE_p is Mcal/kg and EE is %DM.

For fat supplements, ME_p (Mcal/kg) = DE_p (Mcal/kg)

2.11.1.5 Estimating NE_L at actual intake

Vermorel and Coulon (1998) using equation NE_L (Mcal/kg) = 0.0245 × TDN (%) - 0.12, a feed with 40% TDN (DE = 1.76 Mcal/kg) has an efficiency of converting DE to NE_{L3X} of 0.49 and for a feed with a TDN of 90% (DE = 3.97 Mcal/kg), the efficiency is 0.53. That range in efficiencies is less than would be expected among feeds when DE is converted to NEL. To overcome this problem, an equation derived by Moe and Tyrrell (1972) to convert ME_p to NE_L at production levels of intake (NE_{Lp}) was chosen to replace the previous TDN-based NEL equation.

$$NE_{LP} = [0.703 \times ME_P \text{ (Mcal/kg)}] - 0.19 \quad \text{(Moe and Tyrell, 1972)}$$

A modification was made to adjust for improved metabolic efficiency of fat. The average efficiency of converting ME from fat to NE_L is 0.80 [(sd = 0.05; N=3); (Andrews et al., 1991; Romo et al., 1996)]. The same approach as discussed above to adjust ME_p for fat content was used to account for increased efficiency of converting ME from fat to NE_L. The resulting term was : (0.097 × ME_p + 0.19)/97 increase in NE_L per percentage unit increase in feed EE content above 3%. For feeds with less than 3% EE, Equation is used to calculate NEL_p.

$$NE_{LP} = ([0.703 \times ME_P \text{ (Mcal/kg)}] - 0.19) + ([(0.097 \times ME_P + 0.19)/97] \times [EE - 3])$$

where ME_p is Mcal/kg and EE is %DM.

For fat supplements, NE_{Lp} (Mcal/kg) = 0.8 × MEp (Mcal/kg)

2.11.1.4 Estimating net energy of feeds for maintenance and gain

The equations used to estimate the net energy for energy for maintenance (NE_M) and net energy for gain (NE_G) used for beef cattle (NRC, 1996) were retained. The NE_M and NE_G content of feeds assumed dry matter intake at 3 times maintenance and are calculated by multiplying DE_{1X} (described above) by 0.82 to obtain ME (NRC, 1996). That ME value is then converted to NE_M and NE_G using the following relationships (Garrett, 1980) :

$$NE_M = 1.37\ ME - 0.138\ ME^2 + 0.0105\ ME^3 - 1.12$$

$$NE_G = 1.42\ ME - 0.174\ ME^2 + 0.0122\ ME^3 - 1.65$$

where ME, NE_M, and NE_G are expressed in Mcal/kg.

Those equations are not appropriate for fat supplements. For those feeds, $ME_p = DE_p$, and the same efficiency (0.80) of converting ME to NE_L was used to convert ME to NE_M. The efficiency of converting ME to NE_G was set at 0.55 for fat supplements.

2.11.2 Protein requirement of dairy cattle

Previous NRC (1985, 1989) requirements for MP were based on the factorial method. The same approach is used in this edition. The protein requirement includes that needed for maintenance and production. The maintenance requirement consists of urinary endogenous N, scurf N (skin, skin secretions, and hair), and metabolic fecal N. The requirement for production includes the protein needed for the conceptus, growth, and lactation.

$$MP_R = MP_M \pm MP_G + MP_L$$

where MP_R(g/d) = Metabolizable protein requirement

MP_M(g/d) = Metabolizable protein requirement for maintenance

MP_G(g/d) = Metabolizable protein requirement for growth

MP_L(g/d) = Metabolizable protein requirement for lactation

2.11.2.1 Metabolizable protein requirement for maintenance (MP_M)

The protein system used in NRC (2001) is based on metabolizable protein (MP).

$$MP_M\ (g) = MP_U + MP_{SH} + MP_{MFP}$$

where MP_U is MP requirement for Endogenous urinary protein (UPN)

$MP_U = UPN/0.67$

$UPN\ (g/day) = 2.75 \times (\text{Live weight})^{0.5}$

$MP_U = 4.1 \times (\text{Live weight})^{0.5}$

MP_{SH} is MP requirement for Scurf and hair (SPN; skin, skin secretion, hair)

$MP_{SH} = SPN/0.67$

$SPN = 0.2 \times (\text{Live weight})^{0.60}$

$MP_{SH} = 0.3 \times (\text{Live weight})^{0.60}$

MP_{MFP} is MP requirement for metabolic fecal protein

MP_{MFP} = MFP - (bacteria + bacterial debris in cecum, large intestine + keratinized cell + others)

$MFP\ (g/day) = 30 \times$ Dry Matter Intake (kg.)

$MP_{MFP} = [(DMI \times 30) - 0.50((Bact\ MP/0.8) - Bact\ MP)] + Endogenous\ MP/0.67$

2.11.2.2 Protein requirement for growth (MP_G)

$$MP_G = NP_G/EffMP\_NP_G$$

where $NP_G = SWG \times (268-(29.4 \times (RE/SWG)))$

RE = $0.0635 \times EQEBW^{0.75} \times EQEBG^{1.097}$

EQEBW = $0.891 \times EQSBW$

EQEBG = $0.956 \times SWG$

EQSBW = $SBW \times (478/MSBW)$

MSBW = 500 kg

SBW = 0.96 BW

If (EQSBW (Equivalent shrunk BW) $\leq$ 478 kg then efficiency of use of MP for growth :

$$EffMP\_NP_G = (83.4-(0.114 \times EQSBW))/100$$

If (EQSBW (Equivalent shrunk BW) $\geq$ 478 kg then efficiency of use of MP for growth :

$$EffMP\_NP_G = 0.28908$$

2.11.2.1 Protein requirement for lactation (MP_L)

The equation to calculate MP requirement for lactation (MP_L).

$$MP_L \text{ (g/d)} = (Y \text{ Protein}/0.67) \times 1000$$

where Y Protein = milk production (kg/d) × (milk true protein/100)

2.12 References

AbuGhazaleh, A. A., and Holmes, L. D. (2007). Diet supplementation with fish oil and sunflower oil to increase conjugated linoleic acid levels in milk fat of partially grazing dairy cows. **J. Dairy Sci.** 90 : 2897–2904.

Agricultural and Food Research Council. (1992). Nutritive requirements of ruminant animals : Protein. **Nutr. Abstr. Rev. Ser. B** 62 : 787–835.

Allen, M. S. (2000). Effects of diet on short-term regulation of feed intake by lactating dairy cattle. **J. Dairy Sci.** 83 : 1598-1624.

Andrews, S. M., Tyrrell, H. F., Reynolds, C. K., and Erdman, M. D. (1991). Net energy for lactation of calcium soaps of long-chain fatty acids for cows fed silage-based diets. **J. Dairy Sci.** 74 : 2588.

Baird, L. G., Dawson, L. E. R., Young, I. S. and O'Connell, N. E. (2010). Lipid content and fatty acid composition of the digital cushion of bulls offered different amounts of linseed. **J Anim. Sci.** 88 : 2403-2409.

Bauchart, D. (1993). Lipid absorption and transport in ruminants. **J. Dairy Sci.** 76(12) : 3864-3881.

Bauman, D. E., and Lock, A. L. (2006). Concepts in lipid digestion and metabolism in dairy cows. **Proc. Tri-State. Dairy Nutr. Conf.** 1-14.

Bayrak, A., Kiralan, M., Ipek, A., Arslan, N., Cosge B., and Khawar, K.M. (2010). Fatty acid compositions of linseed (Linum usitatissimum L.) genotypes of different origin cultivated in Turkey. **J. Biotechnol & Biotechnological. Equipment.** 24(2) : 1836-1842.

Bu, D. P., Wang, J. Q., Dhiman, T. R., and Liu, S. J. (2007). Effectiveness of oils rich in linoleic and linolenic acids to enhance conjugated linoleic acid in milk from dairy cows. **J. Dairy Sci**. 90 : 998-1007.

Burroughs, W., Trenkle, A. H., and Vetter, R. L.. (1974). A system of protein evaluation for cattle and sheep involving metabolizable protein (amino acids) and urea fermentation potential of feedstuffs. **Vet. Med. Small Anim. Clin.** 69 : 713-722.

Chilliard, Y., Martin, C., Rouel, J., and Doreau, M. (2009). Milk fatty acids in dairy cows fed whole crude linseed, extruded linseed, or linseed oil, and their relationship with methane output. **J. Dairy Sci.** 92 : 5199-5211

Chilliard, Y., Glasser, F., Ferlay, A., Bernard, L., Rouel, J., and Doreau, M. (2007). Diet,

rumen biohydrogenation and nutritional quality of cow and goat milk fat. **Eur. J. Lipid Sci. Technol.** 109 : 828-855.

Corazzin, M., Bovolenta, S., Sepulcri, A., and Piasentier, E. (2012). Effect of whole linseed addition on meat production and quality of Italian Simmental and Holstein young bulls. **Meat Sci.** 90(1) : 99-105.

Crampton, E. W., Lloy, L. E., and Mackay, V. G. (1957). The calorie value of TDN. **J. Anim. Sci.** 16 : 541-552.

Dai, X. J., Wang, C., and Zhu, Q. (2011). Milk performance of dairy cows supplemented with rapeseed oil, peanut oil and sunflower seed oil. **Czech J. Anim. Sci.** 56(4) : 181-191.

Dawson, L. E. R., Fearon, A. M., Moss, B. W., and Woods, V. B. (2007). Effect of substitution of a proportion of the concentrate in grass silage/concentrate-based diets with extruded linseed on performance and meat quality of dairy bulls. **Brit. Soc. Anim. Sci.,** Southport, UK. Page 10 in Proc.

Diane H. M. (2007). **Flax - A health and nutrition primer.** 4th Edition. 134p. http : //www.flaxcouncil.ca/english/index.jsp?p=primer&mp=nutrition

Doreau, M., and Ferlay, A. (1994). Digestion and utilisation of fatty acids by ruminants. **Anim. Feed Sci. Technol.** 45 : 379-396.

Doreau, M., Laverroux, S., Normand, J., Chesneau, G., and Glasser F. (2009). Effect of linseed fed as rolled seeds, extruded seeds or oil on fatty acid rumen metabolism and intestinal digestibility in cows. **Lipids.** 44 : 53-62.

Etherton, P. M., Harris, W.S., and Appel, L. J. (2002). AHA Scientific statement – fish consumption, fish oil, omega-3 fatty acids, and cardiovascular disease. **J. Am. Heart Assoc.** 106 : 2747-2757.

Filleau, H. B., A, Kokkonen T, Lampi A. M, Toivonen. V., Shingfield, K. J, and

Vanhatalo, A. (2011). Effect of plant oils and camelina expeller on milk fatty acid composition in lactating cows fed diets based on red clover silage. **J. Dairy Sci.** 94(9) : 4413-30.

Flowers, G., Ibrahim, S.A., and Ghazaleh, A. A. (2008). Milk fatty acid composition of grazing dairy cows when supplemented with linseed oil. **J. Dairy Sci.** 91 : 722-730.

Fuentes, M.C., Calsamiglia, S., Fievez, V., Blanch, M., and Mercadal, D. (2011). Effect of pH on ruminal fermentation and biohydrogenation of diets rich in omega-3 or omega-6 fatty acids in continuous culture of rumen fluid. **Anim. Feed Sci. Tech.** 169(1) : 35-45.

Garrett, W.N. (1980). Energy utilization by growing cattle as determined in 72 comparative slaughter experiments. **Energy Metab. Proc. Symp.** 26 : 3-7.

Garrett, W. N., Meyer, J. H., and Lofgreen, G. P. (1959). The comparative energy requirements of sheep and cattle for maintenance and gain. **J. Anim. Sci.** 18 : 528-547.

Gebauer, S. K.., Psota, T.L., Harris, W. S., and Etherton, P. M. (2006). N3 Fatty acid dietary recommendations and food sources to achieve essentiality and cardiovascular benefits. **Am. J. Clin. Nutr.** 83(suppl) : 1526-1535.

Glasser, F., Doreau, M., Ferlay, A., Loor, J., and Chilliard, Y. (2007). Milk fatty acids : Mammary synthesis could limit transfer from duodenum in cows. **Eur. Lipid Sci. Technol.** 109 : 817-827.

Glasser, F., Ferlay, A., and Chilliard, Y. (2008). Oilseed lipid supplements and fatty acid composition of cow milk : A meta-analysis. **J. Dairy Sci.** 91 : 4687-4703.

Goyens, P.L.L., Mary, E. S., Peter, L. Z., Martijn, B. K., and Ronald, P. M. (2006). Conversion of α-linolenic acid in humans is influenced by the absolute amount

of α-linolenic acid and linoleic acid in the diet and not by their ratio. **Am. J. Clin. Nutr.** 84 : 44-53.

Grummer, R. R. (1988). Influence of prilled fat and calcium salt of palm oil fatty acids on ruminal fermentation and nutrient digestibility. **J. Dairy Sci.** 7111- 7123.

Guebre E. F., Rabasa L. R., Bonnet, F., Bastard, J.P., Desage, M., Skilton, M. R., Vidal, H., and Laville, M. (2008). Nutritional intervention to reduce the n6/n3 fatty acid ratio increases adiponectin concentration and fatty acid oxidation in healthy subjects. **Eur J. Clin Nutr.** 62(11) : 1287-1293.

He, M., and Armentano, L. E. (2011). Effect of fatty acid profile in vegetable oils and antioxidant supplementation on dairy cattle performance and milk fat depression. **J. Dairy Sci.** 94 : 2481-2491.

Herdmann, A., Martin, J., Nuernberg, G., Dannenberger, D., and Nuernberg, K. (2010). Effect of dietary n-3 and n-6 PUFA on lipid composition of different tissues of German Holstein bulls and the fate of bioactive fatty acids during processing. **J. Agri. Food Chem.** 58 : 8314-8321.

Hernandez-Calva, L. M., He, M., Juarez, M., Aalhus, J. L., Dugan, M. E. R., and McAllister, T. A. (2011). Effect of flaxseed and forage type on carcass and meat quality of finishing cull cows. **Can. J. Anim. Sci.** 91 : 613-622.

Hocquette, G. F., Bas, P., Bauchart, D., Vermorel M., and Geay, Y. (1999). Fat partitioning and biochemical characteristics of fatty tissues in relation to plasma metabolites and hormones in normal and double-muscled young growing bulls. **Comp. Biochem. Physiol.** 122 : 127-138

Hocquette, J.-F., and Bauchart, D. (1999). Intestinal abosrption, blood transport and hepatic and muscle metabolism of fatty acids in preruminant and ruminant animals. **Reprod. Nutr. Dev.** 39(1) : 27-48.

Hocquette, J.F., Gondret, F., Baéza, E., Médale, F., Jurie, C., and Pethick, D.W. (2010). Intramuscular fat content in meat-producing animals : development, genetic and nutritional control, and identification of putative markers. **Inter. J. Anim. Bio. Sci.** 4(2) : 303-319.

Institut National de la Recherche Agronomique. (1988). Alimentation des Bovins, Ovins, et Caprins. R. Jarrige, ed. Paris : **Institut National de la Recherche Agronomique.**

Institute of Medicine. (2002). **Dietary Reference Intakes for Energy, Carbohydrate, Fiber, Fat, Fatty Acids, Cholesterol, Protein, and Amino Acids.** National Academies Press. Washington. DC. 8 : 1-97.

Jenkins, T. C., Wallace, R. J., Moate P. J., and Mosley, E. E. (2008). Board-invited review : Recent advances in biohydrogenation of unsaturated fatty acids within the rumen microbial ecosystem. **J. Anim. Sci.** 86 : 397-412.

Kapoor, R. and Patil, U. K. (2011). Mini Review : Importance and production of omega-3 fatty acids from natural sources. **Int. Food Res. J.** 18 : 493-499.

Kashiwagi, S., and Huang, P. L. (2012). Dietary supplements and cardiovascular disease : what is the evidence and what should we recommended? Edited by Gasparyan, A. Y. 498 p.

Katare, C., Saxena, S., Agrawal, S., Prasad, G., and Bisen, P. S. (2012). Flax seed : A potential medicinal Food. **J. Nutr. Food Sci.** 2 : 1-8

Kim, C. M., Kim, J. H., Oh, Y. K., Park, E. K., Ahn, G. C. , Lee, G. I., and Park, K. K. (2009). Effects of Flaxseed Diets on Performance, Carcass Characteristics and Fatty Acid Composition of Hanwoo Steers. **Asian-Aust. J. Anim. Sci.** 22(8) : 1151-1159.

Kronberg, S. L., Scholljegerdes, E. J., Lepper, A. N., and Berg, E. P. (2011). The effect

of flaxseed supplementation on growth, carcass characteristics, fatty acid profile, retail shelf life, and sensory characteristics of beef from steers finished on grasslands of the northern Great Plains. **J Anim. Sci.** 89 : 2892-2903.

Kromhout, D., Satoshi, Y., Geleijnse, J. M., and Shimokawa, H. (2012). Fish oil and omega-3 fatty acids in cardiovascular disease : do they really work? **Eur. eart J.** l33 : 436-443.

Kuang, C. C. (2001). New York. Routledge Publishing. **Fatty Acid in Foods and Their Health Implications.**

Leat, W. M. (1978). Factors affecting the fatty acid composition of depot fats of cattle and other ruminants. Page 231 in **Patterns of Growth and Development in Cattle, Current Topics in Veterinary Medicine.** Vol. 2. H. De Boer and J. Martin, ed. Martinus Nijhoff, The Hague, the Netherlands.

Lidefelt, J. O. (2007). **Handbook Vegetable Oils and Fats**. Alfaprint, Karishamn. 96-97.

Lofgreen, G. P., and Garrett, W. N. (1968). A system for expressing net energy requirements and feed values for growing and finishing cattle. **J. Anim. Sci.** 27 : 793-806.

Loor, J. J., Ferlay, A., Ollier, A., Doreau, M., and Chilliard, Y.. (2005). Relationship among *trans* and conjugated fatty acids and bovine milk fat yield due to dietary concentrate and linseed oil. **J. Dairy Sci.** 88 : 726-740.

Mach, N., Devant, M., Bach, A., Díaz, I., Font, M., Oliver, M. A., and García, J. A. (2006). Increasing the amount of omega-3 fatty acid of meat from intensively fed young Holstein bulls through nutrition. **J. Anim. Sci.** 84 : 3039-3048.

Manynard, L. A., Loosli, J. K., Hintz, H. F., and Warner. R. G. (1979). **Animal nutrition.** 7th. McGraw-Hill, Inc., New York, NY.

Mirajkar, R. N., Jamadar, S. A., Patil, A. V., and Mirajkar, N. S. (2011). Omega 3 fatty acids. **Clin. Imp. Inter. J. of Chem. Tech. Res.** 3(2) : 724-732.

Moe, P. W., and Tyrrell, H. F. (1972). The net energy value of feeds for lactation. **J. Dairy Sci.** 55 : 945-985.

Nassu, R. T., Dugan, M. E. R., He, M. L., McAllister, T. A., Aalhus, J. L., Aldai, N., and Kramer. J. K. G. (2011). The effects of feeding flaxseed to beef cows given forage based diets on fatty acids of *longissimus thoracis* muscle and backfat. **Meat Sci.** 89 : 469-477.

Noci, F., Freach, P., Monahan, F. J., and Moloney, A. P. (2007). The fatty acid composition of muscle fat and subcutaneous adipose tissue of grazing heifers supplemented with plant oil-enriched concentrates. **J. Anim. Sci.** 85 : 1062-1073.

NRC . (1978). **Nutrient Requirements of Dairy Cattle.** 5th Rev. Ed. Washington, D. C. : National Academy Press.

NRC. (1984). **Nutrient Requirements of Beef Cattle**. 6th Rev. Ed. Washington, D.C. : National Academy Press.

NRC. (1985). **Ruminant Nitrogen Usage.** Washington, D.C. : National Academy Press.

NRC. (1989). **Nutrient Requirements of Dairy Cattle**, 6th Rev. Ed. Update. Washington, D.C. : National Academy Press.

NRC. (1996). **Nutrients Requirements of Beef Cattle.** 7th Ed. National academy press. Washington D.C.

NRC. (2000). **Nutrient Requirements of Beef Cattle.** 8th Rev. Ed. Update Washington, D.C. : National Academy Press.

NRC. (2001). **Nutrients Requirements of Dairy Cattle.** 7th Ed. National academy

press. Washington D.C. 340 p.

Nuernberg, K., Nuernberg, G., Ender, K., Lorenz, S., Winkler, K., Rickert, R., and Steinhart, H. (2002). N-3 fatty acids and conjugated linoleic acids of longissimus muscle in beef cattle. **Eur. J. Lipid Sci. Technol.** 104 : 463-471.

Palmquist, D. L. (1991) .Influence of source and amount of dietary fat on digestibility in lactating cows. **J. Dairy Sci.** 74 : 1354-1360.

Palmquist, D. L., Lock, A. L., Shingfield, K. J., and Bauman, D. E. (2005). Biosynthesis of conjugated linoleic acid in ruminants and humans. **Adv. Food Nutr. Res.** 50 : 179-217.

Partida, J. A., Olleta, J. L., Sañudo, C., Albertí, P., and Campo, M. M. (2007). Fatty acid composition and sensory traits of beef fed palm oil supplements. **Meat Sci.** 76(3) : 444-454.

Preston, R. L. (1982). Empirical value of crude protein systems for feedlot cattle. **Protein Requirements of Cattle : Proceedings.** 201-217

Raes, K., De Smet, S., and Demeyer, D. (2004). Effect of dietary fatty acids on incorporation of long chain polyunsaturated fatty acids and conjugated linoleic acid in lamb, beef and pork meat. **Anim. Feed Sci. Technol.** 113(1-4) : 199-221.

Razminowicz, R. H., Kreuzer, M., Leuenberger, H. M., and Scheeder, R. L. (2008). Efficiency of extruded linseed for the finishing of grass-fed steers to counteract a decline of omega-3 fatty acids in the beef. **Livest. Sci.** 114 : 150-163.

Rego, O.A., Alves, S.P., Antunes, L. M., Rosa, H.J., Alfaia, C.F., Prates, J. A., Cabrita, A.R., Fonseca, A.J., and Bessa, R. J. (2009). Rumen biohydrogenation derived fatty acids in milk fat from grazing dairy cows supplemented with rapeseed, sunflower, or linseed oils. **J. Dairy Sci.** 92(9) : 4530-40.

Rego, O. A., Rosa, H. J. D., Portugal, P. V., Corderio, R., Borba, A. E. S., Vouzela C. M., and Bessa, R. J. B. (2005). Influence of dietary fish on conjugated linoleic acid, omega-3 and other fatty acids in milk fat from grazing dairy cows. **Livest. Prod. Sci.** 95 : 27-33.

Romo, G. A., Casper, D. P., Erdman, R. A., and Teter, B. B. (1996). Abomasal infusion of cis or trans fatty acid isomers and energy metabolism of lactating dairy cows**. J. Dairy Sci.** 79 : 2005-2015.

Scholljegerdes, E. J., and Kronberg, S. L. (2010). Effect of supplemental ground flaxseed fed to beef cattle grazing summer native range on the northern Great Plains. **J. Anim. Sci.** 88 : 2108-2121.

Schroeder, G. F., Gagliostro, G. A., Bargo, F., Delahoy, J. E., and Muller, L. D. (2004). Effect of fat supplementation on milk production and composition by dairy cows on pasture : A review. **Livest. Prod. Sci.** 86 : 1-18.

Schroeder, G. F., Gagliostro, G. A., Villalobos, B. D., and Mengido, I. L. (2002). Supplementation with partially hydrogenated oil in grazing dairy cows in early lactation. **J. Dairy Sci.** 85 : 580-594.

Scollan, N. D., Choi, N. J., Kurt, E., Fisher, A. V., Enser, M., and Wood, J. D. (2001). Manipulating the fatty acid composition of muscle and adipose tissue in beef cattle. **Br. J. Nutr.** 85 : 115-124.

Serhan, C.N., Hong, S., Gronert, K., Colgan, S.P., Devchand, P.R., Mirick, G., and Moussignac, R.L. (2002). Resolvins : A family of bioactive products of omega-3 fatty acid transformation circuits initiated by aspirin treatment that counter pro-inflammation signals. **J. Exp. Med.** 196 : 1025-1037.

Simopoulos, A. P. (2002). The importance of the ratio of omega-6/omega-3 essential fatty acids. Bio. Pharma. 56(8) : 365-379.

Simopoulos, A. P. (2006). Evolutionary aspects of diet, the omega-6/omega-3 ratio and genetic variation : nutritional implications for chronic diseases. **Biomed. Pharmacother. J.** 60 : 502-507.

Spicer, L. A., Theurer, C.B., Some, J., and Noon, T. H. (1986). Ruminal and post-ruminal utilization of nitrogen and starch from sorghum grain-, corn-, and barley-based diets by beef steers. **J. Anim. Sci.** 62 : 521-530.

Swift, B. W. (1957). The caloric value of TDN. **J. Anim. Sci.** 16 : 1055-1059.

Tanaka, K. (2005). Occurrence of conjugated linoleic acid in ruminant products and its physiological functions. **Anim. Sci. J**. 76 : 291-303.

Tyrrell, H. F., and Moe, P. W. (1975). Effect of intake on digestive efficiency. **J. Dairy Sci.** 58 : 1151-1163.

Uauy, R.., and Valenzuala, A. (2000). Marine oils : the health benefits of n-3 fatty acids. **Nutrition**. 16 : 680-684.

Vermorel, M., and Coulon, J. B. (1998). Comparison of the National Research Council energy system for lactating cows with four European systems. **J. Dairy Sci.** 81 : 846-855.

Wagner, D. G., and Loosli, J. K. (1967). Studies on the energy requirements of high producing cows. Memoir 400. **Cornell Univ. Agr. Exp. Sta.**

Wang, Y., Li, D., Wang, L. J., Chiu, Y. L., Chen, X. D., Mao, Z. H., and Song, C. F. (2008). Optimization of extrusion of flaxseeds for in vitro protein digestibility analysis using response surface methodology. **J. Food Eng.** 85(1) : 59-64.

Weiss, W. P., Conrad, H. R. and Pierre, N. R. S. (1992). A theoretically based model for predicting total digestible nutrient values of forages and concentrates. **Anim. Feed Sci. Technol.** 39 : 95-110.

World Health Organization. (2003). **Diet, Nutrition and the Prevention of Chronic**

Diseases. Report of the Joint WHO/FAO Expert Consultation; WHO Technical Report Series No. 916; WHO : Geneva, Switzerland.

Wilkerson, V. A., Klopfenstein, T. J., Britton, R. A., Stock, R. A., and Miller. P. S. (1993). Metabolizable protein and amino acid requirements of growing beef cattle. **J. Anim. Sci.** 71 : 2777-2784.

CHAPTER III

THE STUDY OF LINSEED OIL SUPPLEMENTATION ON PERFORMANCES, CARCASS QUALITY AND n-6/n-3 FATTY ACID RATIO IN BEEF

3.1 Abstract

The objective of this study was to determine the effect of linseed oil supplementation on performance, carcass quality and n-6/n-3 fatty acid ratio in beef. Twenty fattening steers (87.5% Brahman crossbred), approximate 2 years old, were stratified by their LW into 4 groups and each group was randomly assigned to four dietary treatments. All steers were fed 14% CP concentrate and free access to clean water and were individually housed in a free-stall unit. The treatments were 1) concentrate approximately 7 kg/d with *ad libitum* rice straw; 2) concentrate approximately 4 kg/d plus 200 g/d of palm oil (PO) with *ad libitum* fresh grass (FG); 3) concentrate 4 kg/d plus 100 g/d of PO and 100 g/d of linseed oil (LSO) with *ad libitum* FG; 4) concentrate 4 kg/d plus 200 g/d of LSO with *ad libitum* FG**.** Dietary treatment had no effect on nutrient intake. Oil supplement decreased DMI, while ruminal pH, NH_4-N VFA concentrations and protozoa count were not changed. LSO did not negatively affect carcass quality, sensory perceptions but increased the n-3 FA and lowered the n-6/n-3 ratio in beef with increasing amount of LSO supplement.

Key words : beef fatty acid, linseed oil, carcass quality

3.2 Introduction

Thailand imports large quantites of beef especially in frozen form. Recently, high quality product of beef imported is about 2,000 tons/year (Angkuro, 2003; DLD, 2004). Consumer interest includes beef quality, high quality product for healthy and product from natural sources. Beef consumption in the country from Brahman crossbred is approximately 98.5% and approximately 81.2% of beef from farmers raises on no-concentrate feeding system (DLD, 2012). As a result, beef quality and nutritional value of the products is of low quality. Fat and fatty acids are of major importance for beef quality and nutritional value for consumer's health (Wood et al., 2004). Beef contains approximately 50% of saturated fatty acid (SFA) content, which is the result of the process of rumen biohydrogenation (Schollan et al., 2001). Saturated fatty acids (SFA) have been recognized by the international dietary authorities as primary targets for diet reduction (WHO, 2003). SFA is a major factor causing chronic diseases in the Western world; cardiovascular disease and colon cancer probably (McAfee et al.2010). The fatty acid composition of beef (including muscle and subcutaneous adipose tissue) can be influenced, at least in part, by fatty acid composition of the diet (Glaser et al., 2004; Noci et al., 2007). Most of the research aimed at improving dietary quality of beef has been focused on manipulation of animal feed with attempts to increase the intramuscular n-3 PUFA content accomplished by feeding n-3 PUFA rich in ruminants' diets (Scollan et al., 2006). In addition, low PUFA n-6/n-3 ratio aids in the prevention of many chronic diseases. Increasing the content of PUFA and reducing SFA with the net effect of increasing PUFA/SFA and reducing n-6/n-3 ratio are priorities (Scollan et al., 2006). Linseed oil is a natural source of C18:3n3, and it may afford polyunsaturated fatty acids (PUFAs).

It is also a precursor of eicosanoids, which play an important antithrombotic and anti-inflammatory roles (Palmquist, 2009). In general, previous studies reported the effect of different linseed form and concentration on performance and on FA composition of muscle and adipose tissue in beef cattle (Mach et al., 2006; Raes et al., 2004). Herdmann et al. (2010) found significant increases in the concentrations of n-3 fatty acids (alpha linolenic acid, C20:5n3 and C22:5n3 and C22:6n3 in meat from German Holstein Bulls fed 3% linseed oil and 12% rapeseed cake.Thus, the objective of this study was to examine the effect of linseed oil supplementation on quality characteristics and n-6/n-3 fatty acid ratio in beef.

3.3 Objective

The objective of this experiment was to investigate the effect of linseed oil supplementation on quality characteristics and n-6/n-3 fatty acid ratio in beef.

3.4 Materials and methods

3.4.1 Animals and feeding

Twenty steers (87.5% Branhman crossbred), averaging of 337±54 kg live weight (LW) and approximate 2 years old, were stratified by their LW into 4 groups and each group was randomly assigned to four dietary treatments. All steers were fed 14% CP concentrate and free access to clean water and were individually housed in a free-stall unit. The treatments were 1) control, concentrate approximately 7 kg/d with *ad libitum* rice straw (HC); 2) control concentrate approximately 4 kg/d plus 200 g/d of palm oil with *ad libitum* fresh grass (200 g/d PO); 3) control concentrate 4 kg/d plus 100 g/d of palm oil and 100 g/d of linseed oil with *ad libitum* fresh grass (200 g/d MO); 4) control concentrate 4 kg/d plus 200 g/d of linseed oil

with *ad libitum* fresh grass (200 g/d LSO). The experiment lasted for 84 days, with 14 days was the adjustment period, followed by 70 days (5 periods of 14 d), of measurement period.

3.4.2 Fattening steers and slaughter procedures

The experiment lasted for 84 days. At the end of feeding trial the animals were weighed, and 3 animals per treatment were randomly sampled and transported to a commercial abattoir and then slaughtered at Nakhon Ratchasima slaughterhouse, Nakhon Ratchasima, Thailand, following procedures outlined by Jaturasitha (2004). All experimental procedures were carried out following the animal welfare standards of Department of Livestock Development, Ministry of Agriculture and Coopperative, Rayal Thai Government. Muscle samples were cut from outside *Longissimus dorsi* (LD; 6-12th rib) muscle and *Semimembranosus* (SM) muscle were prepared from the left carcass side in order to study beef quality in muscles.

3.4.3 Laboratory analyses

Feed offered and left after eating of individual steer were weighed on 2 consecutive days weekly to calculate DM intakes. Samples were taken and dried at 60°C for 48 hours and at the end of the experiment. Feed samples were pooled to make representative samples for proximate and detergent analyses. Samples were ground through 1 mm screen and analyzed for chemical composition. Dry matter (DM) was determined by hot air oven at 60°C for 48 h while crude protein (CP) was analyzed by Kjeldahl method (AOAC, 1995). Ether extract (EE) was determined by 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°C for 3 h. The chemical analysis

was expressed on the basis of the final DM. Fatty acid composition of concentrates, fresh grass and rice straw were determined by Gas chromatography.

Meat pH (pH meter model UB-5, Denver Instrument, Germany) was determined in LD and SM at 45 min and 24 h. After dissection, the LD and SM samples were cut in to 2.5 cm thick slices, put into polyethylene bags, chilled at 4 °C for 48 h and then stored in the refrigerator outside of the bag for 1 h (‘blooming’) before conducting color measurements using a hunter lab (Color Quest XE, Kable, United Kingdom).

Water-holding capacity (WHC) was assessed via sample losses occurring during different procedures. Thawing and cooking losses were determined in the 2.5 cm thick slices of LD and SM frozen in polyethylene bags at -20 °C. Thawing was performed over 24 h at 4 °C. Before weighing, the sample surfaces were dried with soft paper. Afterwards, samples were sealed in heat-resistant plastic bags to be boiled in water bath (WNE 29, Memmert, Germany) at 80 °C until an internal temperature of 70 °C was reached. Samples were cooled to ambient temperature and weighed after drying the surfaces with soft paper. For the determination of the grilling loss, 2.5 cm thick slices were grilled in a convection oven (model 720, Mara, Taipei, Taiwan) at 150 °C until an internal temperature of 70 °C was reached. In the LD, additionally drip loss according to Honikel (1987) was determined. In the boiled samples, shear forces were measured after cooling and drying. A steel hollow-core device with a diameter of 1.27 cm was punched parallel to the muscle fibers to obtain six pieces from each muscle sample. Measurements were carried out on a material testing machine by Texture analyzer (TA-TX2 Texture Analyzer, Stable Micro Systems, UK) using a Warner–Bratzler shear. A crosshead speed of 200 mm/min and a 5 kN load cell calibrated to read over a range of 0x100 N were applied.

Samples of the LD and SM were minced and analyzed in duplicate for moisture, fat and protein contents according to AOAC (1995). Cholesterol content was measured on LD and SM muscle. The cholesterol was extracted from approximately 5 g of each LD or SM muscle samples using 20 ml of methanol-isopropanol (90 : 5 : 5, v/v/v) and 5 ml of 60% KOH according to the method of Rowe (1999). The cholesterol was analyzed by using gas chromatography (Hewlett-Packard 6890 series GC system, USA) with a capillary column (HP 19091A-112, 25 m × 0.32 mm × 0.52 μm film thickness) and a flame ionization detector. The temperatures of the injector and the detector were 260 and 300°C, respectively. Separation was carried out at 300 °C with helium gas flow rate of 1 ml/min.

Fatty acids in feed and beef samples were extracted using a modified method used by Folch et al. (1957) and Metcalfe et al. (1966). Before the extraction, feed and beef 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 then further homogenized for 2 min with 30 ml of chloroform. Then, each sample was separated in separating funnel and 30 ml of deionized water and 5 ml of 0.58% NaCl was added. The under layer of fatty acid methyl esters (FAME) was removed and placed in screw-cap test tube and stored at -20ºC until methylation. Fatty acid methyl esters (FAME) were prepared by the procedure described by Ostrowska et al. (2000). The procedure involved placing approximately 30 mg of the extracted oil into a 15 ml reaction tube fitted with a teflon-lined screw cap. One and a half ml of 0.5 M sodium hydroxide in methanol was added. The tubes were flushed with nitrogen, capped, heated at 100 ºC for 5 min with occasional shaking and then cooled to room temperature. One ml of C17:0 internal

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

Quantitative descriptive analysis (QDA) was used for sensory evaluation (Stone et al 1974), a test panel was selected from a number of students and faculty members of the School of Animal Production Technology, Suranaree University of Technology, who had undergone sensory evaluation training following the methods of Viriyajare (1992). Grilled 2.5-cm slices of LD and SM were cut into pieces of 1.3× 1.3 × 1.9 cm and served warm. Panelists were asked to grade samples for tenderness, juiciness, flavor and overall acceptability and assessments were given individually using a structured line graph and determined on a straight line. Thus, each point on a linear scale to represent the quantity that can be measured with a ruler. Samples were served subsequently in a randomized order with respect to group and animal. The 24 samples (from 12 animals and two muscles) were tested by 8 persons.

Susceptibility of the lipids to oxidation was assessed by the 2-thiobarbituric acid (TBARS, thiobarbituric acid reactive substances) method (Rossell,

1994). Briefly, samples of 10 g of LD and SM stored for 0and 6 days in the refrigerator at 4 °C were mixed with 30 ml distilled water for 2 min by a Moulinex household blender. Sixty five ml of distilled water was then added, the pH was adjusted to 1.44 with 2.5 ml of 4 M HCl and drops of an antifoaming agent were added. Afterwards the flask containing the sample was connected with the distillation apparatus. Fifty ml of the distillate was collected within 15-20 min. Five ml of the distillate were allowed to react with 5 ml of TBA reagent. The solution was cooled at room temperature and the absorbance was measured against a blank at 538 nm. The TBARS were calculated by multiplying the absorbance by 7.8. Results were given as concentrations of malondialdehyde in the beef.

Approximately 200 ml of ruminal fluid was collected on d 0, 30 and 41 of each periods. The rumen fluid was sampled from 3 steers per treatment by using a stomach tube with a strainer and a vacuum pump, and filtered through 4 layers of cheesecloth at 0 (pre feeding), 2, 4, 6 h post feeding. One portion of rumen fluid was immediately analyzed for pH (pH meter model UB-5, Denver Instrument, Germany). Ruminal volatile fatty acids (VFA) and ammonia N were determined in rumen fluid samples by taking 20 ml of rumen fluid and was then combined with 5 ml 6N HCl, kept frozen for analysis of VFA and ammonia N. The samples were later thawed at 4 °C and centrifuged at 3,000 rpm for 15 min. The supernatant was analyzed for ammonia N by Kjeldahl and concentrations of VFA were determined by GC (Hewlett Packard GC system HP6890 A; Hewlett Packard, Avondale, PA) equipped with a 30 m × 0.32 mm × 0.15 μm film fused silica capillary column (HP_Innowax, AB 002, Agient, USA). Injector and detector temperatures were 250 °C. The column temperature was kept at 80 °C for 5 min, then increased at 10 °C/min to 170 °C and then increased at 30 °C/min to 250 °C and held at 250 °C for 5 min. Protozoa

populations were counted by Hematocytometer in rumen fluid samples which preserved with 10% formal saline solution.

The fatty acid in rumen fluid sample was extracted using a modified method used by Romeu-Nadal et al. (2004). From a well-mixed aliquot of rumen fluid, 3ml was placed in 50 ml centrifuge tubes. We then added 27 ml of a dichloromethane–methanol solution (2 : 1, v/v) to each tube. The mixture was shaken mechanically for 15 min and centrifuged at 2500 × *g* for 8 min at 4 °C. Approximately 8ml of distilled water was pipetted into each tube and, after shaking for a further 15 min, the sample was, again centrifuged at 2500 × *g* for 8 min at 4 °C. As much of the upper aqueous fraction as possible was carefully removed with a pipette. The organic layer was washed with 8ml of a saturated solution of the sodium chloride, and finally mixed mechanically for 15 min and then centrifuged for 8 min at 2500 × *g* at 4 °C. Again, the upper aqueous fraction was carefully removed with a pipette. The organic fraction was carefully transferred to a separating funnel and filtered through 1PS paper (Whatman, Maidstone, UK) containing anhydrous sodium sulfate, and 3–5 ml of dichloromethane was passed through the filter. The fat solution was taken in pre-weighed conical flask. Finally the extract was concentrated by removing dichloromethane in a rotator evaporator and dried under a gentle stream of nitrogen. The weight difference of the conical flask before/after was assumed to be fat. The fat was stored at -20 °C and redissolved in dichloromethane (3%, w/v) intermediately before analyzing by gas chromatography (GC) (7890A GC System, Agilent Technology, USA)

3.4.5 Statistical analysis

All data were statistically analyzed as Completely Randomized Design

using ANOVA procedure of SAS (SAS, 2001).

3.4.6 Experimental location

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

3.4.7 Experimental period

The experiment was from August 2012 to December 2012.

3.5 Result and discussion

3.5.1 Feed Composition and performance

The nutrient composition and fatty acid composition of the concentrate, forage sources and oil supplement are summarized in Table 3.1 and 3.2, respectively. Lipids from fresh grass provided high proportions of C18:3n-3 and PUFA and lowered proportions of C18:2n-6 and MUFA compared to 14% CP concentrate and rice straw. LSO had the highest proportion of PUFA while PO had the highest proportion of SFA. In all concentrates, the main SFA was C16:0, whereas C18:1n-9 was the main MUFA in PO, C18:2n-6 was the main PUFA in 14% CP concentrate, C18:3n-3 was the main PUFA in LSO, and MO, respectively (Table 3.2).

Animal performances and nutrient intake of the steers are summarized in Table 3.3. The total dry matter intake, DMI (kg/d) in the HC treatment was significantly higher ($P<0.01$) which is the result of the higher concentrate DMI compared with other treatments. According to Jenkins and McGuire (2006), the main effects of the addition of lipids on intake reduction are related to modifications in rumen fermentation. Specifically, a reduction in the digestibility of fiber in the rumen leads to growth, NE_g (Mcal/d) of experimental steers were not significantly different

oil an increase in the retention time of the NDF, which results in greater rumen fill. While the results of total crude protein intake, CPI (g/d) and net energy intake for treatments. Diets also had similar levels of net energy for maintenance and growth at 1.98-2.15 and 1.33-1.49 Mcal/kg DM, respectively. Consequently, final body weight, ADG, energy gain, Feed : Gain ratio were unaffected by dietary treatments. Therefore, the oil supplements did not affect performance and nutrient intakes. The results of this study are similar to other studies. For example, Noci et al. (2007) reported that 150 g/d sunflower oil and 150 g/d linseed oil supplementation did not affect final LW and ADG. This is partially because total net energy (Mcal/d) consumption was balanced by treatment. Furthermore, He et al. (2011) supplemented mixture of flaxseed oil and sunflower oil at 5% of diet and reported no significant effects of supplementation ($P>0.05$) on DMI, BW, ADG and gain per unit feed. Similar results were reported by Muller et al. (2004), who used sources of n-3 FA (flaxseed) and n-6 FA (rumen protected fat) (62 g/kg EE in the total diet) in confined crossbred heifers and did not observe differences in DM, organic matter, and NDF and ADF intakes (kg/d and g/kg of BW), thus supporting the results obtained in this experiment.

Table 3.1 Chemical compositions of the experimental diets

Items	14%CP	PO/LO	RS	FG
Dry matter	93.91	-	92.31	12.50
	----------% DM----------			
Ash	7.00	-	10.85	12.40
Crude protein	14.63	-	4.00	10.07
Ether extract	4.07	100	0.81	1.78
Crude fiber	17.13	-	39.79	36.04
Neutral detergent fiber	42.59	-	76.31	64.42
Neutral detergent insoluble N	1.09	-	0.51	0.32
Acid detergent fiber	26.33	-	52.34	34.83
Acid detergent insoluble N	0.89	-	0.41	0.35
Acid detergent lignin	10.95	-	6.34	2.62
TDN_{1x}(%)[1]	60.23	184.15	46.14	55.05
DE_{1x} (Mcal/kg)[2]	2.79	7.71	2.03	2.51
ME_p (Mcal/kg)[3]	2.74	5.79	2.03	2.08
NE_m (Mcal/kg)[4]	1.44	4.23	0.77	1.23
NE_g (Mcal/kg)[5]	0.86	3.11	0.23	0.66

[1]Total digestible nutrients, TDN_{1X} (%) = tdNFC + tdCP + (tdFA x 2.25) + tdNDF – 7 (NRC, 2001)

[2]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

[3]Metabolisable energy, ME = 0.82 x DE (NRC, 1996)

[4]Net energy for maintenance, Ne_m = 1.37ME – 0.138ME2 + 0.0105ME3 – 1.12 (NRC, 1996)

[5]Net energy for growth, Ne_g = 1.42ME – 0.174ME2 + 0.0122ME3 – 1.65 (NRC, 1996)

Table 3.2 Fatty acid compositions of the experimental diets

Fatty acid (% of total FA)	**14%CP**	**FG**	**RS**	**PO**	**MO**	**LSO**
C8:0	0.74	ND	ND	0.05	0.03	0.05
C10:0	1.14	ND	ND	0.02	ND	ND
C12:0	17.96	1.42	ND	0.19	0.10	ND
C14:0	6.38	0.74	1.28	0.96	0.49	0.06
C16:0	17.85	19.66	47.49	38.29	21.11	4.91
C18:0	2.71	3.18	8.57	4.42	3.96	3.46
C18:1n9c	31.90	6.55	16.76	40.61	29.26	17.88
C18:2n6c	20.33	19.03	19.88	13.66	15.76	16.73
C20:0	0.00	0.54	0.00	0.04	0.14	ND
C18:3n3	0.35	48.89	6.03	0.26	27.87	55.87
C18:3n6	0.66	ND	ND	0.11	0.17	0.24
SFA[1]	46.77	25.53	57.34	44.05	25.94	8.70
MUFA[2]	31.90	6.55	16.76	41.07	29.61	17.96
PUFA[3]	21.34	67.92	25.91	14.89	44.45	73.34
total n3[4]	0.35	48.89	6.03	0.43	28.09	56.20
total n6[5]	20.99	19.03	19.88	14.46	16.30	17.04
PUFA:SFA	0.46	2.66	0.45	0.34	1.72	8.43
n6/n3	60.01	0.39	3.30	33.69	0.58	0.30

[1] SFA = Sum of saturated fatty acid from C4:0 – C20:0

[2] MUFA = Sum of monounsaturated fatty acid from C14:1 – C22:1

[3] PUFA = Sum of polyunsaturated fatty acids from C18:2 – C22:6

[4] Sum of n6 fatty acids C18:2n-6 – C22:4n-6

[5] Sum of n3 fatty acids C18:3n-3 – C22:6n-3

Table 3.3 Effect of linseed oil supplementation on performance and nutrient intake of steers

Item	Treatments				SEM	Pr<F
	HC	200 g/d PO	200 g/d MO	200 g/d LSO		
Initial body weight, kg	337	336	338	338	11.17	0.998
Final body weight, kg	430	402	409	402	10.91	0.956
Average daily gain, kg/d	1.3	0.9	1.0	0.9	0.06	0.783
Energy gain	6.22	4.13	4.63	4.04	0.06	0.730
Feed : Gain ratio	0.12	0.15	0.13	0.16	0.01	0.606
Dry matter intake, kg/d						
Concentrate	6.46	3.67	3.65	3.66	-	-
Roughage	4.76	5.64	5.59	5.91	0.02	0.279
Oil	-	0.2	0.2	0.2	-	-
Total	11.22[a]	9.52[b]	9.44[b]	9.76[b]	0.32	0.038
DMI, g/$BW^{0.75}$	131.19[a]	110.97[b]	109.19[b]	113.51[b]	2.81	0.011
Crude protein intake , g/d						
Concentrate	945[a]	537[b]	533[b]	534[b]	2.21	<0.01
Roughage	190[b]	568[a]	563[a]	594[a]	14.13	<0.01
Total	1,135	1,107	1,097	1,130	14.99	0.769
Ether extract intake, g/d						
Concentrate	263[a]	150[b]	148[b]	149[b]	0.61	<0.01
Roughage	39[b]	100[a]	100[a]	105[a]	2.57	<0.01
Oil	-	200	200	200	-	-
Total	301[b]	450[a]	448[a]	454[a]	0.29	<0.01
NE_g intake, Mcal/d						
Concentrate	5.56[a]	3.16[b]	3.15[b]	3.14[b]	0.01	<0.01
Roughage	1.19[b]	3.72[a]	3.69[a]	3.90[a]	0.09	<0.01
Oil	-	0.63	0.63	0.63	-	-

[a, b] Mean within row which different superscripts differ (P<0.01), SEM is standard error of mean

3.5.2 Carcass quality traits.

At slaughter, live weight, hot carcass weight, % hot carcass and dressing percentage were not significantly different among treatments (Table 3.4). Similarly, addition of 5.5% and 11% SFO to the 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 the diets did not affect carcass weight, dressing percentage, back fat thickness and LM area. Similary, He and Armentano (2011) stated that the oil diets had none effects on carcass parameters. The composition of growth can determine the efficiency and nutritional requirements of different animal categories. Body composition is important for evaluating growth performance, with the aim of producing carcasses with a higher proportion of muscle and adequate amounts of fat (Bonilha et al., 2008). According to Ferreira et al. (1998), the carcass composition can be modified by altering the energy intake. The increased fat deposition in the group fed rumen-protected fat appeared to increase the cold carcass yield. Adipose tissue is the most variable tissue in the animal body, and fat partition to the carcass can contribute to increased carcass yield (Berg and Butterfield, 1976).

Loin eye area and 12th rib fat thickness were not significantly different among treatments (Table 3.4). The eye muscle area can be used as a representative measure of the quantity, quality, and distribution of the muscle mass. Late-maturing muscles are used to represent the muscle tissue development rate. Thus, the *longissimus* is the most suitable muscle for analysis because, in addition to its late maturation, it is easy to measure. The values found for eye muscle area and fat

thickness cover were above 29 cm^2/100 kg and 3 mm, respectively, which are the minimum values for eye muscle area and fat thickness cover. Ngidi et al. (1990) observed that the use of rumen-protected fat at a rate of 0-60.0 g/kg in the dietary dry matter for fattening steers did not affect carcass eye muscle area and fat thickness cover. In addition, Zinn et al. (2000) did not observe effects on eye muscle area and fat thickness cover using Holstein steers fed diets containing protected fat or animal fat as a lipid source at up to 60.0 g/kg. Oliveira et al. (2011) reported that the physiological maturity of the steers at slaughter was similar and there was no difference in subcutaneous back fat thickness among treatments.

Initial (45 min post slaughter) and final pH (24 hour post slaughter) values were not different among the treatments (Table 3.4). The initial pH was considered ideal, and should vary between 6.9 and 7.2 (Geay et al., 2001). Final pH values were also found in the interval considered to be normal (5.4 to 5.8) for beef (Mach et al., 2008). The final pH corresponds to the accumulation of lactic acid resulting from the production of ATP from glucose encountered in the form of glycogen reserves. In general, cattle supplemented with grains possess a greater availability of glycogen at the time of slaughter and a lower final pH in the beef (Neath et al., 2007). The final pH values suggested that there was no elevated stress prior to slaughter, because acidification of the muscle occurred as expected, and that the level of substitution of oil supplement evaluated did not affect the final pH.

Beef color remained mostly unaffected by treatment with the exception of higher redness on SM originating from the 200 g/d LSO supplement than other groups (Table 3.4). Beef coloration changing from bright red to brown, due to the oxidation of the oxymyoglobin to myoglobin. Moreover, they can be damaged due to lipid oxidation in the intramuscular fat content. Both types of oxidation are intimately

related and are responsible for the appearance of smells and strange flavours of fat (Kanner and Harel, 1985) that can cause rejection by the consumer. According to Muchenje et al. (2009), values encountered in literature for L*, a* and b* was used to measure beef colour in the CIELAB space (Lightness, L*; redness, a*; yellowness, b* (CIE, 1978) being in the following ranges of variation : 33 to 41, 11.1 to 23.6 and 6.1 to 11.3, respectively. The highest values of L*, lowest value of a* found in this experiment which LSO containing high PUFA made greater concentrations of oxidizable PUFA and reduced color stability over a 7-d period of aerobic storage in darkness at 4°C. Beefs were all vacuum packaged and aged 47 d at 0 °C, they had similar color stability values over a 7-d period of aerobic storage in darkness at 4°C (Yang et al., 2002). However, Realini et al. (2004) reported that pasture-fed beef was redder and yellower than concentrate-fed beef after 5 d of display (at 2°C under light), regardless of vitamin E supplementation. Ladeira et al. (2014) reported probably due to the higher oxidation of the myoglobin pigment caused by the increased susceptibility to lipid oxidation of the LD muscle from these animals, as they exhibited higher polyunsaturated fatty acid concentrations. Radicals generated by lipid oxidation can promote the accumulation of metmyoglobin (Faustman et al., 2010). This fact is consistent with the statement that the effect of nutrition on beef color, especially the redness index (a*), is associated with the instability of heme pigments (Mancini and Hunt, 2005) in the secondary products (alpha- and beta-aldehydes) of lipid oxidation, causing the decreased stability of oxymyoglobin redox (Lynch and Faustman, 2000). According to Zakrys et al. (2008), changes in the a* and the oxymyoglobin values appear to be driven by lipid oxidation and are strongly correlated with the TBARS values. Furthermore, Faustman and Cassens (1990) also report a strong relationship between lipid oxidation and myoglobin oxidation.

For the storage period, an increase was observed in the L*, a*, and b* values, independent of diet treatment, until the 12th day of storage, when the values began to decrease. As time passes, deterioration of mitochondria reduces their competition with myoglobin for dissolved oxygen, resulting in a higher concentration of oxymyoglobin (Hood, 1980). More oxymyoglobin is formed at low pH values, which are conditions that increase the solubility of oxygen and inhibit activity of the enzymes that consume oxymyoglobin (Ledward, 1992), increasing the evaluated colour indices. Moreover, internal reflection of the meat increases as the pH decreases, which is caused by denaturing sarcoplasmatic proteins (Seth et al., 1991) and decreasing the spatial distribution of myofilaments (Bendall and Swatland, 1988). Thus, the penetration of light diminishes by dispersion of its bands as a result of the increase in water outside themyofibrillar space induced by the decrease in pH during glycolysis (Lindahl et al., 2001).

The decrease in the values after day 12 is uncommon, but may also be related to alterations in pH. The pH values can increase during vacuum storage triggered by an increase in nitrogenated compounds, such as amines (Lee and Yoon, 2001), resulting from the proteolysis of endogenous enzymes and microbial metabolism favouring formation of metamyoglobin. Therefore, changes in values of a*, b* and C*, resulting from the storage time, may be related to changes in the form of meat myoglobin (Lindahl et al., 2001).

Table 3.4 Effect of linseed oil supplementation on carcass quality traits and colour traits of *Longgissimus dorsi* (LD) and *Semimembranosus* (SM) muscle

Item	Treatments				SEM	Pr<F
	HC	200 g/d PO	200 g/d MO	200 g/d LSO		
Live weight (kg)	484	374	373	426	17.58	0.226
Hot carcass weight (kg)	264	205	202	224	8.12	0.154
Hot carcass (%)	54.58	54.76	54.21	52.66	0.36	0.294
Dressing (%)	52.95	53.12	52.59	51.09	0.35	0.292
Loin eye area (cm^2)	59.90	61.00	62.00	60.60	0.33	0.301
12^{th} rib fat	0.85	0.25	0.43	0.60	0.11	0.343
pH						
45 min						
LD	6.26	6.22	6.37	6.48	0.06	0.512
SM	6.58	6.89	6.68	6.52	0.11	0.670
24 hr						
LD	5.61	5.57	5.51	5.60	0.04	0.845
SM	6.13	5.69	5.74	5.71	0.05	0.073
Colour trait						
*Lightness, L**						
LD	44.42	40.40	37.56	50.42	2.89	0.511
SM	45.36	42.67	42.79	39.68	0.85	0.277
*Redness, a**						
LD	8.01	6.32	9.22	6.31	0.63	0.404
SM	8.21^{b}	6.00^{c}	6.65^{c}	9.85^{a}	0.19	<0.01
*Yellowness, b**						
LD	5.97	3.23	7.02	8.79	0.88	0.293
SM	6.32	5.94	4.02	7.21	0.48	0.263

^{a,b,c} Mean within row which different superscripts differ (P<0.01); SEM is standard error of mean

Table 3.5 Effect of linseed oil supplementation on Water-holding capacity, Warner-Bratzler shear force (N) and texture-related properties of *Longgissimus dorsi* (LD) and *Semimembranosus* (SM) muscle

Item	Treatments				SEM	Pr<F
	HC	200 g/d PO	200 g/d MO	200 g/d LSO		
Water-holding capacity						
Drip loss, %						
LD	6.44[c]	7.78[b]	8.93[a]	8.96[a]	0.10	<0.01
SM	5.23	6.04	6.79	7.00	0.05	0.024
Boiling loss, %						
LD	32.69	32.88	32.63	32.46	0.28	0.639
SM	33.62	33.31	33.72	33.92	0.24	0.838
Thawing loss, %						
LD	4.34	4.48	4.32	4.43	0.34	0.952
SM	5.62	5.76	5.53	5.60	0.16	0.959
Grilling loss, %						
LD	31.89	31.82	31.97	31.62	0.29	0.681
SM	34.34	34.10	34.17	33.64	0.26	0.804
Warner-Bratzler shear force (N)						
LD	3.27	3.49	3.68	3.86	0.09	0.260
SM	6.95[a]	4.77[b]	4.55[b]	4.09[b]	0.02	<0.01
TBARS (mg)						
Day 0						
LD	0.18[a]	0.26[b]	0.31[c]	0.43[d]	0.02	<0.01
SM	0.46	0.48	0.54	0.56	0.01	0.137
Day 6						
LD	0.22[a]	0.28[b]	0.39[c]	0.47[d]	0.02	<0.01
SM	0.46	0.58	0.63	0.69	0.02	0.060

[a,b,c] Mean within row which different superscripts differ (P<0.01); SEM is standard error of mean

No treatment effects were found in water holding capacity (WHC), with the exception of higher drip loss percentage in LD muscle from the 200 g/d LSO supplement than 200 g/d MO, 200 g/d PO and HC, respectively ($P<0.01$) (Table 3.5). The cooking loss corresponds to the loss of water plus a small portion of fat, protein and minerals. Cooking loss values are related to several factors, such as pH, slow post-mortem glycolysis, rapid cooling of the carcass before the onset of *rigor mortis* and storage. These factors, in turn, may also influence the WHC, given that up to one-third of the loss of WHC is caused by decreases in pH (Fiorentini et al., 2012). In the report of Oliveira et al. (2012) who observed that the meat of bulls fed with linseed oil had the highest WHC value ($P<0.05$) in comparison to the soybean oils and linseed oil of protection. Fernandes et al. (2009) who examined Nellore bulls of the same age and genetic pattern found smaller WHC values and fluid losses due to cooking, possibly due to the high pH of their samples.

The shear force in SM muscle of HC treatment was significantly higher than other treatments ($P<0.01$) (Table 3.5). Beef tenderness is a trait considered to be of great relevance for consumers. Shear force is an objective measure of tenderness. According to Swan et al. (1998), bovine meat is considered to have an acceptable tenderness if its shear strength values are below 8 N. The beef in the report of Santana et al. (2014) was considered tender regardless of the lipid supplementation adopted because the average values obtained were 7.5 N. Similar values were obtained by Fiorentiniet al. (2012), with an average value of 7.6 N. Other studies with heifers showed results varying from 3.0 to 6.2 N (Aferri et al., 2005; Restle et al., 2001). Such variations in the shear force values may be caused by differences in the thicknesses of the blades utilized in the analysis. According to Silva et al. (1999), a 1-mm-thick blade is more sensitive to detecting differences in beef tenderness. In the present study, we

utilized a 1.27 mm Warner-Bratzler blade. Silva et al. (1999) reported low (1.9%) intramuscular fat was not correlated with tenderness.

Furthermore, levels of TBARS increased with storage time and LD muscle in 200 g/d LSO treatment was the highest compared with other treatments ($P<0.01$) (Table 3.5). The TBARS values were used as an index of oxidation of muscle lipids, at a biochemical level. While TBARS measurement is widely used in this context, it is recognised that it is a less sensitive index of lipid oxidation than direct measurement of the end-products of fatty acid oxidation. The susceptibility of PUFA to oxidation increases with increasing degree of unsaturation (Yang et al., 2002). For example, compared to C18:2n-6, the susceptibility of C18:3n-3 to peroxidation is more than two-fold higher (Shahidi, 1992). In addition, Campo et al. (2006) proposed that TBARS values increase in beef that has previously been frozen due to damage of some cellular structures thus leading to oxidation. Lipid oxidation in muscle systems is believed to be initiated at the membrane level in the highly unsaturated polar lipid fraction (Gray and Pearson, 1987). Increasing the muscle concentration of long chain PUFA as occurred in the present experiment, may therefore result in significant increases in lipid oxidation.

Habeanu et al. (2014) made separation and analysis of muscle lipid classes by HPLC and light-scattering detection clearly show preponderance of triglycerides (TG) in total lipids for all muscles and diets considered, especially in *Longissimus thoracis* muscle (79.4% of total lipids) compared to that in *semitendinosus* muscle (72.6%) ($P<0.001$). Higher contents in total lipids and triglycerides observed in *Longissimus thoracis* muscle compared to that in *Semitendinosus* muscle were linked to the metabolic oxidative-glycolytic activity of their fibres, *Longissimus thoracis* muscle being known to be more oxidative than

Semitendinosus muscle (Chriki et al., 2012).

3.5.3 Fatty acid composition of beef

The chemical compositions of beef composed of moisture, protein, fat and cholesterol were not significantly different (P>0.05) among treatments (Table 3.6). In the meat composition, fat is the component that presents greatest variations. In general, the quantity of fat deposited is the result of the balance between energy intake and energy consumption by the animal. If energy intake is higher than its metabolic demands, this excess will be storage as fat (Johnson et al., 2003). The greater supply of lipids in the diet was not enough to increase the deposition of fat in the muscle and back fat (P>0.05), likely because the metabolizable energy of the diets (Table 3.1) and net energy intake for growth (Table 3.3) were similar. The literature suggests that the total protein content is less variable in bovine meat, with values of approximately 20% observed in the *Longissimus dorsi* (LD) muscle without the fat cover, and this is independent of food, breed, the genetic group, and the physiological condition (Marques et al., 2006)

The fatty acid composition of fat extracted from LD and SM muscle are presented in Table 3.7 and 3.8. Total PUFA in LD and SM muscles was unaffected by dietary treatments (P >0.05). There was more total n-6 PUFA than total n-3 PUFA, and C18:2n-6 was the most concentrated PUFA across treatment. The percentage of total and individual n-6 PUFA in LD and SM muscle lipids were not different among diets (P>0.05). Feeding 200 g/d LSO increased total n-3 PUFA (P<0.01) in LD and SM muscle and C18:3n-3 in SM muscle (P<0.01) compared with HC treatment. Overall feeding 200 g/d LSO led to a triple of total n-3 PUFA versus HC in LD and SM muscle. The lack of dietary effects on PUFA in LD and SM indicates that LSO supplement had no effect on rates of lipolysis in the rumen. Also the finding of no

difference in total and individual n-6 PUFA in LD and SM lipids, given the differences amount in dietary treatment supply of C18:2n-6 (greater in concentrate, fresh grass and rice straw), indicates a high efficiency of biohydrogenation in the rumen. In contrast, the higher total n-3 PUFA found in LD and SM when feeding 200 g/d LSO may indicate that either the rate of lipolysis and/or the initial step in C18:3n-3 biohydrogenation were reduced and these desirable effects confirm previous observations when feeding linseed (Mach et al., 2006). Feeding 200 g/d LSO increased C22:5n-3 (DPA) content when compared with HC. The lack of diet effects on C22:6n-3 (DHA) in LD relates to the limited capacity for the last steps in the n-3 PUFA elongation and desaturation pathway (Raes, et al., 2004). The accumulation of UFA in the lumen of the rumen may inhibit the complete biohydrogenation (Beam et al., 2000). Therefore, supplementing bovines with unsaturated fatty acids can increase their passage to the small intestine, which allows more absorption and the possibility of changing the fatty acid profile of beef. Rates of lipolysis and biohydrogenation will depend on the amount and type of lipid source supplied to the animals (Van Nevel et al., 1996) and the ruminal pH (Bauman et al., 2005). The average degree of ruminal biohydrogenation is 70%, and it can vary from 60 to 90% (Whigham et al., 2000). Furthermore, C18:3n-3 is less effective in down-regulating SCD activity than C18:2n-6 as suggested earlier by Jacobs et al. (2011). The present report confirms the result of Noci et al. (2005) that the potential of addition of PUFA-rich plant oils or oilseeds to concentrate rations is to increase the PUFA content of ruminant meat. Baird et al. (2010) report no significant difference in the total C18:3n-3 across treatment, as linseed supplementation increased and there was a linear increase in C18:3n-3 as a proportion of total PUFA increased.

The n-6/n-3 ratio in LD and SM was strongly reduced when feeding

the LSO diet (P<0.01, Table 3.7 and 3.8). It is of interest to note that the improvement in the n-6/n-3 ratio in LD and SM muscle was entirely due to increases in n-3 PUFA, as the n-6 PUFA content did not change. In present experiment, the strong reduction in the n-6/n-3 ratio in LD and SM when feeding 200 g/d LSO brought it into the range recommended for human health (4 : 1) (BDH, 1994).

The n-6/n-3 ratios for the linseed-containing diets were lower than the ratio values of 14.7, 9.0, and 6.3 recorded for Holstein bulls fed concentrate containing 3.6%, 11.2%, and 18.0% linseed (Mach et al., 2006). Furthermore, the n-6/n-3ratio in the linseed-containing dietary groups was lower than the ratio recorded in Holstein bulls fed concentrate supplemented with mixed lard and tallow or with palm oil compounds or standard commercial concentrate (de la Fuente et al., 2009; Partida et al., 2007). However, while adding linseed lowered the n-6/n-3 ratio to close to or less than 5 compared with a commercial fattening diet, the ratio was not as low as for animals fed supplemented grass (French et al., 2000), grass or grass silage with different flax or fish oil supplements (Noci et al., 2007; Scollan et al., 2001; Warren et al., 2008), or corn silage supplemented with linseed (Maddock et al., 2006; Raes et al., 2004).

The percentage of total CLA (cis 9,t11 C18:2) in LD and SM was not affected by dietary treatments (P>0.01, Table 3.7 and 3.8). Across most studies, absolute increases in CLA have been limited (<1.0%), and this is likely due to extensive biohydrogenation of PUFA to C18:0, and potentially to reduced delta-9 desaturase activity when feeding PUFA rich oils (Waters, et al., 2009).

Treatment had no effect on total or individual SFA in LD and SM (P>0.01, Table 3.7 and 3.8). The predominant SFA across all diets in LD and SM was C16:0, followed by C18:0 and C14:0. These results could again suggest that C18:3n-3

and its biohydrogenation intermediates were less effective at down-regulating SCD activity than C18:2n-6. Also SFAs relate to changes in endogenous FA synthesis that may not have been differentially affected by diet (Mapiye et al., 2013). Oliveira et al. (2012) when feeding different oils, they reported lower SFA percentages (about 45%). Current health recommendations are to reduce SFA intake, particularly FA with less than 18 carbons, due to their effects on plasma LDL (low density lipoprotein) and cholesterol (Williams, 2000).

The PUFA/SFA ratios in LD and SM were unaffected (P>0.05, Table 3.9 and 3.10) by treatments. The average PUFA/SFA ratio in LD and SM found in this experiment was lower than the recommended ratio of 0.45 (BDH, 1994).

3.5.4 Sensory grading of beef

The sensory perception were unaffected by treatments (Table 3.11). In another study, German Holstein and Simmental bulls finished on grass or fed a concentrate of silage, barley, and cracked linseed produced beef that had a higher n-3 PUFA content than did beef from animals fed a grass-based diet, but the sensory profiles did not differ, except that meat from grass-finished beef had higher bloody and fishy notes (Nuernberg et al., 2005).

On the other hand, when steers were fed diets that had similar base components, but the diets differed in the amount or composition of fatty acids through the addition of different oils, lipid and colour stability were more closely associated with fatty acid composition and greater abnormal flavours and rancidity scores (Scollan et al., 2006). Scheeder et al. (2001) evaluated the beef of bulls fed different sources of fat and found that the beef of animals fed with linseed oil tended to be juicier and to possess a more agreeable aroma. These results may be due to the higher proportions of n-3 PUFA in these animals, triggering odor precursors that are

activated by oxidation during heating. However, changes in PUFA concentrations in the present experiment would not likely have been large enough to affect taste panel assessments.

Table 3.6 Effect of linseed oil supplementation on chemical composition (%) of *Longgissimus dorsi* (LD) and *Semimembranosus* (SM) muscle.

Chemical composition (%)	**Treatments**				**SEM**	**Pr<F**
	HC	**200 g/d PO**	**200 g/d MO**	**200 g/d LSO**		
Moisture						
LD	72.38	72.59	71.75	72.54	0.31	0.789
SM	72.08	72.43	71.69	71.92	0.11	0.251
Protein						
LD	22.39	21.94	22.05	22.43	0.23	0.426
SM	21.84	20.87	21.43	21.52	0.17	0.013
Fat						
LD	3.39	3.21	3.03	3.30	0.29	0.662
SM	4.33	4.28	4.32	4.09	0.06	0.545
Cholesterol (g/100 g beef)						
LD	59.57	61.47	60.13	52.38	0.28	0.648
SM	61.38	68.73	70.83	73.76	2.39	0.410

SEM is standard error of mean

Table 3.7 Effect of linseed oil supplementation on fatty acid composition of the *Longgissimus dorsi* (LD) muscle

Fatty acid (% of total FA)	Treatments				SEM	Pr<F
	HC	200 g/d PO	200 g/d MO	200 g/d LSO		
C10:0	0.22	0.07	0.05	0.03	0.04	0.362
C12:0	0.31	0.63	0.53	0.19	0.04	0.027
C14:0	6.34	6.71	6.27	6.19	0.19	0.930
C15:0	0.95	0.87	1.15	0.91	0.10	0.921
C16:0	33.84	33.30	32.47	33.40	0.33	0.122
C16:1	0.20	0.29	0.27	0.17	0.02	0.260
C18:0	19.63	20.02	18.49	15.95	0.80	0.291
C18:1n9c	34.24	34.95	36.38	38.23	0.94	0.394
C18:2n6t	0.13	0.30	0.30	0.16	0.04	0.121
C18:2n6c	2.42	1.42	1.94	1.63	0.24	0.191
C20:1	0.08	0.12	0.12	0.14	0.01	0.322
C18:3n3	0.13	0.13	0.13	0.17	0.01	0.072
C9,T11	0.23	0.10	0.20	0.42	0.05	0.095
T10,C12	0.15	0.26	0.30	0.31	0.03	0.198
C22:0	0.36	0.13	0.14	0.35	0.06	0.757
C20:3n6	0.00	0.04	0.08	0.01	0.02	0.448
C20:4n6	0.71	0.63	0.99	1.15	0.11	0.162
C20:5n3	0.04^{c}	0.05^{ab}	0.16^{bc}	0.46^{a}	0.04	0.010
C22:6n3	ND	ND	0.05	ND	0.01	0.589
SFA[1]	61.11	61.15	58.45	56.15	0.94	0.235
MUFA[2]	34.51	33.35	36.76	38.67	0.94	0.370
PUFA[3]	3.80	2.93	4.15	4.31	0.42	0.168
tatal n-6[4]	3.63	2.75	3.82	3.68	0.40	0.071
total n-3[5]	0.17^{b}	0.23^{b}	0.42^{b}	0.64^{a}	0.04	0.007
PUFA /SFA	0.06	0.05	0.07	0.08	0.01	0.166
n-6/n-3	24.12^{a}	12.32^{b}	10.73^{bc}	6.11^{c}	1.23	0.002

^{a,b,c} Mean within row which different superscripts differ (P<0.01); SEM = Standard error of mean

[1] SFA = Sum of saturated fatty acid from C4:0 – C20:0; [2] MUFA = Sum of monounsaturated fatty acid from C14:1 – C22:1; [3] PUFA = Sum of polyunsaturated fatty acids from C18:2 – C22:6; [4] Sum of n6 fatty acids C18:2n-6 – C22:4n-6; [5] Sum of n3 fatty acids C18:3n-3 – C22:6n-3

Table 3.8 Effect of linseed oil supplementation on fatty acid composition of the *Semimembranosus* (SM) muscle

Fatty acid (% of total FA)	Treatments				SEM	Pr<F
	HC	200 g/d PO	200 g/d MO	200 g/d LSO		
C10:0	0.05	ND	0.01	0.03	0.01	0.664
C12:0	0.48	0.38	0.38	0.36	0.03	0.295
C14:0	6.25	5.44	5.44	5.77	0.24	0.584
C15:0	1.22	1.15	1.13	1.60	0.07	0.518
C16:0	32.51	31.54	31.71	33.21	0.55	0.649
C16:1	0.21	0.19	0.16	0.06	0.03	0.076
C18:0	15.99	15.89	16.45	15.04	0.50	0.660
C18:1n9c	38.32	37.43	37.28	39.67	0.65	0.504
C18:2n6t	0.11	0.07	0.10	ND	0.03	0.002
C18:2n6c	2.73	3.90	3.42	0.96	0.40	0.676
C20:1	0.06	0.10	0.08	0.01	0.01	0.117
C18:3n3	0.16^{b}	0.33^{b}	0.32^{ab}	0.42^{a}	0.03	0.025
C9,T11	0.10^{b}	0.33^{a}	0.24^{ab}	0.14^{b}	0.04	0.017
T10,C12	0.25	0.30	0.23	0.46	0.04	0.695
C22:0	0.13	0.20	0.16	ND	0.02	0.012
C20:3n6	0.00	0.18	0.30	0.12	0.06	0.264
C20:4n6	1.28	2.11	2.10	1.55	0.19	0.543
C20:5n3	0.15	0.46	0.45	0.27	0.05	0.245
C22:6n3	ND	0.04	0.02	0.32	0.05	0.250
SFA[1]	55.85	53.76	54.48	55.08	0.92	0.782
MUFA[2]	38.59	37.71	37.53	39.95	0.65	0.564
PUFA[3]	4.79	7.71	7.18	4.24	0.59	0.662
tatal n-6[4]	4.48	6.89	6.39	3.23	0.56	0.679
total n-3[5]	0.31^{c}	1.01^{bc}	1.09^{ab}	1.12^{a}	0.08	0.005
PUFA / SFA	0.09	0.14	0.14	0.08	0.01	0.714
n-6/n-3	14.50^{a}	7.36^{b}	6.23^{c}	2.89^{c}	0.49	<0.01

[a,b,c] Mean within row which different superscripts differ (P<0.01); SEM = Standard error of mean; [1] SFA = Sum of saturated fatty acid from C4:0 – C20:0; [2] MUFA = Sum of monounsaturated fatty acid from C14:1 – C22:1; [3] PUFA = Sum of polyunsaturated fatty acids from C18:2 – C22:6; [4] Sum of n6 fatty acids C18:2n-6 – C22:4n-6; [5] Sum of n3 fatty acids C18:3n-3 – C22:6n-3

Table 3.9 Effect of linseed oil supplementation on sensory grading of *Longgissimus dorsi* (LD) and *Semimembranosus* (SM) muscle

Item	Treatments				SEM	Pr<F
	HC	200 g/d PO	200 g/d MO	200 g/d LSO		
Tenderness score						
LD	3.96	4.45	4.33	4.55	0.263	0.879
SM	4.54	3.90	4.69	4.42	0.245	0.724
Juiciness score						
LD	4.08	4.77	4.02	4.23	0.299	0.798
SM	3.71	4.19	4.77	4.71	0.264	0.446
Flavour score						
LD	5.53	6.64	5.44	6.72	0.232	0.092
SM	5.79	6.36	5.99	6.38	0.219	0.728
Off-flavour score						
LD	2.13	2.33	2.41	2.18	0.277	0.983
SM	1.87	2.48	2.08	1.99	0.258	0.853
Overall acceptability						
LD	5.79	6.83	5.62	6.47	0.231	0.206
SM	5.92	6.57	6.00	6.54	0.204	0.543

SEM = Standard error of mean

3.5.5 Rumen fermentation characteristics

Ruminal pH were not affected by oil supplementation (P>0.05) (Table 3.10). Ueda et al. (2003) reported that changes in ruminal pH about 6.78±0.05 due tom LSO were modest and could explain the strong changes in unsaturated fatty acid metabolism in the rumen. Furthermore, in a previous study, Loor et al., 2004 did not observe an effect of concentrate or linseed oil on ruminal pH despite reduced biohydrogenation with the high concentrate diets.

Ammonia nitrogen concentration (NH_3-N) in the rumen did not vary among treatments (P>0.05) (Table 3.10). A decreased protozoa concentration also leads to a decrease in the NH_3-N concentration in the rumen, which is due to a reduction in the proteolytic activity of the protozoa (Doreau and Ferlay, 1995). The effect of dietary supplementation with lipids is highly variable, as it depends on the lipid source and the diets used. In the present experiment, steer supplied the LSO had a higher NH_3-N concentration (20.68 mg/L) than the HC, PO and MO (16.15, 18.32 and 19.21 mg/L, respectively). The NH_3-N concentrations were adequate to support bacterial growth, according to the minimum value of 50 mg NH_3-N/L, as reported by Satter and Slyter (1974). Confirming these results, Eugène et al. (2004) used data obtained from the literature on faunated and defaunated animals in a meta-analysis study and observed that defaunation increased the concentration of rumen ammonia to 50.3 mg NH_3-N/L.

The protozoa concentration in rumen fluid was also not affected by dietary treatments (P>0.05) (Table 3.10). Several studies showed that dietary lipids reduced protozoa concentrations in the rumen (Firkins et al., 2007), because unsaturated fatty acids are toxic to rumen ciliate protozoa. Williams and Coleman (1997) supported that the toxicity of high dietary lipid concentrations to rumen protozoa is due to their limited ability to absorb and transform lipids, resulting in enlargement and cause break of the protozoa cells. However the concentration of the dietary lipids must be sufficient to affect the rumen protozoa population. In the present experiment, the concentration of lipid supplements might be not enough to affect the rumen concentration of protozoa. Furthermore, Machmüller et al. (2000) and Ueda et al. (2003) reported that when the large amounts of linseed oil were fed to cattle, protozoa almost disappeared which could be the cause of the decrease in butyrate

proportion. In addition, effect on protozoa concentration in the present study was consistent with the absence of change in butyrate proportion. Furthermore, both *in vitro* and *in vivo* studies have demonstrated that protozoa are sensitive to linolenic (C18:3), linoleic (C18:2), and oleic (C18:1) unsaturated fatty acids, in this order (Hristov et al., 2004), and to capric (C8:0) and myristic (C14:0) saturated fatty acids (Dohme et al., 1999), with greater defaunation effects observed with medium chain saturated lipids (Machmüller et al., 2000).

The increasing level of LSO supplementation decreased acetate molar concentration ($P<0.05$) at 4 h (post feeding), while the molar proportion of propionate was increased ($P<0.05$) at 2 h (post feeding), resulting in a decreased acetate: propionate ratio ($P<0.05$, Table 3.12). Oil supplementation led to lower VFA concentration, especially a decrease in acetate : propionate ratio in the rumen. It is suggested that unsaturated fatty acid from oil could have interfered with ruminal fermentation resulting in greater gut fill and reduction in residual organic matter digestion (Yang et al., 2009). Onetti et al. (2001) reported that when feeding supplemental lipid the molar proportion of ruminal acetate was decreased and of propionate was increased; resulting, in decreased acetate : propionate ratio. Furthermore, a high supply of linseed oil has been shown in the literature to increase propionic acid at the expense of acetic and butyric acid (Sutton et al., 1983). Ruminal digestibility was not reduced by a supply of linseed oil, while propionate was increased at the expense of either butyrate (Machmüller et al., 2000) or acetate (Gonthier et al., 2004).

A reduction in the acetate to propionate ratio often improves the efficiency of feed utilization, since relatively higher propionate production is associated with less of energy in from of gas (Machmueller et al., 2000). In this study, high molar proportion

of propionate has been found in cow fed with 200 g/d MO and 200 g/d LSO, resulting in low acetate : propionate ratio.

Table 3.10 Effect of linseed oil supplementation on ruminal pH, NH_3-N, protozoa population in rumen fluid grass

Item	Treatments					
	HC	200 g/d PO	200 g/d MO	200 g/d LSO	SEM	Pr<F
pH						
0 hr	7.51	7.46	7.15	7.31	0.048	0.084
2 hr	7.06	6.80	6.90	6.78	0.034	0.123
4 hr	7.25	7.19	7.13	6.87	0.119	0.078
6 hr	7.16	7.35	7.17	7.09	0.057	0.497
NH_3-N (mg/L)						
0 hr	8.87	12.31	12.71	12.81	0.376	0.054
2 hr	16.15	18.32	19.21	20.68	0.425	0.078
4 hr	11.45	12.32	16.06	17.63	0.690	0.090
6 hr	7.78	9.16	9.26	13.69	0.464	0.088
Protozoa (x10^6 cells/ml)						
0 hr	8.75	4.50	5.00	7.25	0.805	0.340
2 hr	3.00	3.00	3.25	3.00	0.763	0.257
4 hr	4.25	4.50	4.25	4.00	0.916	0.668
6 hr	6.25	5.50	6.00	4.75	1.365	0.863

SEM is standard error of mean

Table 3.11 Effect of linseed oil supplementation on Volatile fatty acid (VFA) in rumen fluid

Item	Treatments					
	HC	**200 g/d PO**	**200 g/d MO**	**200g/d LSO**	**SEM**	**Pr<F**
VFA (mol/100 mol)						
Acetate, C2						
0 hr	73.85	73.83	73.30	71.99	0.306	0.251
2 hr	73.01	73.33	69.83	70.57	0.664	0.298
4 hr	73.43[a]	72.90[a]	70.26[b]	70.66[b]	0.276	0.034
6 hr	73.26	73.97	70.90	71.46	0.394	0.133
Propionate, C3						
0 hr	14.92	13.95	14.89	15.89	0.280	0.257
2 hr	16.35[b]	15.82[b]	17.93[a]	17.43[a]	0.081	0.028
4 hr	15.26	14.62	17.20	16.89	0.349	0.145
6 hr	14.70	14.97	16.12	15.59	0.467	0.724
Butyrate, C4						
0 hr	11.24	12.23	11.82	12.13	0.134	0.174
2 hr	10.64	10.85	12.25	12.01	0.619	0.745
4 hr	11.31	12.48	12.55	12.45	0.172	0.162
6 hr	12.05	11.06	12.99	12.95	0.709	0.752
Acetate:Propionate						
0 hr	4.94	5.30	4.93	4.54	0.112	0.267
2 hr	4.47[a]	4.64[a]	3.90[b]	4.05[b]	0.057	0.028
4 hr	4.82	4.99	4.11	4.19	0.338	0.095
6 hr	4.98	5.00	4.42	4.59	0.143	0.470

[a,b,] Mean within row which different superscripts differ (P<0.05); SEM = Standard error of mean

3.5.6 Rumen fluid fatty acid profiles

Fatty acid concentrations in the rumen fluid were varied depending on the time after feeding and oil supplements in the diet as shown in Table 3.13, 3.14, 3.15 and 3.16. The C16:0 percentage was similar between HC, MO and LSO at 0 h (pre feeding), 2 and 4 h (post feeding) ($P>0.01$), however oil supplement decreased C16:0 at 6 h (post feeding) ($P<0.01$). Dietary LSO and MO resulted in markedly lower C18:0 at 2 h (post feeding) ($P<0.01$), and increased percentages of C18:0 at 6 h (post feeding) ($P<0.05$). The percentage of C18:1 was higher in HC treatment than in PO, MO, and LSO at 0 h (pre feeding), 4 h and 6 h (post feeding) ($P<0.01$). Both HC and LSO resulted in greater 18:2n6 at 4 h (post feeding) than did PO and MO. Feeding LSO also resulted in greater percentages of C18:3n3 at 2, 4, 6 h (post feeding) ($P<0.01$). Feeding MO and LSO increased percentages of SFA at 0 h (pre feeding), 2 and 6 h (post feeding) ($P<0.01$), and LSO increased percentages of PUFA at 2 and 4 h (post feeding) ($P<0.01$). In addition, LSO supplement resulted in lower n-6/n-3 ratio than MO, PO and HC, respectively. Harvatine and Allen (2006) completed an *in vivo* experiment with lactating dairy cows to determine rates of fatty acid (FA) biohydrogenation (BH) of fat supplements with different grades of unsaturation, and developed a kinetic model of ruminal BH. Based on their results, they showed that passage rates of C16:0, C18:0 and total C18 carbon FA linearly decreased as UFA increased. Increasing UFA increased the extent of C18:2 and C18:3 biohydrogenation, and decreased the extent of C18:1 BH. Gulati, et al. (2000) reported that the concentration of C18:0 indicated a shift of the BH of UFA to the accumulation of C18:1 in the rumen. In contrast to sunflower oil and rapeseed oil, the higher concentration of C18:0 with LSO indicated a shift of the BH of UFA to a lower mean concentration of C18:1. The accumulation of C18:1 is probably due to an excess of

free fatty acids that inhibited the final hydrogenation of C18:1 to C18:0. According to Lock and Garnsworthy (2003), possible reasons for increases in the concentration of C18:1 include an increased intake of substrates (C18:2n6 and C18:3n3) and/or a decrease in the final hydrogenation step from C18:1 to C18:0 in the rumen. A higher concentration of c9,t11 CLA in the rumen fluid in this experiment was observed with PO to maximum 0.26 % of total FA (2 h after feeding) whilst with HC, MO, and LSO the maximum c9,t11 CLA concentration was 0.18, 0.16, 0.14 % of total FA, respectively (2 h after feeding).

The report by Loor et al. (2004) was enhancement of c9, t11 CLA in the rumen fluid of cows fed a high concentrate diet. The lower concentration of these isomers with LSO was accompanied by the higher concentration of C18:3n3 in the rumen fluid compared to PO, MO and HC. The input of C18:3n3, when hydrogenation is incomplete may result in an enhanced ruminal outflow of C18:2 and C18:1 (Loor et al., 2004). According to Loor et al. (2002) LSO may increase an endogenous synthesis of c9, t11 CLA in tissues by enhancing the post absorptive availability of C18:1. It is evident that the differences in c9, t11 CLA concentration between oil supplements are influenced by the level of C18:2n6 in the original oils used to produce CLA (Szolloskei et al., 2005). Greater C18:3n3 hydrogenation with LSO and greater C18:2n6 BH with HC was documented by Loor et al. (2005). Therefore, high concentration of linoleic acid in the diet would reduce biohydrogenation and increase the postruminal flow of this unsaturated fatty acid (Beam et al., 2000).

Table 3.12 Effect of linseed oil supplementation on percentage of fatty acids in ruminal fluid from beef steers at 0 h (pre feeding)

Item	HC	200 g/d PO	200 g/d MO	200 g/d LSO	SEM	Pr<F
C12:0	1.66	1.27	1.41	1.61	0.1	0.572
C14:0	2.98	2.59	2.35	2.65	0.28	0.888
C16:0	33.05	34.1	35.23	31.86	0.42	0.166
C18:0	52.21	52.73	54.22	56.52	0.54	0.149
C18:1	7.39[a]	6.57[a]	4.91[b]	4.38[b]	0.18	0.012
C18:2	1.84	1.87	1.55	1.15	0.11	0.23
C18:3	0.01	0.05	0.35	0.2	0.02	0.078
C20:0	0.8	0.75	0.81	1.08	0.02	0.031
C9,T11	0.08[a]	0.09[a]	0.03[b]	0.04[b]	0.01	<0.01
SFA	89.90[b]	90.68[b]	93.20[a]	92.64[a]	0.23	0.018
PUFA	2.72	2.75	2.42	2.46	0.13	0.726
PUFA/SFA	0.03	0.03	0.03	0.03	0.001	0.479
n-6/n-3	271.00[a]	64.10[b]	76.20[b]	12.90[b]	8.16	<0.01

[a,b] Mean within row which different superscripts differ (P<0.01)

SEM is standard error of mean

Table 3.13 Effect of linseed oil supplementation on percentage of fatty acids in ruminal fluid from beef steers at 2 h (post feeding)

Item	HC	200 g/d PO	200 g/d MO	200 g/d LSO	SEM	Pr<F
C12:0	6.05	5.87	4.15	4.00	0.27	0.106
C14:0	9.6	11.03	11.24	10.77	0.27	0.272
C16:0	27.81	33.29	34.17	36.20	0.69	0.06
C18:0	34.83[a]	30.23[b]	25.82[c]	24.86[c]	0.92	0.049
C18:1	15.74	17.75	12.06	12.98	0.58	0.077
C18:2	2.72	2.69	2.52	2.49	0.04	0.259
C18:3	0.03[d]	1.55[c]	2.79[b]	4.71[a]	0.11	<0.01
C20:0	2.42	2.3	2.33	2.65	0.05	0.203
C9,T11	0.18[b]	0.26[a]	0.16[b]	0.14[b]	0.01	0.081
T10,C12	0.64	0.41	0.37	0.28	0.03	0.05
SFA	78.93	75.45	80.15	77.05	0.73	0.252
PUFA	5.34[c]	6.80[b]	7.80[b]	9.98[a]	0.15	<0.01
PUFA/SFA	0.07[c]	0.09[c]	0.10[b]	1.13[a]	0.01	<0.01
n-6/n-3	221.25[a]	3.42[b]	1.80[b]	1.13[b]	10.81	<0.01

[a,b,c] Mean within row which different superscripts differ (P<0.01)

SEM is standard error of mean

Table 3.14 Effect of linseed oil supplementation on percentage of fatty acids in ruminal fluid from beef steers at 4 h (post feeding)

Item	HC	200 g/d PO	200 g/d MO	200 g/d LSO	SEM	Pr<F
C12:0	5.01	5.58	4.38	5.74	0.24	0.034
C14:0	12.02^{b}	12.54^{b}	13.80^{a}	14.97^{a}	0.15	<0.01
C16:0	32.94	32.83	34.1	35.07	0.66	0.625
C18:0	31.02	30.5	33.08	28.38	0.93	0.45
C18:1	12.07^{a}	12.96^{a}	8.19^{b}	8.18^{b}	0.16	<0.01
C18:2	2.81^{a}	2.48^{b}	2.50^{b}	2.73^{a}	0.02	0.021
C18:3	ND	0.1^{b}	0.36^{b}	1.13^{a}	0.04	<0.01
C20:0	3.47^{a}	2.67^{b}	3.37^{a}	3.52^{a}	0.04	<0.01
C9,T11	0.08^{a}	0.07^{ab}	0.03^{c}	0.02^{bc}	0.01	0.046
T10,C12	0.61	0.3	0.21	0.28	0.03	0.023
SFA	81.58^{b}	81.73^{b}	85.56^{a}	84.43^{a}	0.18	<0.01
PUFA	6.35^{b}	5.31^{c}	6.25^{b}	7.41^{a}	0.09	<0.01
PUFA/SFA	0.08^{ab}	0.07^{c}	0.08^{bc}	0.09^{a}	0.002	0.032
n-6/n-3	ND	52.83^{a}	17.93^{b}	5.58^{b}	2.23	<0.01

^{a,b,c} Mean within row which different superscripts differ (P<0.01)

SEM is standard error of mean

Table 3.15 Effect of linseed oil supplementation on percentage of fatty acids in ruminal fluid from beef steers at 6 h (post feeding)

Item	HC	200 g/d PO	200 g/d MO	200 g/d LSO	SEM	Pr<F
C12:0	6.51	6.8	4.69	5.52	0.08	0.121
C14:0	11.37	11.57	11.34	12.42	0.24	0.436
C16:0	39.78^{a}	34.20^{b}	33.94^{b}	36.43^{b}	0.33	0.01
C18:0	30.29^{b}	34.05^{b}	40.62^{a}	34.59^{b}	0.7	0.028
C18:1	7.73^{b}	9.22^{a}	5.29^{d}	6.71^{c}	0.07	<0.01
C18:2	1.69	1.84	1.82	1.73	0.04	0.56
C18:3	ND	0.03^{b}	0.06^{b}	0.13^{a}	0.01	<0.01
C20:0	2.11^{b}	2.15^{b}	2.17^{b}	2.45^{a}	0.02	0.022
C9,T11	0.03^{b}	0.35^{a}	0.04^{b}	0.35^{a}	0.01	<0.01
T10,C12	0.51^{a}	0.12^{b}	0.04^{b}	ND	0.02	<0.01
SFA	88.45^{b}	86.73^{c}	90.63^{a}	88.96^{b}	0.08	<0.01
PUFA	3.83	4.06	4.08	4.34	0.06	0.127
PUFA/SFA	0.04	0.04	0.04	0.05	0.01	0.381
n-6/n-3	ND	134.17^{a}	78.47^{b}	33.18^{c}	5.12	<0.01

^{a,b,c,d} Mean within row which different superscripts differ (P<0.01)

SEM is standard error of mean

3.6 Conclusions

Feeding dietary treatments including HC and 200 g/d of oil PO, MO or LSO did not negatively affect any of performance and carcass quality of steers. The overall feed consumption of the steers was decreased when dietary oil was provided, leading to improvement in efficiency of growth performance. LSO supplement increased % drip loss, TBARS values and reduced beef color stability (a*), beef tenderness and had no impact on sensory perceptions. LSO increased the percentage of n-3 fatty acids (mainly C18:3n3) in the intramuscular fat and lowered the n-6/n-3 ratio in beef. Thus, it can be concluded that 200 g/d LSO can be safely supplemented to low concentrate and fresh grass diets of steers to enrich beef with potential health beneficial FA, without causing any detrimental effect on rumen fermentation function.

3.7 References

Aferri, G., Leme, P. R., Silva, S. L., Putrino, S. M., and Pereira, A. S. C. (2005). Performance and carcass characteristics of steers fed diets containing different fat sources. **Rev. Bras. Zootec.** 34 : 1516-3598.

Andrae, J.G., Hunt, C.W., Duckett, S.K., Kennington, L.R., Feng, P., Owens, F.N., and Soderlung, S. (2001). Effects of high-oil corn on growth performance, diet digestibility, and energy content of finishing diets fed to beef cattle. **J. Anim. Sci**. 78 : 2257-2262.

Angkuro, S. (2003). Beef cattle situation. pp. 1–9. **In Thai Beef Cattle : Development and the Impact of Free Trade Agreement Asia Hotel.** Bangkok, Thailand.

Association of Official Analytical Chemists. (1995). **Official Methods of Analysis**. 16th ed. AOAC, Arlington, VA, USA. 1110p.

Baird, L. G., Dawson, L. E. R., Young, I. S. and O'Connell, N. E. (2010). Lipid content and fatty acid composition of the digital cushion of bulls offered different amounts of linseed. **J Anim. Sci.** 88 : 2403-2409.

Bauman, D. E., Lock, A. L., Corl, B. A., Ip, C., Salter, A. M., and Parodi, P. M. (2005). Milk fatty acids and human health : potential role of conjugated linoleic acid and trans fatty acids. **In Ruminant Physiology : 529-561.** Digestion, Metabolism and Impact of Nutrition on Gene Expression, Immunology and Stress. Edited by K. Serjrsen, T. Hvelplund and M. O. Nielsen. Wageningen, The Netherlands : Wageningen Academic Publishers.

Beam, T. M., Jenkins, T. C. and Moate, P. J. (2000). Effects of amount and source of fat on the rates of lipolysis and biohydrogenation of fatty acids in ruminal contents. **J Dairy Sci.** 83 : 2564-2573.

Bendall, J. R. and Swatland, H. J. (1988). A review of the relationships of pH with physical aspects of pork quality. **Meat Sci.** 24 : 85-126.

Berg, R. T., and Butterfield, R. M. (1976). **New Concepts of Cattle Growth.** Sydney University Press, Sydney, N.S.W. 215p.

British Department of Health (1994). Nutritional aspects of cardiovascular diseases. **Report on Health and Social Subjects No. 46.** London : H.M. Stationary Office.

Bonilha, S. F. M., Tedeschi, L. O., Packer, I. U., Razook, A. G., Alleoni, G. F., Nardon, R. F., and Resende, F. D. (2008). Evaluation of carcass characteristics of *Bos indicus* and tropically adapted *Bos taurus* breeds selected for post weaning weight. **J. Anim. Sci.** 86 : 1770–1780.

Campo, M. M., Nute, G. R., Hughes, S. I., Enser, M., Wood, J. D., and Richardson, R. I. (2006). Flavour perception of oxidation in beef. **Meat Sci.** 72 : 303-311.

Chriki, S., Picard, B., Jurie, C., Reichstadt, M., Micol, D., Brun, J. P., Journaux, L., Hocquette, F. (2012). Meta-analysis of the comparison of the metabolic and contractile characteristics of two bovine muscles : *Longissimus thoracis* and *Semitendinosus*. **Meat Sci.** 91 : 423-429.

CIE. (1978). International commission on illumination, recommendations on uniform color spaces, color difference equations, psychometric color terms. **Supplement No. 15 to CIE publication No. 15 (E-1.3.1) 1971/ (TO-1.3).** Bureau Central de la CIE, Paris, France.

de la Fuente, J., Díaz, M. T., Álvarez, I., Oliver, M.A., Furnols, M. F, Sañudo, C., Campo, M. M., Montossi, F., Nute, G. R., and Cañeque, V. (2009). Fatty acid and vitamin E composition of intramuscular fat in cattle reared in different production systems. **Meat Sci.** 82(3) : 331-337.

DLD (Department of Livestock Development). (2004). **Economic Data of Import Meat and Meat Product in 2003.** Bangkok, Thailand : Ministry of Agriculture and Cooperative Royal Thai Government.

DLD (Department of Livestock Development). (2012). **Beef Strategies (2012-2516).** Bangkok, Thailand : Ministry of Agriculture and Cooperative, Royal Thai Government.

Dohme, F., Machmüller, A., Estermann, B. L., and Kreuzera, M., (1999). The role of the rumen ciliate protozoa for methane suppression caused by coconut oil. **Lett. Appl. Microbiol.** 29 : 87-92.

Doreau, M., and Ferlay, A. (1995). Effect of dietary lipids on nitrogen metabolism in the rumen : A review. **Livest. Prod. Sci.** 43 : 97-110.

Eugène, M., Archimède, H., and Sauvant, D. (2004). Quantitative meta-analysis on the effects of defaunation of the rumen on growth, intake and digestion in ruminant.

Livest. Prod. Sci. 85 : 81-97.

Faustman, C., and Cassens, R. G. (1990). The biochemical basis for discoloration in freshmeat : a review. **J. Muscle Foods.** 1 : 217-243.

Faustman, C., Sun, Q., Mancini, R., and Suman, S. P. (2010). Myoglobin and lipid oxidation interactions : Mechanistic bases and control. **Meat Sci.** 86 : 86-94.

Ferreira, M. A., Valadares Filho, S. C., Coelho da Silva, J. F., Paulino, M. F., Valadares, R. F. D., Cecon, P. R., and Muniz, E. B. (1998). Body composition and liquidrequirements of protein and energy for weight gain of F1 Simental × Nellore bulls. **Rev. Bras. Zootec.** 28 : 352-360.

Fernandes, A. R. M., Sampaio, A. A. M., Henrique,W., Tullio, R. R., Oliveira, E. A., and Silva, E. M. (2009). Composição química e perfil de ácidos graxos da carne de bovinos de diferentes condições sexuais recebendo silagem de milho e concentrado ou cana-deaçúcare concentrado contendo grãos de girassol. **Rev. Bras. Zootec.** 38 : 705-712.

Fiorentini, G., Berchielli, T. T., Santana, M. C. A., Dian, P. H. M., Reis, R. A., Sampaio, A. M. M., and Biehl, M. V. (2012). Qualitative characteristics of meat from confined crossbred heifers fed with lipid sources. **Sci. Agric.** 69 : 336-444.

Folch, J., Lees, M., and Sloane-Stanley, G.H. (1957). A simple method for the isolation and purification of total lipids from animal tissues. **J. Bio. Chem**. 226 : 495-509.

Firkins, J. L., Yu, Z., and Morrison, M., (2007). Ruminal nitrogen metabolism : perspectives for integration of microbiology and nutrition for dairy. **J. Dairy Sci.** 90 : E1-E16.

French, P., Stanton, C., Lawless, F., O'Riordan, E. G., Monahan, F. J., Caffrey, P. J.,

and Moloney, A. P. (2000). Fatty acid composition, including conjugated linoleic acid, of intramuscular fat from steers offered grazed grass, grass silage or concentrate-based diets. **J. Anim. Sci.** 78(11) : 2849-2855.

Geay, Y., D. Bauchart, J. F. Hocquette, and J. Culioli. (2001). Effect of nutritional factors on biochemical, structural and metabolic characteristics of muscles in ruminants, consequences on dietetic value and sensorial qualities of meat. **Reprod. Nutr. Dev.** 41 : 1-26.

Glaser, K. R., Wenk, C., and Scheeder, M. R. L. (2004). Evaluation of pork backfat firmness and lard consistency using several different physicochemical methods. **J. Sci. Food Agric.** 84 : 853-862.

Gonthier, C., A. F. Mustafa, R. Berthiaume, H. V. Petit, R. Martineau, and D. R. Ouellet. (2004). Effects of feeding micronized and extruded flaxseed on ruminal fermentation and nutrient utilization by dairy cows. **J. Dairy Sci.** 87 : 1854-1863.

Gray, J. I., and Pearson, A. M. (1987). Rancidity and warmed-over flavour. **Adv. Meat Res.** 3 : 221-269.

Gregory J., Foster, K., Tyler, H.,Wiseman, M. (1990). **The Dietary and Nutritional Survey of British Adults.** HMSO. London.

Gulati, S. K., Kitessa, S. M., Ashes, J. R., Fleck, E., Byers, E. B., Byers, Y. G., and Scott, T. W. (2000). Protection of conjugated linoleic acids from ruminal biohydrogenation and their incorporation into milk fat. **Anim. Feed Sci. Technol.** 86 : 139-148.

Habeanu, M., Thomas, A., Bispo, E., Gobert, M., Gruffat, D., Durand, D., and Bauchart, D. (2014). Extruded linseed and rapeseed both influenced fatty acid composition of total lipids and their polar and neutral fractions in *longissimus thoracis* and

semitendinosus muscles of finishing Normand cows. **Meat Sci.** 96 : 99-107.

Harvatine, K.J., and Allen, M.S. (2006). Effects of fatty acid Supplements on ruminal and total tract nutrient digestion in lactating dairy cows. **J. Dairy Sci**. 89 : 1092-1103.

He, M., and Armentano, L. E. (2011). Effect of fatty acid profile in vegetable oils and antioxidant supplementation on dairy cattle performance and milk fat depression. **J. Dairy Sci.** 94 : 2481-2491.

Herdmann, A., Martin, J., Nuernberg, G., Dannenberger, D., Nuernberg, K. (2010). Effect of dietary n3 and n-6 PUFA on lipid composition of different tissues of German Holstein bulls and the fate of bioactive fatty acids during processing. **J. Agri. Food Chem.**58 : 8314–8321.

Honikel, K. O. (1987). How to measure the water-holding capacity of meat? (pp. 129–142).Recommendation of the standardized method. In Eikelenboom, G., Tarrant, P. V., and Monin, G. (Eds.). **Evaluation and control of meat quality in pigs.** The Hague, The Netherland : Martinus Nijhoff Publishers.

Hood, D. E. (1980). Factors affecting the rate of metmyoglobin accumulation in pre-packaged beef. **Meat Sci.** 4 : 247-265.

Hristov, A. N., Ivan, M., and McAllister, T. A. (2004). *In vitro* effects of individual fatty acids on protozoal numbers and on fermentation products in ruminal fluid from cattle fed a high concentrate, barley-based diet. **J. Anim. Sci.** 82 : 2693-2704.

Jacobs, A. A. A., van Baal, J., Smits, M. A., Taweel, H. Z. H., Hendriks, W. H., van Vuuren, A. M., and Dijkstra, J. (2011). Effects of feeding rapeseed oil, soybean oil, or linseed oil on stearoyl-CoA desaturase expression in the mammary gland of dairy cows. **J. Dairy Sci.** 94(2) : 874-887.

Jaturasitha, S. (2004). **Meat Management**. Chiang Mai, Thailand : Mingmuang Press.

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

Johnson, D. E., Ferrell, C. L. and Jenkins, T. G. (2003). The history of energetic efficiency research : Where have we been and where are we going? **J. Anim. Sci.** 81(E. Suppl. 1) : E27-E38.

Kanner, J., and Harel, S. (1985). Initiation of membranal lipid peroxidation by activated metmyoglobin and methemeglobin. **Arch. Biochem. Biophys**. 237 : 314-319.

Ladeira, M. M., Santarosa, L. C., Chizzotti, M. L., Ramos, E. M., Machado, Netoa O. R., Oliveira, D. M., Carvalhoa, J. R. R., Lopes, L. S., and Ribeiro, J. S. (2014). Fatty acid profile, color and lipid oxidation of meat from young bulls fed ground soybean or rumen protected fat with or without monensin. **Meat Sci.** 96 : 597-605.

Ledward, D. A. (1992). Colour of raw and cooked meat. In : Johnston, D. E., M. Knight, and D. A. Ledward. **The Chemistry of Muscle Based Foods.** Cambridge : Royal Society of Chemistry. 128–144.

Lee, K. T. and Yoon, C. S. (2001). Quality changes and shelf-life of imported vacuum-packaged beef chuck during storage at 0ºC. **Meat Sci.** 59 : 71-77.

Lindahl, G., Lundstrom, K.., and Tornberg, E. (2001). Contribution of pigment content, myoglobin forms and internal reflectance to the colour of pork loin and ham from pure breed pigs. **Meat Sci.** 59 : 141-151.

Lock, A. L. and Garnsworthy, P. C. (2003). Seasonal variation in milk conjugated linoleic acid and Δ9-desaturase activity in dairy cows. **Livest. Prod. Sci.** 79 : 47-59.

Loor, J. J., Ferlay, A., Doreau, M., and Chilliard, Y. (2002). Conjugated linoleic acids (CLA), trans fatty acids, and lipid content in milk from Holstein cows fed a high- or lowconcentrate diet with two levels of linseed oil. **J. Dairy Sci.** 85 : 1188 pp.

Loor, J. J., Ueda, K., Ferlay, A., Chilliard, Y., and Doreau M. (2004). Short communication : Diurnal profiles of conjugated linoleic acid and trans fatty acid in ruminal fluid from cows fed a high concentrate diet supple mented with fish oil, linseed oil, or sunflower oil. **J. Dairy Sci.** 87 : 2468-2471.

Lynch, M. P., and Faustman, C. (2000). Effect of aldehyde lipid oxidation products on myoglobin. **J. Agri. Food Chem.** 48 : 600-604.

Mach, N., Devant, M., Bach, A., Díaz, I., Font, M., Oliver, M. A., and García, J. A. (2006). Increasing the amount of omega-3 fatty acid of meat from intensively fed young Holstein bulls through nutrition. **J. Anim. Sci.** 84 : 3039-3048.

Mach, N., A. Bach, A. Velarde, and M. Devant. (2008). Association between animal, transportation, slaughterhouse practices, and meat pH in beef. **Meat Sci.** 78 : 232-238.

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

Maddock, T. D., Bauer, M. L., Koch, K. B., Anderson, V. L., Maddock, R. J., Barcelo-Coblijn, G., Murphy, E. J., and Lardy, G. P. (2006). Effect of processing flax in beef feedlot diets on performance, carcass characteristics, and trained sensory panel ratings. **J. Anim. Sci.** 84(6) : 1544-1551.

Mancini, R. A., and Hunt, M. C. (2005). Current research in meat color. **Meat Sci.** 71 : 100-121.

Mapiye, C., Aalhus , J. L., Turner, T. D, Rolland, D. C., Basarab, J. A., Baron, V. S., McAllister, T. A., Block, H.C. Uttaro, B., Lopez-Campos, O., Proctor, S. D., and Dugan, M. E. R.(2013). Effects of feeding flaxseed or sunflower-seed in high-forage diets on beef production, quality and fatty acid composition. **Meat Sci**. 95 : 98-109.

Marques, J. A., Prado, I. N., Moletta, J. L., Prado, I. M., Prado, J. M., Macedo, L. M. A., Souza, N. E., and Matsushita, M. (2006). Características físicoquímicas da carcaça e da carne de novilhas submetidas ao anestro cirúrgico ou mecânico terminadas em confinamento. **Rev. Bras. Zootec.** 35 : 1514-1522.

McAfee, A. J., McSorley, E. M., Cuskelly, G. J., Moss, B. W.,Wallace, J.M.W., Bonhama, M. P., and Fearon, A. M. (2010). Red meat consumption : An overview of the risks and benefits. **Meat Sci.** 84 : 1-13.

Metcalfe, L. D., Schmitz, A. A. and Pelka, J. R. (1966). Rapid preparation of fatty acid esters from lipids for gas chromatographic analysis. **Anal. Chem**. 38 : 514-515.

Muchenje, V., K. Dzama, M. Chimonyo, J. G. Raats, and P. E. Strydom. (2009). Some biochemical aspects pertaining to beef eating quality and consumer health : a review. **J. Agric. Food Chem.** 112 : 279-289.

Muller, M., Prado, I. N., Lobo Jr., A. R., Capovilla, L. C. T., Rigolon, L. P. (2004). Fat sources of omega-3 and omega-6 on the apparent digestibility of heifers confined. **Acta Sci. Anim. Sci.** 26 : 393-398.

Ngidi, M. E., Loerch, S. C., Fluharty, F. L., and Palmquist, D. L. (1990). Effects of calcium soaps of long-chain fatty acids on feedlot performance, carcass characteristics and ruminal metabolism of steers. **J. Anim. Sci.** 68 : 2555-2565.

Noci, F., Freach, P., Monahan, F. J., and Moloney, A. P. (2007). The fatty acid composition of muscle fat and subcutaneous adipose tissue of grazing heifers supplemented with plant oil-enriched concentrates. **J. Anim. Sci.** 85 : 1062-1073.

Noci, F., O'Kiely, P., Monahan, F.J., Stanton, C., and Moloney, A.P. (2005). Conjugated linoleic acid concentration in *M. Longissimus dorsi* from heifersoffered sunflower oil-based concentrates and conserved forages**. Meat Sci**. 69 : 509-518.

Nuernberg, K., Dannenberger, D., Nuernberg, G., Ender, K., Voigt, J., and Scollan, N. (2005). Effect of a grass-based and a concentrate feeding system on meat quality characteristics and fatty acid composition of *longissimus* muscle in different cattle breeds. **Livest. Prod. Sci**. 94 : 137-147.

Oliveira, D. M., Ladeira, M. M., Chizzotti, M. L., Machato Neto, O. R., Ramos, E. M., Gonçalves, T. M., Bassi, M. S., Lanna, D. P. D., and Ribeiro, J. S. (2011). Fatty acid profile and qualitative characteristics of meat from Zebu steers fed with different oilseeds. **J. Anim. Sci.** 89 : 2546-2555.

Oliveira, E. A., Sampaio, A. A. M., Henrique, W., Pivaro, T. M., Rosa, B. L. A., Fernandes, R. M., and Andrade, A. T. (2012). Quality traits and lipid composition of meat from Nellore young bulls fed with different oils either protected or unprotected from rumen degradation. **Meat Sci.** 90 : 28-35.

Onetti, S. G., Shaver, R. D., McGuire, M. A., and Grummer, R. R. (2001). Effect of typeand level of dietary fat on rumen fermentation and performance of dairy cows fed corn silage-based diets. **J. Dairy Sci.** 84 : 2751-2759.

Ostrowska, E., Dunshea, F.R., Muralitharan, M., and Cross, R.F. (2000). Comparison of silver-ion high-performance liquid chromatographic quantification of free

and methylated conjugated linoleic acids. **Lipids**. 35 : 1147-1153.

Palmquist, D. L. (2009). Omega-3 fatty acids in metabolism, health, and nutrition and for modified animal product foods. **Prof. Anim. Sci.** 25(3) : 207-249.

Partida, J. A., Olleta, J. L., Sañudo, C., Albertí, P., & Campo, M. M. (2007). Fatty acid composition and sensory traits of beef fed palm oil supplements. **Meat Sci.** 76(3) : 444-454.

Realini, C. E., Duckett, S. K., Brito, G. W., Rizza, M. D, and Mattos, D. D. (2004). Effect of pasture vs. concentrate feeding with or without antioxidants on carcass characteristics, fatty acid composition, and quality of Uruguayan beef. **Meat Sci.** 66 : 567–577.

Raes, K., De Smet, S.,and Demeyer, D. (2004). Effect of dietary fatty acids on incorporation of long chain polyunsaturated fatty acids and conjugated linoleic acid in lamb, beef and pork meat. **Anim. Feed Sci. Technol.** 113(1-4) : 199-221.

Restle, J., Cerdótes, L., Vaz, F. N., and Brondani, I. L. (2001). Carcass and meat characteristics of charolais and 3/4 charolais 1/4 Nellore cull heifers, finished infeedlot. **Rev. Bras. Zootec.** 30 : 1065-1075.

Romeu-Nadal, M., Morera-Pons, S., Castellote, A.I. and Lopez-Sabater, M.C. (2004). Comparison of two methods for the extraction of fat from human milk. **Anal. Chim. Acta.** 513 : 457-461.

Rossell, J. B. (1994). Measurement of rancidity. In Allen J. C., and Hamilton, R. J. (Eds.), **Rancidity in foods** (pp. 22–53). London, UK : Blackie Academic and Professional.

Rowe, A., Macedo, F.A.F.,Visentainer, J. V., Souza, N.E., and Matsushita, M. (1999). Muscle composition and fatty acid profile in lambs fattened in drylot or

pasture. **Meat Sci.**51 : 283-288, 1999.

SAS. (2001). Institute Inc., SAS/STAT Software : Changes and Enhancements, Release 8.2, Cary, NC. USA.

Santana, M. C. A., Fiorentinia, G., Diana, P. H. M., Canesina, R. C., Messanaa, J. D., Oliveira, R. V., Reisa, R. A., and Berchielli, T. T. (2014). Growth performance and meat quality of heifers receivingdifferent forms of soybean oil in the rumen. **Anim. Feed Sci. Technol.** Article in press. 9 pp.

Satter, L., and Slyter, L. L. (1974). Effect of ammonia concentration on rumen microbial protein production in vitro. **Br. J. Nutr.** 32 : 199-208.

Scheeder, M. R. L., Casutt, M. M., Roulin, M., Escher, F., Dufey, P. A., and Kreuzer, M. (2001). Fatty acid composition, cooking loss and texture of beef patties from meat of bulls fed different fats. **Meat Sci.** 58 : 321-328.

Scollan, N. D., Choi, N. J., Kurt, E., Fisher, A. V., Enser, M., and Wood, J. D. (2001). Manipulating the fatty acid composition of muscle and adipose tissue in beef cattle. **Br. J. Nutr.** 85 : 115-124.

Scollan, N. G., Hocquette, J. F., Nuernberg, K., Dannenberger, D., Richardson, R. I., and Moloney, A. P. (2006). Innovations in beef production systems that enhance the nutritional and health value of beef lipids and their relationship with meat quality. **Meat Sci.** 74 : 17-33.

Seth, G., E. Tornberg, and B. M. Moller. (1991). The influence of constant rigor temperature and storage time on the internal reflectance (FOP) in pork muscle. **In : International Congress of Meat Sci. Technol. Proceedings.** Germany : Kulmbach. 37 : 481-484.

Shahidi, F. (1992). Prevention of lipid oxidation in muscle foods by nitrite and nitrite free compositions. In A. J. St. Angelo (Ed.), **Lipid Oxidation in Food** (pp.

161-182). Washington DC, USA.

Silva, J. A., Patarata, L., and Martins, C. (1999). Influence of ultimate pH on bovine meat tenderness during ageing. **Meat Sci.** 52 : 453-459.

Steel, R. G. D. and Torries, J. H. (1980). **Principles and Procedures of Statistics : a Biometeric Approach (2nd ed.)** McGrowHill, New York.

Stone, H., Sidel, J., Oliver, S., Woolsey, A., &Singleto, R. C. (1974). Sensory evaluation by quantitative descriptive analysis. **Food Technol**. 28 : 24-34.

Sutton, J. D., Knight, R., McAllan, A. B., and Smith, R. H. (1983). Digestion and synthesis in the rumen of sheep given diets supplemented with free and protected oils. **Br. J. Nutr.** 49 : 419-432.

Swan, J. E., Esguerra, C. M., and Farouk, M. M. (1998). Some physical, chemical and sensory properties of chevon products from three New Zealand goat breeds. **Small Rum. Res.** 28 : 273-280.

Szolloskei, G., Wagner, L., Nemeth, S., and Husveth, F. (2005). *In sacco* studies of conjugated linoleic acid productionfrom various oils in the rumen of sheep. **Acta Vet. Hung.** 53 : 411-423.

Ueda, K., Ferlay, A., Chabrot, J., Loor, J.J., Chilliard, Y., and Doreau, M. (2003). Effect of linseed oil supplementation on ruminal digestion in dairy cows feddiets with different forage : concentrate ratios. **J. Dairy Sci.** 86 : 3999-4007.

Van Soest P. J., Robertson J. B., and Lewis B. A. (1991). Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. **J. Dairy Sci.** 74 : 3583-3597.

Viriyajare, P. (1992). **Experiment and Analysis of Organoleptic Test.** Thailand : Department of Food Science and Technology, Faculty of Agriculture,

ChiangMai University. 275 p.

Waters, S. M., Kelly, J. P., O'Boyle, P., Moloney, A. P., and Kenny, D. A. (2009). Effect of level and duration of dietary n-3 polyunsaturated fatty acid supplementation on the transcriptional regulation of Δ9-desaturase in muscle of beef cattle. **J. Anim. Sci.** 87(1) : 244-252.

Warren, H. E., Scollan, N. D., Enser, M., Hughes, S. I., Richardson, R. I., and Wood, J. D. (2008). Effects of breed and a concentrate or grass silage diet on beef quality in cattle of 3 ages. I : Animal performance, carcass quality and muscle fatty acid composition. **Meat Sci.**78(3) : 256-269.

Whigham, L. D, Cook, M. E., and Atkinson, R. L. (2000). Conjugated linoleic acid : Implications for human health. **Pharm Res.** 42 : 503-510.

Williams, A. G., and Coleman, G. S. (1997). The rumen protozoa. In : Hobson, P.N., Stewart, C.S. (Eds.). **The Rumen Microbial Ecosystem. Blackie.** Academic and Professional, London, UK. pp.73-139.

Williams, C. (2000). Dietary fatty acids and human health. **Ann Zootech J**. 49(3) : 165-180.

Wood, J. D., Richardson, R. I., Nute, G. R., Fisher, A. V., Campo, M. M., Kasapidou, E., Sheard, P.R.,and Enser, M. (2004). Effects of fatty acids on meat quality : a review. **Meat Sci.** 66(1) : 21-32.

World Health Organization. (2003). **Diet, Nutrition and the Prevention of Chronic Diseases.** Report of the Joint WHO/FAO Expert Consultation; WHO Technical Report Series No. 916; WHO : Geneva, Switzerland.

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

Yang, A., Lanari, M. C., Brewster, M., and Tume, R. K. (2002). Lipid stability and meat colour of beef from pasture- and grain-fed cattle with or without vitamin E supplement. **Meat Sci.** 60 : 41–50.

Yang, S. L., Bu, D. P., Wang, J. Q., Hu, Z. Y., Li, D., Wei, H. Y., Zhou, L. Y., and Loor, J. J. (2009). Soybean oil and linseed oil supplementation affect profiles of ruminal microorganisms in dairy cows. **Anim.** 3 : 1562.1569.

Zakrys, P. I., Hogan, S. A., O'Sullivan, M. G., Allen, P., and Kerry, J. P. (2008). Effects of oxygen concentration on the sensory evaluation and quality indicators of beef muscle packed under modified atmosphere. **Meat Sci.** 79 : 648-655.

Zinn, R. A., Alvarez, E. G., Montaño, M. F., and Ramirez, J. E. (2000). Interaction of protein nutrition and laidlomycin on feedlot growth performance and digestive function in Holstein steers. **J. Anim. Sci.** 78 : 1768-1778.

CHAPTER IV

THE EFFECT OF LINSEED OIL SUPPLEMENTATION ON MILK PRODUCTION, MILK COMPOSITION AND n-6/n-3 FATTY ACID RATIO IN MILK

4.1 Abstract

The effects of linseed oil supplementation on milk production, milk composition, and n-6/n-3 ratio of dairy cow's milk were studied. Twenty four Holstein Friesian crossbred lactating dairy cows were assigned into a 2 × 2 Factorial arrangement. All cows were fed approximately 6 kg/d of 21% CP concentrate. Treatments were : 1) concentrate plus 300 g/d of palm oil (PO) together with *ad libitum* corn silage (CS); 2) concentrate plus 300 g/d of linseed oil (LSO) together with *ad libitum* CS; 3) concentrate plus 300 g/d of PO together with *ad libitum* fresh grass (FG); and 4) concentrate plus 300 g/d of LSO together with *ad libitum* FG. Supplementation with LSO had no effect on DMI, milk production and milk composition. Milk fat content was not affected by LSO supplementation. However, the milk C18:3n3 percentage increased by LSO supplementation. It was concluded that the milk FA composition can be altered by 300 g/d LSO supplementation with increasing concentrations of potentially health beneficial FA and decreasing concentrations of SFA. Finally, LSO supplemented with *ad libitum* FG lowed n-6/n-3 FA ratio in dairy cow's milk.

Key words : milk fatty acid, linseed oil, dairy cow's milk, fresh grass, corn silage

4.2 Introduction

The nutritional contribution of milk and the potential health effects of its main components (fat, protein, antioxidants, vitamins, and minerals) have been reviewed extensively, most recently by Haug et al. (2007) and Steijns (2008). The protein, antioxidants/vitamins, minerals, and some mono- (MUFA) and poly- unsaturated (PUFA) fatty acids in milk are considered beneficial on human health. However, milk contains a high proportion of saturated fatty acids (SFA) because of extensive biohydrogenation of dietary unsaturated fatty acids (UFA) in the rumen and *de novo* synthesis of short- and medium chain saturates in the mammary gland (Shingfield et al., 2008). Due to the incomplete biohydrogenation (BH) of UFA in the rumen, *trans* fatty acid intermediates accumulate, which can be incorporated into milk fat triglyceride following digestion and absorption in the small intestine (Chilliard et al., 2007). SFA and *trans* fatty acids in milk fat are generally considered to have negative effects on human health. The effect of SFA and *trans* fatty acids on the relative proportions of high and low density lipoprotein cholesterol results in coronary heart disease (CHD) (Hu et al., 2001; WHO, 2003). Thus, the milk industry aims to improve the nutritional quality of milk fat by reducing SFA and increasing the content of n-3 series FA, including alpha linolenic acid (ALA), which is recognized as minimizing the risk of cardiovascular disease and is equally essential for the functional development of the central nervous system (ANC, 2001). The fatty acid distribution in milk fat is dependent on dietary composition (Dewhurst et al., 2003). It is now well established that supplementation of cow's diet with UFA affects milk FA profiles (Chilliard et al., 2000; Harvatine et al., 2009). The main sources of unsaturated lipids are oilseed lipids, among which linseed, rapeseed, soybean, and sunflower seeds (Glasser et al., 2008). Linseed oil contains the essential alpha-

linolenic acid (ALA), which the body converts into eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), the n-3 FA found in fish oil. Linolenic acid, usually from fish oil, has been shown to reduce inflammation and help to prevent certain chronic diseases, such as heart disease and arthritis. Linseed oil (LSO) is oil from flaxseed (*Linum usitatissimum)* produced predominantly in the northern Great Plains and Canada (Berglund and Zollinger, 2002). LSO supplementation caused a quadratic increase in milk fat and protein contents and supplementing grazing dairy cow diets with algae and LSO at up to 510 g/d can improve the nutritional value of milk without compromising milk composition or cow performance (Flowers et al., 2008). Previous studies compared the effects of TMR containing mixtures of fish oil and different sources of UFA (AbuGhazaleh et al., 2003), or concentrate : forage ratio and LSO, the results suggested that the nature of supplemental PUFA added to high-concentrate diets likely altered the profile and amount of hydrogenation intermediates available for secretion in milk (Loor et al., 2005). Thus, the objective of this study was to determine the effects of LSO supplementation on milk production, milk composition, and n-6/n-3 ratio of dairy cow's milk.

4.3 Objective

The objective of this experiment was to investigate the effects of linseed oil supplementation on milk production, milk composition, and n-6/n-3 ratio of dairy cow's milk

4.4 Materials and methods

4.4.1 Animals and treatments

Twenty four Holstein Friesian crossbred lactating dairy cows, averaging 106 ± 43 days in milk, 12.1 ± 3.0 kg of milk and 387 ± 41 kg body weight, were blocked by parity first and then stratified random balanced for milk yield, milking days and body weight into four groups of 6 cows each. They were then assigned into a 2 × 2 Factorial arrangement. All cows were fed approximately 6 kg/d of 21%CP concentrate. Treatments were : 1) concentrate plus 300 g/d of palm oil (PO) together with *ad libitum* corn silage (CS); 2) concentrate plus 300 g/d of LSO together with *ad libitum* CS; 3) concentrate plus 300 g/d of PO together with *ad libitum* fresh grass (FG); and 4) control concentrate plus 300 g/d of LSO together with *ad libitum* FG. All cows also had free access to clean water and were individually housed in a free-stall unit and individually fed according to treatments. The experiment lasted for 40 days (8 periods of 5 d), with the first 2 periods (10 days) was the adjustment period, followed by 30 days (6 periods) of measurement period.

4.4.2 Laboratory analyses

Feed offered and left after eating of individual cow were collected on 2 consecutive days of each period and dried at 60 °C for 48 h. At the end of the experiment, feed samples were pooled to make representative samples for proximate and detergent analyses. Samples were ground through 1 mm screen and analyzed for chemical composition. Dry matter (DM) was determined by hot air oven at 60°C for 48 h. The crude protein (CP) was determined by Kjeldahl analysis (AOAC, 1990). Ether extract (EE) was determined using petroleum ether in a Soxtec System (AOAC, 1990). 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°C for 3 h. The chemical analysis was expressed on the basis of the final DM. Fatty acids composition of concentrates, fresh grass and corn silage were extracted using a modified of the method used by Folch et al. (1957) and Metcalfe et al. (1966) for analyzing by gas chromatography (GC) (7890A GC System, Agilent Technology, USA) (See chapter III).

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. Milk samples from both the morning and evening milking were collected on 2 consecutive days of each period and stored at 4 °C with a preservative until analyzed for fat, protein, lactose and solid not fat content using Milkoscan FT 6000® (Foss Electric, 2000; Hillerod, Den-mark) at Veterinary Research and Development Center (Lower Northeastern Region), Muang, Surin, Thailand. In addition, milk samples were collected on day 0, 10, 20 and 30 of the experiment and stored at -20 °C until analyzed for fatty acids. Milk samples of each period were extracted for fatty acid using a modified method used by Romeu-Nadal et al. (2004). From a well-mixed aliquot of milk, 3 ml was placed in 50 ml centrifuge tubes. Then added 27 ml of a dichloromethane–methanol solution (2 : 1, v/v) to each tube. The mixture was shaken mechanically for 15 min and centrifuged at 2500 × *g* for 8 min at 4 °C. Approximately 8 ml of distilled water was pipette into each tube and, after shaking for a further 15 min, the sample was, again centrifuged at 2500 × *g* for 8 min at 4 °C. As much of the upper aqueous fraction as possible was carefully removed with a pipette. The organic layer was washed with 8ml of a saturated solution of the sodium chloride, and finally

mixed mechanically for 15 min and centrifuged for 8 min at 2500 × *g* at 4 °C. Again, the upper aqueous fraction was carefully removed with a pipette. The organic fraction was carefully transferred to a separating funnel and filtered through 1PS paper (Whatman, Maidstone, UK) containing anhydrous sodium sulfate, and 3-5 ml of dichloromethane was passed through the filter. The fat solution was taken in pre-weighed conical flask. Finally the extract was concentrated by removing dichloromethane in a rotatory evaporator and dried under a gentle stream of nitrogen. The weight difference of the conical flask before/after was assumed to be fat. The fat was stored at -20 °C and redissolved in dichloromethane (3%, w/v) intermediately analyzing by gas chromatography (GC) (7890A GC System, Agilent Technology, USA).

4.4.3 Statistical analysis

Measured data of intake, milk production, milk composition, and body weight change were analyzed by ANOVA for 2 × 2 Factorial in randomized complete block design using the Statistical Analysis System (SAS, 1996). Significant differences among treatment were assessed by Duncan's new multiple range test. A significant level of $p<0.05$ was used (Steel and Torrie, 1980).

4.4.4 Experimental location

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

4.4.5 Experimental Period

The experiment was from February 2012 to April 2012.

4.5 Result and discussion

4.5.1 Feed chemical composition

Chemical compositions of the control concentrate, corn silage (CS), and fresh grass (FG) used in the experiment are show in Table 4.1. The average EE content and energy values of the oil supplement were higher than the concentrate diet, CS and FG, respectively.

The fatty acid compositions of the concentrate, FG, CS, PO and LSO used in the experiment are shown in Table 4.2. C18:2n6 was the major fatty acid in the FG and LSO accounting for approximately 48.89% and 56.20% of total fatty acid, respectively. The second major fatty acid in the FG and LSO was C18:2n6 accounting for 19.03% and 17.04% of total fatty acid, respectively. FG and CS had a greater proportion of C16:0, MUFA, PUFA and n-3FA than the concentrate. The LSO had similar proportion of n-3 FA to FG, but n-3 FA was almost absent from the PO. The concentrate contained higher C18:1, than the FG and CS and this accounted for the higher proportion of PUFA in the concentrates. Flower et al. (2008) reported the pasture grasses accounting for n-3FA approximately 40.8% higher than control concentrate (3.72%) and LSO supplement with control concentrate (37.43%) of total fatty acid. According to Shingfield et al. (2011), measurements of fatty acid composition indicated that maize silage and concentrates contained relatively high proportions of *cis*-9 18:1 and 18:2 n-6, 18:3 n-3 predominated in LSO (20.6, 30.6, 57.8% of total FA, respectively).

Table 4.1 Chemical compositions of the experimental diets.

Item	21% CP	PO/LSO	CS	FG
Dry matter	94.40	-	24.09	12.50
Ash	7.66	-	7.43	12.40
Crude protein	20.50	-	6.69	10.07
Ether extract	2.80	100	0.90	1.78
Crude fiber	12.67	-	29.28	36.04
Neutral detergent fiber	45.88	-	61.64	64.42
Neutral detergent insoluble N	1.51	-	0.46	0.32
Acid detergent fiber	22.79	-	26.51	34.43
Acid detergent insoluble N	0.81	-	0.54	0.35
Acid detergent lignin	7.17	-	3.41	2.62
TDN_{1x}(%)[1]	62.01	184.15	57.67	55.05
DE_{1x} (Mcal/kg)[2]	2.96	7.71	2.55	2.51
DE_p (Mcal/kg)[3]	2.87	5.79	2.54	2.51
ME_p (Mcal/kg)[4]	2.45	5.79	2.12	2.08
NE_{lp} (Mcal/kg)[5]	1.53	4.63	1.30	1.27

[1]Total digestible nutrients, TDN_{1X} (%) = tdNFC + tdCP + (tdFA x 2.25) + tdNDF – 7 (NRC, 2001)

[2]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

[3]DE_P (Mcal/kgDM) = DE_{1X} x Discount (NRC, 2001)

[4]Metabolisable energy, ME_p = [1.01 x (DE_p) – 0.45] + [0.0046 x (EE – 3)] (NRC, 2001)

[5]Net energy for lactation, NElp = ([0.703 x MEp (Mcal/kg)] – 0.19) + ([(0.097 x MEp + 0.19)/97] x [EE– 3]) (NRC, 2001)

Table 4.2 Fatty acid compositions of control concentrate, fresh grass (FG), corn silage (CS), linseed oil (LSO) and palm oil (PO).

% of total FA	21% CP	FG	CS	PO	LSO
C8:0	1.03	ND	ND	0.05	0.05
C10:0	1.09	ND	ND	0.02	ND
C12:0	15.89	1.42	1.14	0.19	ND
C14:0	5.75	0.74	2.09	0.96	0.06
C16:0	16.26	19.66	19.03	38.29	4.91
C18:0	2.92	3.18	5.56	4.42	3.46
C18:1n9c	30.11	6.55	2.52	40.61	17.88
C18:2n6c	25.29	19.03	14.21	13.77	16.97
C20:0	ND	0.54	3.00	0.04	ND
C18:3n3	0.34	48.89	8.00	0.26	55.87
SFA[1]	42.93	25.53	30.81	44.05	8.70
MUFA[2]	30.11	6.55	2.52	41.07	17.96
PUFA[3]	26.97	67.92	66.67	14.89	73.34
Total n6[4]	26.63	19.03	58.66	14.46	17.04
Total n3[5]	0.34	48.89	8.00	0.43	56.20
PUFA/SFA	0.63	2.66	2.16	0.34	8.43
n-6/n-3	79.49	0.39	7.33	33.69	0.30

[1] SFA = Sum of saturated fatty acid from C4:0 - C20:0

[2] MUFA = Sum of monounsaturated fatty acid from C14:1 - C22:1

[3] PUFA = Sum of polyunsaturated fatty acids from C18:2 - C22:6;

[4] Sum of n6 fatty acids C18:2n-6 - C22:4n-6

[5] Sum of n3 fatty acids C18:3n-3 -C22:6n-3

4.5.2 Intake and live weight

The average values of nutrient intake, live weight, and live weight change of lactating dairy cows are presented in Table 4.3. No interactions of main treatment effects occurred for intake of nutrients. However, the ANOVA of main effects showed that cows fed diets based on corn silage consumed more total dry matter intake (DMI) than fresh grass ($P<0.01$) which had greater CF, NDF and ADF compared to corn silage. Tafaj et al. (2007) reported that the DMI decreased linearly with increasing dietary NDF. Forage NDF is a major factor affecting feed intake and rumen fill in high-producing cows (Kendall et al., 2009). Waldo (1986) suggested that diet NDF content is the best single chemical predictor of DMI in dairy cows. Mertens (1987) proposed that voluntary feed intake of dairy cattle is limited by digestive tract fill when high NDF diets are fed.

Others (Schroeder et al., 2003; Boken et al., 2005; Shingfield et al., 2005) have reported similar effects when cow's diets were supplemented with plant oils and grazing dairy cow diets supplemented with different amounts of LSO showed no effect on grain intake (Flower et al., 2008). One of the factors which influences DMI of ruminants was the net energy density of the diet, and a high level of lipid supplementation (7 g/100 g concentrate, DM) was reported to reduce DMI of Hanwoo steers (Song et al., 2010). However DMI in the current experiment (4.92 g/100 g concentrate, DM) was not reduced by oil supplementation.

Crude protein intake (CPI) was unaffected by treatments (Table 4.3). Although, fresh grass contain more total CP than corn silage, cows fed diets based on corn silage consumed more total dry matter intake (DMI) than fresh grass ($P<0.01$). Furthermore, rumen degradable protein (RDP) and rumen undegradable protein (RUP) of roughage were unaffected by treatments.

There was no interaction between oil and roughage sources on ether extract intake (EEI, g/d) and net energy for lactation intake (NE_{LP} intake). However, cows fed diets based on corn silage consumed more EE and NE_{LP} than those cows on fresh grass ($P<0.01$). Final live weight (FLW, kg) and live weight change (LWC, g/d) was reduced by dietary fresh grass ($P>0.05$).

4.5.3 Milk Production and Milk Composition

The results of milk production and milk composition are presented in Table 4.4. Milk production and composition were unaffected by treatments ($P > 0.05$). Literature data on the effects of oil supplementation on milk production have been variable. Loor et al. (2005) did not observe any effect of 3% LSO on milk production when added to either forage based or concentrate based diets of dairy cows. A higher 5% LSO supplementation in a grass hay based diet did not affect milk production either (Roy et al., 2006). In contrast, Bu et al. (2007) observed that supplementing 4% LSO to a forage based diet of dairy cows increased milk production, although it did not affect ECM. On the other hand, Martin et al. (2008) reported that 5.7% LSO supplementation to a corn silage based diet decreased milk production.

From the previous studies cited, it appears that changes in milk yield are closely related to the effects of oil on DMI and diet digestibility. Indeed, the decrease in milk production reported by Martin et al. (2008) was associated with a depression in DMI and diet digestibility due to disturbances in rumen function caused by a high level of LSO intake (>5% of DMI). In contrast, Bu et al. (2007) studies where LSO was supplemented at a level lower than 5%, milk production increased because of a greater DMI. In the present experiment, adding oil to dairy cow diets did not affect milk composition. Inclusion of plant lipids in the concentrate had no effect on milk yield or milk composition in cow fed red cover silage, possibly because the intake

of DM and ME was similar across treatments (Halmemies et al., 2011).

Milk fat content was unaffected by treatments (Table 4.4). Effect of dietary oil supplementation on milk fat content has not been consistent. Bu et al. (2007) and AbuGhazaleh and Holmes (2007) reported no effect of dietary oil supplementations on milk fat content. The effects of lipid supplement on milk yield and milk fat are variable and known to be dependent on inclusion rate, degree of unsaturation, physical form and basal diet composition (Shingfield et al., 2010). Although, Flachowsky et al. (2006) and Chilliard et al. (2009) reported that LSO supplementation decreased milk fat content when added to low NDF diet but not to high NDF diets. According to Bauman and Griinari (2003), large PUFA supplies in ruminant diets inhibit rumen biohydrogenation and generate a lot of long-chain FA biohydrogenation intermediates (trans C18:1 and CLA isomers derived from C18:2n6 and C18:3n3) which then are transferred to milk, where some of them inhibit fat synthesis in the udder. In contrast, Flowers et al. (2008) reported increased milk fat content and Hurtaud et al. (2010) observed an increase in milk production and milk fat yield when rations were supplemented with extruded linseed. However these increases could be attributed to the greater dry matter intake of ration or to the extra energy supply of the supplemented rations compared with the unsupplemented ones.

The present experiment, LSO did not affected milk protein. The effect of oils on milk protein content has also been variable. Bu et al. (2007) reported no effect of LSO addition on milk protein Variability between studies in milk composition response to oil supplementation could be explained not only by the amount of oil added to the diet but also by the composition of the basal diet. Indeed, Loor et al. (2005) reported that milk protein content was decreased in cows fed LSO in a high forage diet, whereas it was increased in cows fed LSO in a high concentrate diet.

Table 4.3 Effect of treatment on nutrient intake of dairy cows

Item	CS		FG		SEM	Pr>F		
	300 g/d PO	300 g/d LSO	300 g/d PO	300 g/d LSO		R	O	R × O
Initial LW (kg)	402	397	374	378	2.84	0.195	0.992	0.790
Final LW (kg)	404	401	369	374	8.20	0.077	0.964	0.822
LW change (g/d)	+67	+128	-150	-150	116	0.300	0.897	0.897
Dry matter intake , kg/d								
Concentrate	6.1	6.1	6.1	6.1	-	-	-	-
Roughage	9.6	9.8	5.8	6.1	0.23	<0.01	0.560	0.990
Oil	0.3	0.3	0.3	0.3	-	-	-	-
Total	16.0	16.3	12.3	12.3	0.30	<0.01	0.658	0.990
DMI, g/$BW^{0.75}$	174.9	180.4	141.4	143.3	3.31	<0.01	0.589	0.789
Crude protein intake, g/d								
Concentrate	1,280	1,280	1,280	1,280	-	-	-	-
Roughage	639	657	588	616	19.90	0.259	0.575	0.899
Total	1,920	1,937	1,869	1,896	51.05	0.656	0.826	0.960
Ether extract intake, g/d								
Concentrate	171	171	171	171	-	-	-	-
Roughage	86	88	103	109	3.31	<0.01	0.561	0.981
Oil	300	300	300	300	-	-	-	-
Total	558	560	518	520	7.14	0.195	0.801	0.930
NE_{LP} intake, Mcal/d								
Concentrate	9.4	9.4	9.4	9.4	-	-	-	-
Roughage	10.4	12.8	7.4	7.8	0.53	<0.01	0.223	0.364
Oil	1.39	1.39	1.39	1.39	-	-	-	-
Total	21.2	23.5	18.1	18.5	0.64	<0.01	0.310	0.451

SEM is standard errer of mean

R = roughage source (corn silage and fresh grass); O = oil source (palm oil and linseed oil)

Table 4.4 Effect of treatment on milk yield (MY) and milk composition of dairy cows

Item	CS		FG		SEM	Pr>F[2]		
	300 g/d PO	300 g/d LSO	300 g/d PO	300 g/d LSO		R	O	R × O
MY, kg/d	13.5	12.8	12.1	12.4	0.58	0.447	0.831	0.686
3.5% FCM	14.59	13.41	12.86	13.28	0.49	0.361	0.706	0.430
Fat								
%	4.07	3.88	3.93	4.01	0.13	0.987	0.829	0.604
g/d	549	497	476	497	18.05	0.367	0.649	0.322
Protein								
%	3.05	2.98	3.02	3.01	0.05	0.988	0.707	0.775
g/d	412	381	365	373	13.54	0.323	0.713	0.586
Lactose								
%	4.8	4.78	4.7	4.63	0.04	0.209	0.645	0.747
g/d	648	612	569	574	27.98	0.319	0.737	0.767
SNF								
%	8.57	8.46	8.44	8.36	0.08	0.484	0.578	0.927
g/d	1157	1083	1021	1037	45.21	0.333	0.724	0.683
TS								
%	12.64	12.39	12.38	12.35	0.18	0.688	0.702	0.754
g/d	1706	1586	1498	1531	59.24	0.303	0.707	0.564

SEM is sattandard errer of mean

R = roughage source (corn silage and fresh grass); O = oil source (palm oil and linseed oil)

Table 4.5 Effect of treatment on milk fatty acid composition of dairy cows

% of total FA	CS		FG		SEM	Pr>F		
	300 g/d PO	300 g/d LSO	300 g/d PO	300 g/d LSO		R	O	R × O
C4:0	1.58	2.58	1.74	2.57	0.053	0.506	<0.01	0.450
C6:0	1.50	1.53	1.60	1.58	0.042	0.372	0.961	0.760
C8:0	0.68	0.99	0.94	1.17	0.014	<0.01	<0.01	0.164
C10:0	1.55	1.71	1.36	1.36	0.012	<0.01	<0.01	0.003
C12:0	1.88	1.58	1.76	1.36	0.009	<0.01	<0.01	0.010
C14:0	8.08	7.53	7.62	6.80	0.010	<0.01	<0.01	<0.01
C14:1	0.75	0.68	0.67	0.68	0.005	<0.01	<0.01	<0.01
C16:0	28.23	25.30	27.17	23.97	0.068	<0.01	<0.01	0.336
C16:1	1.49	1.40	1.55	1.41	0.010	0.069	<0.01	0.214
C18:0	12.27	12.55	12.55	12.45	0.020	0.036	0.028	<0.01
C18:1n9t	6.22	8.35	6.94	9.55	0.018	<0.01	<0.01	<0.01
C18:1n9c	30.26	29.13	30.22	30.17	0.073	<0.01	<0.01	<0.01
C18:2n6t	0.53	0.85	0.60	1.08	0.017	<0.01	<0.01	0.027
C18:2n6c	2.29	2.10	2.22	2.07	0.028	0.406	<0.01	0.720
C20:0	0.22	0.17	0.20	0.14	0.003	<0.01	<0.01	0.463
C18:3n6	0.01	0.01	0.01	0.01	0.006	0.646	0.640	0.646
C20:1	0.06	0.02	0.04	0.02	0.002	0.013	<0.01	0.013
C18:3n3	0.56	1.06	0.77	1.19	0.013	<0.01	<0.01	0.150
C9,T11	1.15	1.39	1.25	1.71	0.011	<0.01	<0.01	<0.01
C9,C11	0.02	0.02	0.02	0.02	0.002	0.666	0.665	0.665
T9,T11	0.12	0.17	0.14	0.19	0.002	<0.01	<0.01	0.809
C20:2	0.10	0.07	0.11	0.05	0.003	0.451	<0.01	0.011
C22:0	0.11	0.45	0.11	0.05	0.056	0.091	0.218	0.096
C20:3n6	0.02	0.02	0.02	0.02	0.002	0.255	0.817	0.490
C22:1n9	0.02	0.02	0.01	0.02	0.002	0.168	0.639	0.168
C20:3n3	0.01	0.02	0.01	0.02	0.001	0.063	<0.01	0.781
C20:4n6	0.17	0.15	0.16	0.12	0.004	0.014	<0.01	0.420
C22:2	0.02	0.02	0.01	0.01	0.002	0.239	1.000	1.000
C24:0	0.03	0.04	0.04	0.03	0.005	0.931	0.931	0.794
C22:6n3	0.04	0.09	0.12	0.13	0.008	<0.01	0.056	0.246
C20:5n3	0.04	0.04	0.05	0.04	0.003	0.233	0.628	0.469
SFA[1]	56.14	54.42	55.08	51.48	0.081	<0.01	<0.01	<0.01
MUFA[2]	38.80	39.59	39.44	41.85	0.078	<0.01	<0.01	<0.01
PUFA[3]	5.06	5.99	5.48	6.67	0.038	<0.01	<0.01	0.098
n-6[4]	4.31	4.70	4.42	5.23	0.038	<0.01	<0.01	0.011
n-3[5]	0.66	1.22	0.97	1.41	0.017	<0.01	<0.01	0.085
n-6/n-3	6.66	3.87	4.59	3.74	0.114	<0.01	<0.01	<0.01

SEM is sattandard errer of mean

R = roughage source (corn silage and fresh grass); O = oil source (palm oil and linseed oil)

[1]SFA = Sum of saturated fatty acid from C4:0 - C20:0; [2]MUFA = Sum of monounsaturated fatty acid from C14:1 - C22:1; [3]PUFA = Sum of polyunsaturated fatty acids from C18:2 - C22:6

[4]Sum of n6 fatty acids C18:2n-6 - C22:4n-6; [5]Sum of n3 fatty acids C18:3n-3 - C22:6n-3

4.5.4 Milk Fatty Acid Composition

Results of milk FA composition are shown in Table 4.5. Supplementing 300 g/d LSO with *ad libitum* FG significantly increased MUFA, PUFA, n6 FA, n3 FA concentrations, and decreased milk fat SFA concentration, n-6/n-3 ratio ($P<0.01$). The FA composition of milk fat depends on various dietary characteristics including roughage to concentrate ratio (Sterk et al., 2012), FA intake, FA metabolism in the mamary gland (Chilliard et al., 2007).

LSO supplementation with roughage sources significantly increased concentration of C4:0, C8:0 and decreased C12:0 to C16:0 ($P<0.01$). Furthermore, 300 g/d LSO with *ad libitum* FG decreased C14:0 to C16:0 in milk fat ($P<0.01$; Table 4.5). These effects are consistent with the reduction in *de novo* FA synthesis due to feeding unsaturated oils, which occurs as a result of greater uptake and secretion of dietary or ruminally derived FA (Palmquist et al., 1993). Decreases in short- and medium- chain FA percentages have already been reported by Hurtaud et al., (2010) for CS based diets supplemented with increaseing amount up to 4% extrude linseed. Accordingly, Benchaar et al., 2012 reported that feeding increasing levels of LSO linearly decreased milk fat content of short- and medium-chain FA (8:0 to 16:0), and increased the proportion of most 18 carbon FA in milk fat.

LSO supplementation decreased proportions of C15:0 and C17:0 in milk fat ($P<0.01$). The major source of odd chain FA found in milk fat is long chain FA synthesized by ruminal bacteria from odd numbered VFA. However, this rumen microbial synthesis is known to decrease when cows are fed dietary fat because bacteria use especially preformed FA available in the ruminal ecology (Byers and Schelling, 1988).

The increased concentration of C18:0 in milk fat can be expected due

to extensive metabolism of long chain PUFA in the rumen which leads to an increase in the amount of C18:0 for absorption (Jensen, 2002). Recent research has shown that the proportion of forage in the diet is also an important determinant of milk fatty acid composition responses in cows fed linseed oil, with interactions occurring between the roughage and lipid supplementation on the concentration of several fatty acids in milk, including 16:0, 18:0 and cis-9 18:1 (Loor et al., 2005).

The decreased in milk SFA content with LSO supplementation was similar between the CS and FG diets ($P<0.01$; Table 4.5). The faster rate of oil release into the rumen from LSO could result in the higher production of *trans* FA in the rumen and, thus an inhibitory effect on *de novo* mamary lipogenesis (Chillard et al., 2007). Furthurmore, a recent meta-analysis citing a range of studies in which linseed and other oil supplements decreased concentrations of long chain UFA (Glasser et al., 2008).

Feeding LSO increased C18:1n9t, C18:1n9c and CLA ($P<0.01$; Table 4.5) in milk fat. This can be explained by the result from the ruminal BH of C18:2 and C18:3 (Shingfield et al., 2010). In particular, C18:1 could be derived from the reduction of C18:2 and C18:3n3 and during ruminal metabolism (Shingfield et al., 2010), which both increased when the diet was supplemented with LSO (Benchaar et al., 2012).

Increased proportions of C18:1 in milk fat could, therefore, be explained by the higher supply of dietary C18:2 (Table 4.5) or by the overall ruminal UFA load, as suggested by Lock (2010). Duodenal flow and secretion of C18:1 in milk fat was shown to be highly dependent on the composition of the basal diet. Feeding LSO in a diet rich in starch and based on CS as the sole forage increased milk fat content of C18:1 (Chilliard et al., 2009). On the contrary, supplemental LSO

increased C18:1 when the diet was based on grass hay rich in fiber (Roy et al., 2006). In the present study, the roughage source was FG and CS, which led to an increase in C18:1.

Milk fat content of CLA also increased with LSO supplementation. It has been established that a great proportion of this CLA isomer found in the milk is produced endogenously in the mammary gland using C18:1 produced in the rumen as a substrate (Griinari et al., 2000).

Nevertheless, for each type of forage observed that specific FA were correlations with milk fat content. Loor et al. (2005) showed that C18:2 was enhanced in the duodenal digesta in response to natural grassland hay based diets containing LSO as a source of C18:3n3. It is likely that the ruminal C18:1 inhibited de novo FA synthesis because inhibitory effects were observed in cows receiving oil rich diets (Shingfield et al., 2010).

300 g/d LSO with *ad libitum* FG treatment showed in higher C18:3n-3 than other treatment (Table. 4.5). There was a significant effect of oil supplement and roughage source ($P<0.01$) interaction on C18:3n-3 FA in milk fat. The decrease in transfer efficiency observed with increasing supply of LSO could be explained by (1) an increased efficiency in biohydrogenation with higher supply of free LSO in the diet, (2) a lower intestinal digestibility with an increasing amount of C18:3n-3 escaping ruminal fermentation as dietary LSO supply increased, or (3) a lower efficiency of mammary uptake as the arterial concentration of C18:3n-3 increased (Benchaar et al., 2012). In this regard, Loor et al. (2004) observed an increase in intestinal digestibility of C18:3n-3 with dietary supplement of LSO, which does not support a limitation in absorption capacity. At the level of the mammary gland, Enjalbert et al. (1998) reported that extraction of arterial FA, either nonesterified or in

the form of triglycerides, increased with arterial concentration following duodenal infusion of long chain FA. Finally, Khas-Erdene et al. (2010) observed a quite stable efficiency of transfer to milk of duodenally infused C18:3n-3 over a wide range of FA supply.

The decrease in apparent recovery of dietary C18:3n3 can be explained by the efficiency BH process, which has been shown by Loor et al. (2004) to be greater when free LSO was added in the diet. In this case (Loor et al., 2004), the extent of ruminal BH has been assessed by calculating the proportion of dietary PUFA disappearing during their passage in the rumen (not found in the small intestine). However, this calculation does not provide any information about the efficiency of the overall series of reactions (e.g., the final production of C18:0). According to Harfoot (1981), the BH pathway of C18:3n3 involves the production of C18:3n3, C18:2n6t and C18:1n9t, which can all be absorbed and incorporated in to milk fat. Among these intermediates, C18:2n6t and C18:1n9t showed a response to the level of LSO supplementation, with the largest increase observed at 3% of dietary supplementation (Table 4.5). Therefore, it speculates that dietary C18:3n3 rapidly undergoes BH by being reduction of double bonds at carbons 6 and 9 to yield C18:2n6t and C18:1n9t, which accumulate in the rumen as the efficiency of the first step in the pathway increases.

The average concentrations of C18:3n3 and C18:2n6 in milk fat of 300 g/d LSO with *ad libitum* FG cows were 1.17 and 2.04 % of total FA (Table 4.5). The C18:3n3 and C18:2n6 ratio of 3.79 ($P<0.01$) falls within the recommended range, from 1 : 1 to 4 : 1, that is considered to be important for human homeostasis and normal development (Simopolos, 2008). For cows, main sources of C18:3n3 and C18:2n6 were from LSO, grass and corn silage, respectively (Chillard et al., 2001).

C18:3n3 and C18:2n6 that escape rumen BH will propably, as in other mammals, be beta-oxidized (Cunnane et al., 2003), stored in body tissues, or incorporated in milk fat.

In the first step of the n-3 and n-6 pathways, Δ^6- desaturase converts C18:3n3 to C18:4n3 (Not detected) and C18:2n6 to C18:3n6. In milk fat, C18:4n3 (Not detected) was low concentration (<0.02% of total FA), whereas the everage C18:3n6 was 0.01% of total FA. The low concentrations, or even absence, of C18:4n3 (Not detected) is remarkable because the affinity of Δ^6- desaturase is higher for n-3 than n-6 FA (Sprecher, 2002). Desaturation of C18:3n6 into C18:4n3 (Not detected) is, therefore, expect to occur at higher rate than the conversion of the C18:2n6 into C18:3n6. Product inhibition of Δ^6- desaturase by high level of C18:3n6 (Emken et al., 1994) is unlikely to occur due to the low n-6/n-3 raio (Table 4.5). The low concentration of C18:4n3 (Not detected) may be caused by rapid metabolization of C18:4n3 (Not detected) into C20:4n3 (Not detected). The concentration of C20:4n3 is relatively low compared with C20:5n3 (0.04, P=0.47) and C22:5n3 (Not detected) concentration. Desaturation of C20:4n3 causes the formation of C20:5n3, which is subsequently elongated to form C22:5n3. In humans, the main products formed out of C18:3n3 are C20:5n3 and C22:5n3; the latter may have benefical effects (Kaur et al., 2011) and may also serve as a substrate for metabolic retroconversion to C20:5n3 (Russo, 2009). However, the absorption levels of C22:5n3 and the conversion rate to C20:5n3 have never been reported. In the generally accepted n-3 FA partway, C22:5n3 is further converted into C24:5n-3, C24:6n-3, and finally C22:6n3.

The conversion of C22:5n3 is the rate limiting step for the conversion of C18:3n-3 to C22:6n-3 (Arterburn et al., 2006). Unfortunately, the intermediates C24:5n-3 and C22:6n-3 could not be determined in the present study, which makes it

impossible to determine the actual rate-limiting step. Concentrations of C22:6n-3 were lower than 0.20% of total FA of fat in all treatments, which is in agreement with the low conversion rates from C18:3n-3 (<0.1%) that are frequently reported in the literature (Emken et al., 1994; Goyens et al., 2006; Williams and Burdge, 2006).

In the present analyses, we used the average FA content of milk. Milk FA composition is not constant and feeding trials have shown that concentrations of very long chain PUFA may increase significantly due to variation in the diets of dairy cows. For example, the C22:6n-3 concentration may be increased from 0.04 to 0.13 % of total FA upon supplementation of the dairy cow diet with linseed oil (Zachut et al., 2010; Sterk et al., 2012).

4.6 Conclusion

Supplementing 300 g/d oil (PO and LSO) to roughage sources (CS and FG) did not negatively affect on DMI, milk yield, milk production and milk composition. Milk fatty acid proportions of n-3FA increased, whereas total SFA decreased with the addition of LSO in the diet. As a result of changes in these FA, the ratio of n-6 to n-3 was lowered in dairy cow supplemented with LSOFG as compared with other treatment. It can be concluded that 300 g/d LSO can be safely supplemented to forage-based diets of dairy cows to enrich milk with potential health-beneficial FA without causing any detrimental effect on animal performance.

4.7 References

AbuGhazaleh, A. A., and Holmes, L. D. (2007). Diet supplementation with fish oil and sunflower oil to increase conjugated linoleic acid levels in milk fat of partial grazing dairy cows. **J. Dairy Sci.** 90 : 2897-2904

ANC. (2001). Apports Nutritionnels Conseilles pour la Population Francaise. Tec. Et Doc, Paris, France.

Arterburn, L. M., Hall, E. B., and Oken, H. (2006). Distribution, interconversion, and dose response of n-3 fatty acids in humans. **Am. J. Clin. Nutr.** 83 : 1467S-1476S.

Association of Official Analytical Chemists. (1995). **Official Method of Analysis.** Washington D. C. p. 1298.

Bauman, D. E., and Griinari, J. M. (2003). Nutrional regulation of milk fat synthesis. **Annurev. Nutr.** 23 : 203-227.

Benchaar, C., Romero-Pérez ,G. A., Chouinard ,P. Y., Hassanat , F., Eugene M., Petit, H. V., and Côrtes, C. (2012). Supplementation of increasing amounts of linseed oil to dairy cows fed total mixed rations : Effects on digestion, ruminal fermentation characteristics, protozoal populations, and milk fatty acid composition. **J. Dairy Sci.** 95 : 4578-4590.

Berglund, D. R., and Zollinger, R. K. (2002). **Flax Production in North Dakota.** North Dakota State University Extension Service Bulletin. A-1038.

Boken, S. L., Staples, C. R., Sollengberger, L. E., Jenkins, T. C. and Thatcher, W. W. (2005). Effect of grazing and fat supplementation on production and reproduction of Holstein cows. **J. Dairy Sci.** 88 : 4258-4272.

Bu, D. P., Wang, J. Q., Dhiman, T. R. and Liu, S. J. (2007). Effectiveness of oils rich in linoleic and linolenic acids to enhance conjugated linoleic acid in milk from dairycows. **J. Dairy Sci.** 90 : 998-1007

Byers, F. M. and Schelling, G. T. (1988). Lipids in Ruminant Nutrition. in : D. C. Church (Ed.) **The Ruminant Animal Digestive Physiology and Nutrition.** Prentice-Hall, Inc., New Jersey, USA. 298-312

Chilliard, Y., Ferlay, A., and Doreau, M. (2001). Effect of different types of forages, animal fat or marine oils in cow's diet on milk fat secretion and composition, especially conjugated linoleic acid (CLA) and polyunsaturated fatty acids. **Livest. Sci.** 70 : 31-48.

Chilliard, Y., Ferlay, A., Mansbridge, R. M., and Doreau, M. (2000). Ruminant milk fat plasticity : Nutritional control of saturated, polyunsaturated, *trans* and conjugated fatty acids. **Ann. Zoo Tech.** 49 : 181–205.

Chilliard, Y., Glasser, F., Ferlay, A., Bernard, L., Rouel, J., and Doreau, M. (2007). Diet, rumen biohydrogenation and nutritional quality of cow and goat milk fat. **Eur. J. Lipid Sci. Technol.** 109 : 828-855.

Cunnane, S. C., Ryan, M. A., Nadeau, C. R., Bazinet, R. P., Musa-Veloso, K., and McCloy, U. (2003). Why is carbon from some polyunsaturates extensively recycled into lipid synthesis? **Lipids.** 38 : 477-484.

Dewhurst, R. J., Scollan, N. D., Lee, M. R. F., Ougham, H. J., and Humphreys, M. O. (2003). Forage feeding and management to increase the beneficial fatty acid content of ruminant products. **Proc. Nutr. Soc.** 62(2) : 329-336.

Emken, E. A., Adlof, R. O., and Gulley, R. M. (1994). Dietary linoleic acid influences desaturation and acylation of deuterium-labeled linoleic and linolenic acids in young adult males. **Biochem. Biophys. Acta.** 1213 : 277-288.

Enjalbert, F., Nicot, M. C., Bayourthe, C. and Moncoulon, R. (1998). Duodenal infusions of palmitic, stearic or oleic acids differently affect mammary gland metabolism of fatty acids in lactating dairy cows. **J. Nutr.** 128 : 1525-1532.

Ferlay, A., Doreau, M., Martin, C., and Chilliard, Y. (2013). Effects of incremental amounts of extruded linseed on the milk fatty acid composition of dairy cows receiving hay or corn silage. **J. Dairy Sci.** 96 : 6577-6595.

Flachowsky, G., Erdmann, L., Hüther, K., Jahreis, G., Möckel, P., and Lebzien, P. (2006). Influence of roughage/concentrate ratio and linseed oil on the concentration of trans-fatty acids and conjugated linoleic acid in duodenal chyme and milk fat of late lactating cows. **Arch. Anim. Nutr.** 60 : 501–511.

Flowers, G., Ibrahim, S.A., and Ghazaleh, A. A. (2008). Milk fatty acid composition of grazing dairy cows when supplemented with linseed oil. **J. Dairy Sci.** 91 : 722-730.

Folch, J., Lees, M., and Sloane-Stanley, G.H. (1957). A simple method for the isolation and purification of total lipids from animal tissues. **J. Bio. Chem**. 226 : 495-509.

FOSS Electric. (2000). CombiFoss 6000 Type 76100 operator's manual. Hillerod, Denmark.

Glasser, F., Ferlay, A., and Chilliard, Y. (2008). Oilseed Lipid Supplements and Fatty Acid Composition of Cow Milk : A Meta-Analysis. **J. Dairy Sci.** 91 : 4687-4703.

Goyens, P. L. L., Spilker, M. E., Zock, P. L., Katan, M. B., and Mensink, R. P. (2006). Conversion of alpha-linolenic acid in humans is influenced by the absolute amounts of α-linolenic acid and linoleic acid in the diet and not by their ratio. **Am. J. Clin. Nutr.** 84 : 44-53.

Harfoot, C. G. (1981). Lipid metabolism in the rumen. Page 21 in Lipid Metabolism in Ruminant Animals. W. W. Christie, ed. Pergamon Press, New York, NY.

Haug, A., Hostmark, A. T., and Harstad, O. M. (2007). Bovine milk in human nutrition- A review. **Lipids Health Dis.** 6 : 25-41.

Harvatine, K. J., Boisclair, Y. R., and Bauman, D. E. (2009). Recent advances in the regulation of milk fat synthesis. **Animal.** 3 : 40-54.

Hu, F. B., Manson, J. E., and Willett, W. C. (2001). Types of dietaryfat and risk of coronary heart disease : A critical review. **J. Am.Coll. Nutr.** 20 : 5-19.

Hurtaud , C., Faucon, F., Couvreur, S., and Peyraud, J. L. (2010). Linear relationship between increasing amounts of extruded linseed in dairy cow diet and milk fatty acid composition and butter properties. **J. Dairy Sci.** 93 : 1429-1443.

Jensen, R. G. (2002). The composition of bovine milk lipids : January 1995 to December 2000. **J. Dairy Sci.** 85 : 295-350.

Kaur, G., Cameron-Smith, D., Garg, M., and Sinclair, A. J. (2011). Docosapentaenoic acid (22:5n-3) : A review of its biological effects. **Prog. Lipid Res.** 50 : 28-34.

Kendall, C., Leonardi, C., Hoffman, P. C., and Combs D. K. (2009). Intake and milk production of cows fed diets that differed in dietary neutral detergent fiber and neutral detergent fiber digestibility. **J. Dairy Sci.** 92 : 313-323.

Khas-Erdene, Q., Wang, J. Q., Bu, D. P., Wang, L., Drackley, J. K., Liu, Q. S., Yang, G., Wei, H. Y., and Zhou, L. Y. (2010). Responses to increasing amounts of free α-linolenic acid infused into the duodenum of lactating dairy cows. **J. Dairy Sci.** 93 : 1677-1684.

Lock, A. (2010). Update on dietary and management effects on milk fat. Pages 15-26 in **Proc. Tri-State Dairy Nutr. Conf., Fort Wayne, IN.** The Ohio State University, Columbus.

Loor, J. J., Ferlay, A., Ollier, A., Doreau, M., and Chilliard, Y. (2005). Relationship among *trans* and conjugated fatty acids and bovine milk fat yield due to dietary concentrate and linseed oil. **J. Dairy Sci.** 88 : 726-740.

Martin, C., Rouel, J., Jouany, J. P., Doreau, M., and Chilliard, Y. (2008). Methane output and diet digestibility in response to feeding dairy cows crude linseed,

extruded linseed, or linseed oil. **J. Anim. Sci.** 86 : 2642–2650.

Metcalfe, L.D., Schmitz, A.A. and Pelka, J.R. (1966). Rapid preparation of fatty acid esters from lipids for gas chromatographic analysis. **Anal. Chem**. 38 : 514-515.

Mertens, D. R. (1987). Predicting intake and digestibility using mathematical methods of ruminal function. **J. Anim. Sci.** 64 : 1548–1558.

Palmquist, D. L., Beaulieu, A. D., and Barbano, D. M. (1993). Feed and animal factors influencing milk fat composition. **J. Dairy Sci.** 76 : 1753-1771.

Rego, O. A., Rosa, H. J. D., Portugal, P. V., Franco, T., Vouzela, C. M., Borba, A. E. S., and Bessa, R. J. B. (2005). The effects of supplementation with sunflower and soybean oils on the fatty acid profile of milk fat from grazing dairy cows. **J. Anim. Res.** 54 : 17-24.

Romeu-Nadal, M., Morera-Pons, S., Castellote, A.I. and Lopez-Sabater, M.C. (2004). Comparison of two methods for the extraction of fat from human milk. **Anal. Chim. Acta.** 513 : 457-461.

Roy, A., Ferlay, A., Shingfield, K. J., and Chilliard, Y. (2006). Examination of the persistency of milk fatty acid composition responses to plant oils in cows fed different basal diets, with particular emphasis on trans-C18: 1 fatty acids and isomers of conjugated linoleic acid. **J. Anim. Sci**. 82 : 479-492.

Russo, G. L. (2009). Dietary n-6 and n-3 polyunsaturated fatty acids : From biochemistry to clinical implications in cardiovascular prevention. **Biochem. Pharmacol.** 77 : 937-946.

SAS. (1996). **User'Guide : Statistics**. SAS Institute Inc., North Carolina. 231p.

Schroeder, G. F., Delahoy, J. E., Vidaurreta, I., Bargo, F., Gagliostro, G. A. and Muller, L. D. (2003). Milk fatty acid composition of cows fed a total mixed

ration or pasture plus concentrates replacing corn with fat. **J. Dairy Sci.** 86 : 3237-3248.

Shingfield, K. J., Ahvenjarvi, S., Toivonen, V., Vanhatalo, A., Huhtanen, P., and Griinari, J. M. (2008). Effect of incremental level of sunflower-seed oil in the diet on ruminal lipid metabolism in lactating cows. **Br. J. Nutr.** 99 : 971-983.

Shingfield, K. J., Bernard, C. Leroux, C., and Chilliard, Y. (2010). Role of *trans* fatty acids in the nutritional regulation of mammary lipogenesis in ruminants. **Amimal.** 4 : 1140-1160.

Shingfield, K. J., Lee, M. R. F., Humphries, D. J., Scollan,N. D. Toivonen, V., Beever, D. E. and Reynolds, C. K. (2011). Effect of linseed oil and fish oil alone or as an equal mixture on ruminal fatty acid metabolism in growing steers fed maize silage-based diets. **J. Anim. Sci.** 89 : 3728-3741.

Shingfield, K. J, Reynolds, C. K., Lupoli, B., Toivonen, V., Yurawecz, M. P., Delmonte, P., Griinari, J. M., Grandison, A. S., and Beever, D. E. (2005). Effect of forage type and proportion of concentrate in the diet on milk fatty acid composition in cows given sunflower or fish oil. **J. Anim. Sci.** 80 : 225-238.

Simopoulos, A. P. (2008). The importance of the omega-6/omega-3 fatty acid ratio in cardiovascular disease and other chronic diseases. **Exp. Biol. Med.** (Maywood) 233 : 674-688.

Sprecher, H. (2002). The roles of anabolic and catabolic reactions in the synthesis and recycling of polyunsaturated fatty acids. **Prostaglandins Leukot. Essent. Fatty Acids**. 67 : 79-83.

Steijns, J. M. (2008). Dairy products and health : Focus on their constituents or on the matrix? **Int. Dairy J.** 18 : 425-435.

Steel, R. G. D., and Torries, J. H. (1980). **Principles and Procedures of Statistics : a Biometeric Approach (2 nd Ed).** McGrow Hill : New York.

Sterk, A., Vlaeminck, B., van Vuuren, A. M., Hendriks, W. H., and Dijkstra, J. (2011). Effects of feeding different linseed sources on omasal fatty acid flows and fatty acid profiles of plasma and milk fat in lactating dairy cows. **J. Dairy Sci.** 95 : 3149-3165.

Tafaj, M., Zebeli, Q., Baes, Ch., Steingass, H., and Drochner, W. (2007). A meta-analysis examining effects of particle size of total mixed rations on intake, rumen digestion and milk production in high-yielding dairy cows in early lactation : Review. **Anim. Feed Sci. Technol.** 138 : 137-161.

Van Soest, P.J., Robertson, J.B., and Lewis, B.A. (1991). Methods for dietary fiber, neutral detergent fiber and non starch polysaccharides in relation to animal production. **J. Dairy Sci**. 74 : 3583-3597.

Waldo, D. R. (1986). Symposium : Forage utilization by the lactating cow. Effect of forage quality on intake and forage-concentrate interactions. **J. Dairy Sci.** 69 : 617-632.

WHO. (2003). **Diet, Nutrition and the Prevention of Chronic Diseases.** Report of a Joint WHO/FAO Expert Consultation. WHO Technical Report Series No. 916. WHO, Geneva, Switzerland.

Williams, C. M., and Burdge, G. (2006). Long-chain n-3 PUFA : Plant v. marine sources. **Proc. Nutr. Soc.** 65 : 42-50.

Zachut, M., Arieli, A., Lehrer, H., Livshitz, L., Yakoby, S., and Moallem, U. (2010). Effects of increased supplementation of n-3 fatty acids to transition dairy cows on performance and fatty acid profile in plasma, adipose tissue, and milk fat. **J. Dairy Sci.** 93 : 5877-5889.

CHAPTER V

THE EFFECT OF LINSEED OIL SUPPLEMENTATION ON RUMEN DIGESTA FATTY ACID PROFILES IN FISTULATED COWS

5.1 Abstract

The aim of this experiment was to study the effects of linseed oil supplementation on ruminal fermentation, fiber degradability and rumen digesta fatty acid profiles in fistulated crossbred Holstein Friesian cows. Four ruminally fistulated crossbred Holstein Friesian cows were assigned in 4 dietary treatments in a 4 × 4 Latin square design. All cows were fed approximately 3 kg/d of 21% CP concentrate. Treatments were : 1) concentrate plus 300 g/d of palm oil (PO) together with *ad libitum* corn silage (CS); 2) concentrate plus 300 g/d of linseed oil (LSO) together with *ad libitum* CS ; 3) concentrate plus 300 g/d of PO together with *ad libitum* fresh grass (FG); and 4) concentrate plus 300 g/d of LSO together with *ad libitum* FG. Each period in the Latin square design lasted 21 d, with the first 14 d for adaptation. The results demonstrate that feeding LSO at 2 h increased C18: 3n3, but had no effect on C18:0, C18:2 and CLA proportion in rumen digesta. Feeding LSO inhibited BH of C18:2 to C18:0, as indicated by the increased rumen flows and proportions of BH intermediates in rumen digesta. Furthermore, LSO did not negatively influence on ruminal fermentation, DM and NDF digestibilities, and did not change ruminal pH,

NH_3-N, protozoa and VFA concentration.

Key words : linseed oil, ruminal fermentation, rumen digesta, fatty acid

5.2 Introduction

In recent decades, many authors (e.g., Harfoot, 1978; Palmquist and Jenkins, 1980; Jenkins, 1993) have studied the fate of dietary lipids during rumen fermentation, emphasizing the two major processes in which esterified lipids are involved (i.e., lipolysis (LP) and biohydrogenation (BH)). Lipids presented in most feeds used in animal feeding contain high proportions of unsaturated fatty acids (Van Soest, 1994), which affects the permeability of the microbial membrane; in particular, they inhibit activity of Gram-positive bacteria and protozoa and modify rumen fermentation (Nagaraja et al., 1997). The effects of lipids on the rumen and total digestion are difficult to predict and are highly variable because they depend on the nature and concentration of lipids in the diet, the types of chemicals and/or physical treatments added to feeds, and the nature and amounts of forages, concentrates, and minerals (especially calcium) in the diet (Jenkins and McGuire, 2006). Due to these complex interactions, the metabolic effects of lipid supplementation in the diet cannot be analyzed as a simply result of increase in the absorption of intact fatty acids (or transformation by the rumen) from the diet (Oliveira et al., 2007). Thus, when one wants to supply lipids in the diet of ruminants, it is important to evaluate their effects on ingestion and digestion of nutrients so as not to impair the necessary uptake for the desired production (Jenkins and McGuire, 2006). Furthermore, linseed oil (LSO) supplementation in cattle feed can increase *trans*-11C18:1, *cis*-9, *trans*-11 CLA, and 18:3n-3 at the duodenum (Loor et al., 2004; Doreau et al., 2009b), they accumulate in

tissue lipids and milk fat (Destaillats et al., 2005; Akraim et al., 2007).

This study was conducted to evaluate the effects of oil supplementation (PO and LSO) and roughage sources (CS and FG) on ruminal fermentation, nutrient degradability, and rumen digesta fatty acid profiles of fistulated Crossbred Holstein Friesian non-lactating dairy cows.

5.3 Objective

The objective of this experiment was to investigate the effects of effects of oil supplementation (PO and LSO) and roughage source (CS and FG) on ruminal fermentation, fiber degradability, and rumen digesta fatty acid profiles of fistulated Crossbred Holstein Friesian non-lactating dairy cows.

5.4 Materials and methods

5.4.1 Animals and feeding

Four fistulated Crossbred Holstein Friesian non-lactating dairy cows housed in individual pens were assigned to one of four treatments in 4 × 4 Latin squares design. All cows were fed approximately 3 kg/d of 21% CP concentrate. Treatments were : 1) concentrate plus 300 g/d of palm oil (PO) together with *ad libitum* corn silage (CS); 2) concentrate plus 300 g/d of linseed oil (LSO) together with *ad libitum* CS; 3) concentrate plus 300 g/d of PO together with *ad libitum* fresh grass (FG); and 4) concentrate plus 300 g/d of LSO together with *ad libitum* FG. All cows also had free access to clean water and were individually housed in a free-stall unit and individually fed according to treatments. The experiment lasted for 84 days (4 periods) with 21 d in each period, the first of each period 14 d for adaptation to diets followed by 7 d for ruminal sample collection and *in sacco* disappearance trial.

5.4.2 Laboratory Analyses

Concentrate and roughage was ground through a 2 mm screen for *in sacco* ruminal disappearance determination. Approximately 5 g of 2 mm ground samples e were placed into 8 × 11 cm nylon bags with 47 μm pore size. Samples of roughage were suspended in the rumen of each fistulated non-lactating dairy cow for 0 (pre feeding), 2, 4, 6, 12, 24, 48 (concentrate) and 72 h (roughage), and were then removed and washed in water and then dried at 65°C for 48 h. After weighing each bag individually, the residues were subjected to DM, CP determination. The degradability value was obtained by subjecting nutrient losses at arbitrary of time using NEWAY EXCEL (Chen, 1996).

To evaluate ruminal fermentation, on the last day of each experimental period (d 21), ruminal fluid samples were collected from each fistulated non-lactating dairy cow at 0, 2, 4 and 6 h after the morning feeding. Approximately 200 ml of ruminal fluid was collected and filtered through 4 layers of cheesecloth at 0 (pre feeding), 2, 4, 6 h post feeding. One portion of rumen fluid was immediately analyzed for pH (pH meter model UB-5, Denver Instrument, Germany). Ruminal volatile fatty acids (VFA) and ammonia N were determined in rumen fluid samples by taking 20 ml of rumen fluid and was then combined with 5 ml 6N HCl, kept frozen for analysis of VFA and ammonia N. The samples were later thawed at 4°C and centrifuged at 3,000 rpm for 15 min. The supernatant was analyzed for ammonia N by Kjeldahl and concentrations of VFA were determined by GC (Hewlett Packard GC system HP6890 A; Hewlett Packard, Avondale, PA) equipped with a 30 m × 0.32 mm × 0.15 μm film fused silica capillary column (HP_Innowax, AB 002, Agient, USA). Injector and detector temperatures were 250°C. The column temperature was kept at 80 °C for 5

min, then increased at 10 °C/min to 170 °C and then increased at 30 °C/min to 250 °C and held at 250 °C for 5 min. Protozoa populations were counted by Hematocytometer in rumen fluid samples which preserved with 10% normal saline solution.

Rumen digesta (approximately 500 g) was collected by hand from different sites within the ventral sac via the rumen cannulae, mixed immediately and a sub-sample (approximately 200 g) placed in a sealed plastic container, immersed immediately in ice slurry, transferred to the laboratory and then stored at -20 °C until FAs.

5.4.3 Statistical analysis

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

5.4.4 Experimental location

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

5.4.5 Experimental period

The experiment was from March 2013 to August 2013.

5.5 Result and discussion

5.5.1 Ruminal fermentation and rumen degradability

Ruminal pH, NH_3-N, protozoa and VFA concentration was not influenced by treatments (Table 5.1, 5.2). Doreau et al. (2009a) demonstrated that linseed oil did not affect the rumen fermentation pattern. Neveu et al. (2014) reported

that the inconsistent response of ruminal fermentation to grain source can be due to various factors such as grain variety, extent of grain processing, and forage level and source. Furthremore, Messana et al. (2013) suggested that the rumen fermentation depends on the feed intake, feeding frequency and composition of the diet. Similar to the present experiment, Harvatine and Allen (2006) have suggested that the use of saturated and unsaturated lipids has a minor or insignificant effect on ruminal fermentation parameters.

The average ruminal pH values were not affected by treatments (Table 5.1). Messana et al. (2013) reported that animals receiving the highest dietary lipid content (60 g/kg), rumen pH decreased quadratically ($P<0.001$) with an increase in the lipid content. However, in all treatments of the present study, the ruminal pH remained above 6.5; thus, the pH did not have a significant effect on ruminal fermentation. Russell and Wilson (1996) and Mertens (1997) reported that pH levels greater than 6.2 did not affect ruminal fermentation.

The concentration of NH_3-N was not affected by treatments (Table5.1). Van Soest (1994) suggested that a ruminal NH_3-N concentration below 13 mg/L of rumen fluid may affect the availability of nitrogen for microorganisms, which can compromise fiber ingestion and degradability. Thus, the ruminal NH_3-N concentration obtained from cow fed the 300 g/d LSO was below the suggested range. However, Messana et al. (2013), cow fed based on the 20 g lipid /kg diet (1,080 g/d), no relationship between the concentration of NH_3-N and the ruminal availability of fiber could be established.

These results indicated that the populations of protozoa were not affected by the dietary LSO (Table 5.1). In general, fats are not a usable source of energy for anaerobic microorganisms, and they can inhibit microbial growth by altering the

metabolic pathways of gram-positive bacteria. However, no significant differences in the responses to diets with different lipid contents and roughage source were observed in the present experiment.

Ruminal VFA was not affected by treatments (Table 5.2). Starch and carbohydrate sources affected cell wall fermentation due to bacterial activity, partly explained the total VFA concentrations. In the present experiment, the VFA concentration were unaffected by treatments because the animal offer the same amount of concentrate feed. However, Yang et al. (2009) reported that LSO supplementation (40 g/kg DM intake) led to lower total VFA concentration. It is suggested that unsaturated FA from oil could have interfered with ruminal fermentation resulting in greater gut fill. The substitution of oil in the diets coupled with the reduction in cell wall fermentation due to reduced bacterial activity, partly explained the reduction in total VFA concentrations.

The DM and NDF digestibilities were not significantly different among treatments (Table 5.3). Sterk et al. (2012) reported that various PUFAs have negative effects on degradation of NDF in the rumen and fiber degradation. However, the level of dietary PUFA (g/Kg DM) in the current study may not have been detrimental to microbes and diet degradability. Jenkins (1993) reported that the large amounts of unsaturated oils fed to the animals were expected to interfere ruminal fiber degradability. The high fiber content of our diets might have promoted hydrolysis, which creates ideal conditions for rapid growth of the microbes that are responsible for the hydrolysis and hydrogenation of dietary fat. However, it is also possible that the effects of LSO supplementation on ruminal digestion vary with the amount of LSO added to the diet. At a level of LSO supplementation of 2.6% in dairy cows (Doreau et al., 2009a) and 3% in growing steers (Shingfield et al., 2011), no effects

were observed on ruminal degradability, whereas at higher levels (i.e., 6% of dietary DM), Ikwuegbu and Sutton (1982) and Broudiscou et al. (1994) reported significant decreases in ruminal digestion.

Table 5.1 Effect of treatment on rumen pH, NH_3-N and protozoa of cows

Item	Treatments				SEM	Pr<F
	CS		FG			
	300 g/d PO	300 g/d LSO	300 g/d PO	300 g/d LSO		
pH						
0 hr	6.96	6.95	6.95	6.91	0.04	0.960
2 hr	6.47	6.44	6.38	6.53	0.12	0.631
4 hr	6.45	6.42	6.55	6.54	0.02	0.373
6 hr	6.45	6.58	6.52	6.64	0.03	0.410
NH_3-N (mg/L)						
0 hr	12.79	12.29	13.39	12.57	0.15	0.449
2 hr	19.48	18.45	21.01	19.24	0.18	0.341
4 hr	16.55	16.34	16.02	16.37	0.14	0.931
6 hr	14.16	11.81	14.37	11.48	0.07	0.364
Protozoa($x10^6$cells/ml)						
0 hr	3.00	3.75	4.25	4.00	0.32	0.454
2 hr	1.75	2.00	1.50	1.50	0.12	0.455
4 hr	2.00	2.33	2.00	1.67	0.21	0.821
6 hr	2.20	3.40	2.80	2.80	0.15	0.069

SEM is standard error of mean

Table 5.2 Effect of treatment on ruminal volatile fatty acid (VFA) of cows

Item	Treatments				SEM	Pr<F
	CS		FG			
	300 g/d PO	300 g/d LSO	300 g/d PO	300 g/d LSO		
VFA (mol/100 mol)						
Acetate, C2						
0 hr	70.91	72.65	73.78	73.50	0.76	0.576
2 hr	66.85	66.56	67.51	67.20	1.07	0.985
4 hr	69.92	68.11	69.84	68.83	0.51	0.677
6 hr	73.44	71.52	74.57	75.14	0.76	0.303
Propionate, C3						
0 hr	18.63	17.88	16.86	17.61	0.60	0.781
2 hr	20.42	18.10	16.95	17.54	0.59	0.267
4 hr	19.28	19.80	17.91	18.66	0.15	0.092
6 hr	16.17	16.68	16.25	14.98	0.66	0.767
Butyrate, C4						
0 hr	10.46	10.47	9.36	8.89	0.35	0.506
2 hr	12.99	15.35	15.55	12.76	0.83	0.539
4 hr	10.80	12.10	12.25	12.65	0.57	0.782
6 hr	10.39	11.80	9.18	9.88	0.45	0.198
Acetate : Propionate						
0 hr	6.85	8.77	8.03	8.37	0.52	0.615
2 hr	5.44	4.38	4.83	5.48	0.39	0.718
4 hr	6.48	5.94	5.70	5.49	0.29	0.758
6 hr	7.19	6.23	8.30	7.73	0.35	0.175

SEM is standard error of mean

Table 5.3 Effect of treatment on rumen degradability (*dg*) of cows.

Item	Treatments				SEM	Pr<F
	CS		FG			
	300 g/d PO	300 g/d LSO	300 g/d PO	300 g/d LSO		
Rumen degradability						
DM	54.13	54.81	44.67	44.33	0.42	<0.01
NDF	60.97	60.07	60.42	60.41	0.20	0.471

SEM is standard error of mean

5.5.2 Rumen digesta fatty acid profiles

The FA profiles of rumen digesta are summarized in Tables 5.4, 5.5, 5.6 and 5.7. The concentration of C12:0, C14:0 and C16:0 at 0 h (pre feeding), 2 h, 4 h, 6 h (post feeding) in ruminal digesta was unaffected by treatments.

In the present study, the concentration of C18:0 at 2 h post feeding was lower than at 0 h pre feeding in the rumen digesta. In addition, the concentration of C18:0 at 2 h of LSO treatment was lowered than PO treatment (P<0.01, Table 5.5). Increased concentration of C18:3n3 and C18:2n6 were accompanied by decreased C18:0 concentrations in rumen digesta. Increasing PUFA in the rumen caused effect bacteria to hydrogenate C18:1 and/or competition for hydrogen in the simultaneous BH of C18 UFA (Boeckaert et al., 2008). Thus, BH in the rumen showed the limited conversion of C18:1 to C18:0 by bacteria belonging to the *B. proteoclasticus* group (Jenkins et al., 2008; AbuGhazaleh and Jenkins, 2004; Wasowska et al., 2006).

At 2 h, the concentration of C18:1 in ruminal digesta was decreased (P < 0.01) by FG compared with CS (Table 5.5); while, there were no significant differ-

ences between oil supplements. The observed C18:1 with LSO treatments did not significantly differ from that of the PO treatment. However, the concentration of C18:1 at 6 h (post feeding) increased with LSO supplements. The observed C18:1 with the LSO treatment at 6 h was higher than PO treatment (Table 5.5). This increase in C18:1 at 6 h is an indication of incomplete BH of UFA with oil supplements (AbuGhazaleh et al., 2002). Rego et al. (2009) reported that the response of total C18:1 BH isomers was higher for the LSO diet, followed by the sunflower oil and rapeseed, although differences in individual isomers were detectable. The magnitude and pattern of BH differed with the different oils supplemented. LSO increased C18:1, which is consistent with the results obtained *in vitro* with marked C18:1 (Mosley et al., 2002). However, Sterk et al. (2012) reported that apparent ruminal BH of C18:1 was not affected by the linseed treatments.

The LSO treatment did not affect C9, T11 CLA and T10, C12 CLA in ruminal digesta (Table 5.5). The proportion of C9, T11 CLA was only higher in milk fat, which it was not an intermediate in the BH of C18:3n3, but is mainly produced in the mammary gland from C18:1 (Sterk et al., 2012). In addition, Rego et al. (2009) reported that the concentration of CLA did not differ between sunflower oil and LSO.

The concentration of C18:2n6 FA was unaffected by dietary treatment. The LSO diet greatly increased the proportions of almost all C18:2n6 FA as well as CLA. Linoleic acid accounted for 35 to 84% of total nonconjugated C18:2 isomer flow depending on diet. T9, C12 and C9, T13 C18:2 flow was greater ($P < 0.05$) with high concentrate diets and increased ($P < 0.05$) further with linseed oil.

The concentrations of C18:3n3 fatty acids in the ruminal digesta were decreased ($P < 0.01$) at 2 h, 4 h and 6 h post feeding with LSO supplements compared with PO treatment, probably caused by BH of C18:3n3. Wachira et al. (2000) reported

that BH of C18:2 and C18:3 ranged between 80 and 93% when lambs were fed FO and/or whole linseed. Sterk et al. (2010) studied the BH rate fractional and lag time by *in vitro* method according to an exponential model and to calculate effective BH of C18:3n3, assuming a fractional passage rate of 0.060/h. The BH values for C18:2n6 and C18:3n3 are in the range reported by Doreau and Ferlay (1994) of between 0.70 to 0.95 and 0.85 to 1.0, respectively. Furthermore, the apparent ruminal BH of C18:3n3 was high, which confirms the first step of the BH pathway (Sterk et al., 2010). However, Troegeler-Meynadier et al. (2003) examined the effect of pH on BH of C18:2n6 and C18:3n3 and concluded that BH of C18:2n6 and C18:3n3 was inhibited when pH was below 6.0 compared with above 6.5. In addition, data from *in vivo* (Kalscheur et al., 1997) and *in vitro* (Van Nevel and Demeyer, 1996) studies suggested that low ruminal pH reduces rates of ruminal BH. In the present experiment, ruminal pH showed a decrease with time, but the ruminal pH remained above 6.5 for all treatments. Therefore, it is expected that BH of C18:3n3 was not influenced by the pH at all supplemented.

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Table 5.4 Effect of treatment on rumen digesta fatty acid profile of cows at 0 h pre feeding

Item	Treatment				SEM	Pr<F
	CS		FG			
	300 g/d PO	300 g/d LSO	300 g/d PO	300 g/d LSO		
C12:0	0.35	0.40	0.42	0.39	0.02	0.76
C14:0	1.04	0.87	0.99	0.89	0.01	<0.01
C16:0	14.48	12.13	14.64	14.24	0.26	0.05
C18:0	45.95	42.82	39.18	42.86	0.33	0.7
C18:1	36.20	42.02	42.68	40.10	0.16	<0.01
C18:2n6	1.34	1.15	1.71	1.19	0.01	<0.01
C18:3n3	0.02	0.03	0.02	0.03	0.01	0.16
C20:0	0.40	0.36	0.06	0.07	0.01	0.41
C9,T11	0.07	0.06	0.08	0.05	0.01	0.19
T10,C12	0.19	0.18	0.24	0.21	0.01	0.5
SFA[1]	62.21	56.58	55.28	58.44	0.15	0.01
PUFA[2]	1.60	1.41	2.05	1.47	0.01	<0.01
PUFA/SFA	0.03	0.03	0.04	0.03	0.01	0.05
n-6/n-3	99.50	48.50	85.50	49.25	8.73	0.07

SEM is standard error of mean

[1] SFA = Sum of saturated fatty acid from C12:0 – C20:0

[2] PUFA = Sum of polyunsaturated fatty acids from C18:2 – C18:3

Table 5.5 Effect of treatment on rumen digesta fatty acid profile of cows at 2 h post feeding

Item	Treatment				SEM	Pr<F
	CS		FG			
	300 g/d PO	300 g/d LSO	300 g/d PO	300 g/d LSO		
C12:0	0.39	0.44	0.42	0.38	0.01	0.81
C14:0	0.82	0.84	0.87	0.9	0.01	0.47
C16:0	15.4	15.39	14.99	14.68	0.17	0.66
C18:0	42.85	36.11	38.00	36.12	0.47	0.01
C18:1	30.11	33.15	27.81	25.58	0.34	0.59
C18:2n6	8.52	8.23	7.41	8.67	0.10	0.06
C18:3n3	0.38	4.19	9.84	12.96	0.25	<0.01
C20:0	1.3	1.38	0.37	0.4	0.01	0.1
C9,T11	0.08	0.06	0.05	0.06	0.01	0.69
T10,C12	0.18	0.23	0.26	0.27	0.01	0.3
SFA[1]	60.76	54.15	54.64	52.47	0.55	0.02
PUFA[2]	9.15	12.7	17.55	21.95	0.23	<0.01
PUFA/SF	0.16	0.24	0.32	0.42	0.01	<0.01
n-6/n-3	22.98	2.00	0.76	0.67	0.58	<0.01

SEM is standard error of mean

[1] SFA = Sum of saturated fatty acid from C12:0 – C20:0

[2] PUFA = Sum of polyunsaturated fatty acids from C18:2 – C18:3

Table 5.6 Effect of treatment on rumen digesta fatty acid profile of cows at 4 h post feeding

Item	Treatment				SEM	Pr<F
	CS		FG			
	300 g/d PO	300 g/d LSO	300 g/d PO	300 g/d LSO		
C12:0	0.38	0.51	0.43	0.39	0.03	0.47
C14:0	0.92	1.04	0.93	0.91	0.02	0.27
C16:0	13.96	14.4	14.6	14.51	0.10	0.43
C18:0	43.32	48.72	48.05	56.15	0.54	<0.01
C18:1	37.75	30.2	29.03	21.83	0.22	<0.01
C18:2n6	2.78	3.51	4.87	3.9	0.37	0.88
C18:3n3	0.08	1.29	1.66	1.92	0.04	<0.01
C20:0	0.52	0.33	0.20	0.16	0.04	0.14
C9,T11	0.04	0.05	0.06	0.08	0.01	0.16
T10,C12	0.27	0.16	0.19	0.21	0.01	0.16
SFA[1]	59.09	64.99	64.21	72.07	0.47	<0.01
PUFA[2]	3.17	5.00	6.77	6.11	0.37	0.04
PUFA/SFA	0.06	0.08	0.11	0.09	0.01	0.86
n-6/n-3	35.65	2.72	2.97	2.05	1.82	<0.01

SEM is standard error of mean

[1] SFA = Sum of saturated fatty acid from C12:0 – C20:0

[2] PUFA = Sum of polyunsaturated fatty acids from C18:2 – C18:3

Table 5.7 Effect of treatment on rumen digesta fatty acid profile of cows at 6 h post feeding

Item	Treatment				SEM	Pr<F
	CS		FG			
	300 g/d PO	300 g/d LSO	300 g/d PO	300 g/d LSO		
C12:0	0.34	0.42	0.43	0.49	0.04	0.48
C14:0	1.02	0.92	0.89	1.14	0.02	0.11
C16:0	12.98	13.38	13.94	14.52	0.18	0.24
C18:0	44.99	44.27	46.27	39.1	0.31	<0.01
C18:1	37.6	38.27	35.58	41.57	0.46	0.02
C18:2n6	2.43	2.06	2.41	2.68	0.07	0.72
C18:3n3	0.01	0.12	0.17	0.19	0.01	<0.01
C20:0	0.35	0.37	0.07	0.06	0.01	1.00
C9,T11	0.08	0.07	0.07	0.07	0.01	0.40
T10,C12	0.21	0.15	0.19	0.21	0.01	0.46
SFA[1]	59.68	59.34	61.59	55.3	0.42	0.02
PUFA[2]	2.73	2.4	2.83	3.14	0.07	0.95
PUFA/SF	0.05	0.04	0.04	0.06	0.01	0.60
n-6/n-3	24.30	16.75	14.30	14.15	6.04	<0.01

SEM is standard error of mean

[1] SFA = Sum of saturated fatty acid from C12:0 – C20:0

[2] PUFA = Sum of polyunsaturated fatty acids from C18:2 – C18:3

5.6 Conclusion

LSO supplementation (300g/d) with *ad libitum* roughage source in fistulated crossbred Holstein Friesian cows did not negatively influence on ruminal fermentation including ruminal pH, NH_3-N, protozoa and VFA concentration. Supplementation of LSO did not decrease rumen degradability of DM and NDF. At 2 h post feeding of LSO resulted in an inhibition of the complete C18:3n3 BH toward C18:0, as indicated by a low C18:0 proportions in the rumen digesta. However, no difference in C18:2n6 and CLA proportion in rumen digesta was observed.

5.7 References

AbuGhazaleh, A. A., Schingoethe, D. J., and Hippen, A. R. (2002). Feeding fish meal and extruded soybeans enhances the conjugated linoleic acid (CLA) content of milk. **J. Dairy Sci.** 85 : 624-631

AbuGhazaleh, A. A., and Jenkins, T. C. (2004). Disappearance of docosa hexaenoic and eicosapentaenoic acids from cultures of mixed ruminal mi croorganisms. **J. Dairy Sci.** 87 : 645-651.

Akraim, F., Nicot, M. C., Juaneda, P., and Enjalbert, F. (2007). Conjugated linolenic acid (CLnA), conjugated linoleic acid (CLA) and other biohydrogenation intermediates in plasma milk fat of cows fed raw or extruded linseed. **Animal** 6 : 835-843.

Broudiscou, L., Pochet, S., and Poncet, C. (1994). Effect of linseed oil supplementation on feed degradation and microbial synthesis in the rumen of ciliate-free and refaunated sheep. **Anim. Feed Sci. Technol.** 49 : 189-202.

Chen, X.B. (1996). An excel application programme for processing feed digestibility

data, User ManualRowett Research Institute, Bucksburn, Aberdeen, UK.

Doreau, M., and Ferlay, A. (1994). Digestion and utilisation of fatty acids by ruminants. **Anim. Feed Sci. Technol.** 45 : 379-396.

Doreau, M., Aurousseau, E. and Martin, C. (2009a). Effect of linseed lipids fed as rolled seeds, extruded seeds or oil on organic matter and crude protein digestion in cows. **Anim. Feed Sci. Technol.** 150 : 187-196.

Doreau, M., Laverroux, S. Normand, J. Chesneau, G. and Glasser, F. (2009b). Effect of linseed fed as seeds, extruded seeds or oil on fatty acid rumen metabolism and intestinal digestibility in cows. **Lipids.** 44 : 53-62.

Destaillats, F., Trottier, J. P., Galvez, J. M. G., and Angers, P. (2005). Analysis of alpha-linolenic acid biohydrogenation intermediates in milk fat with emphasis on conjugated linolenic acid. **J. Dairy Sci.** 88 : 3231-3239.

Harvatine, K. J., and Allen, M. S. (2006) Effects of fatty acid supplements on ruminal and total tract nutrient digestion in lactating dairy cows. **J. Dairy Sci.** 89 : 1092-1103.

Harfoot, C. G. (1978). Lipid metabolism in the rumen. **Prog. Lipid Res.** 17 : 21–54.

Ikwuegbu, O. A., and Sutton, J. D. (1982). The effect of varying the amount of linseed oil supplementation on rumen metabolism in sheep. **Br. J. Nutr.** 48 : 365-375.

Ivan, M., Mir, P. S., Koenig, K. M., Rode, L. M., Neill, L., Entz, T., and Mir, Z. (2001). Effects of dietary sunflower seed oil on rumen protozoa population and tissue concentration of conjugated linoleic acid in sheep. **Small Rumi. Res.** 41 : 215-227.

Jenkins, T. C. (1993). Lipid metabolism in the rumen. **J. Dairy Sci.** 76 : 3851-3863.

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

Jenkins, T. C., Wallace, R. J., Moate, P. J., and Mosley E. E. (2008). Recent advances

in biohydrogenation of unsaturated fatty acids within the rumen microbial ecosystem. **J. Anim. Sci.** 86 : 397-412.

Loor, J. J., Ueda, K. Ferlay, A. Chilliard, Y., and Doreau, M. (2004). Biohydrogenation, duodenal flow, and intestinal digestibility of trans fatty acids and conjugated linoleic acids in response to dietary forage : concentrate ratio and linseed oil in dairy cows. **J. Dairy Sci.** 87 : 2472-2485.

Mertens, D. R. (1997) Predicting in intake and digestibility using mathematical models of ruminal functions. **J. Anim. Sci.** 64 : 1548-1558.

Messana. J. D., Berchielli, T. T., Arcuri, P. B.., Reis, R. A., Canesin, R. C., Ribeiro, A. F., Fiorentini, G., and Fernandes, J. R. (2013). Rumen fermentation and rumen microbes in Nellore steers receiving diets with different lipid contents. **R. Bras. Zootec.** 42(3) : 204-212.

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

Nagaraja, T. G., Newbold, C. J., Ven Nevel, C. J., and Demeyer, D. I. (1997). Manipulation of ruminal fermentation. In : Hubson, P. N., and Stewart, C. S. (Eds.). **The Rumen Microbial Ecosystem.** Blackie Acad. and Prof., an Imprint of Chapman and Hall, London. 523-632 p.

Neveu, C., Baurhoo, B., and Mustafa, A. (2014). Effect of feeding extruded flaxseed with different grains on the performance of dairy cows and milk fatty acid profile. **J Dairy Sci.** 97 : 1543-1557.

Oliveira, R. L., Assuncao, D. M. P., Barbosa, M. A. A. F., Ladeira, M. M., Silva, M.

M. P., Mascarenhas, A. G., Snel-Oliveira, M. V., and Oliveira, R. L. (2007). Effect of different fat sources on intake, digestibility and blood urea nitrogen of feedlot

water buffalo steers. **Rev. Bras. Zootecn.** 36 : 733-738.

Palmquist, D. L., and Jenkins, T. C. (1980). Fat in lactation rations : review. **J. Dairy Sci.** 63 : 1-14.

Rego O. A., Alves S. P., Antunes M. S., Rosa H. J. D., Alfaia C. F., Prates J. A. M., Cabrita A. R. J., Fonseca A. J. M., and Bessa R. J. B. (2009). Rumen biohydrogenation-derived fatty acids in milk fat from grazing dairy cows supplemented with rapeseed, sunflower, or linseed oils. **J. Dairy Sci.** 92 : 4530-4540.

Russell, J.B., and Willson, D.B. (1996). Why are ruminal cellulolytic bacteria unable to digest cellulose at low pH? **J. Dairy Sci.** 79 : 1503-1509.

Shingfield, K. J., Lee, M. R. F., Humphries, D. J., Scollan, N. D., Toivonen, V. Beever, D. E., and Reynolds, C. K. (2011). Effect of linseed oil and fish oil alone or as an equal mixture on ruminal fatty acid metabolism in growing steers fed maize silage-based diets. **J. Anim. Sci.** 89 : 3728–3741.

Sterk, A., Hovenier, R., Vlaeminck, B., van Vuuren, A. M., Hendriks, W. H., and Dijkstra, J. (2010). Effects of chemically or technologically treated linseed products and docosahexaenoic acid addition to linseed oil on biohydrogenation of C18 : 3n3 *in vitro*. **J. Dairy Sci.** 93 : 5286-5299.

Sterk , A., Vlaeminck , B., van Vuuren , A. M., Hendriks , W. H., and Dijkstra, J. (2012). Effects of feeding different linseed sources on omasal fatty acid flows and fatty acid profiles of plasma and milk fat in lactating dairy cows. **J. Dairy Sci.** 95 : 3149-3165.

Troegeler-Meynadier, A., M., Nicot, C., Bayourthe, C. and Moncoulon, R., and Enjalbert, F. (2003). Effects of pH and concentrations of linoleic and linolenic acids on extent and intermediates of ruminal biohy drogenation in vitro. **J. Dairy**

Sci. 86 : 4054–4063.

Van Nevel, C. J., and Demeyer, D. I. (1996). Influence of pH on lipolysis and biohydrogenation of soybean oil by rumen contents in vitro. **Rep. Nutr. Dev.** 36 : 53-63.

Van Soest, P. J. (1994). **Nutritional Ecology of the Ruminant.** Cornell University Press, Ithaca, NY.

Wachira, A. M., Sinclair, L. A., Wilkinson, R. G., Hallett, K., Enser, M., and Wood, J. D., (2000). Rumen biohydrogenation of n−3 polyunsaturated fatty acids and their effects on microbial efficiency and nutrient digestibility in sheep. **J. Agric. Sci.** 135 : 419-428.

Wasowska, I., Maia, M. R. G., Niedzwiedzka, K. M., Czauderna, M., Ramalho Ribeiro, J. M. C., Devillard, E., Shingfield, K. J., and Wallace, R. J. (2006). Influence of fish oil on ruminal biohydrogenation of C18 unsaturated fatty acids. **Br. J. Nutr.** 95 : 1199-1211.

Yang, H. J., Yue, Q., Cao, Y. C., Zhang D. F., and Wang, J. Q. (2009). Effects of crude feruloyl and acetyl esterase solutions of *Neocallimastix* sp. YQ1 and *Anaeromyces* sp. YQ3 isolated from Holstein steers on hydrolysis of Chinese wildrye grass hay, wheat bran, maize bran, wheat straw and corn stalks. **Anim Feed Sci Technol.** 154 : 218-227.

CHAPTER VI

OVERALL CONCLUSIONS AND IMPLICATION

6.1 Conclusions

The purposes of the present study were to investigate the effect of linseed oil (LSO) supplementation in steer and dairy cattle diets on fatty acid profiles and n-6/n-3 ratio in beef and milk. The present studies were successful.

The first experiment was conducted to determine whether LSO can increase n-3FA accumulation and decrease n-6/n-3 ratio in beef. The results showed that accumulation of n3 FA in both *Longissimus dorsi* (LD) and *Semimembranosus* (SM) muscles were increased and n-6/n-3 ratio was decreased by the addition of LSO. The differences in responses to LSO were probably due to variations in levels of oil supplementation, levels of oil in total ration and amount of linolenic acid in oils. The overall feed consumption of the steers was decreased when dietary oil was provided, leading to improvement in efficiency of growth performance. Although, LSO supplementation increased drip loss percentage, TBARS values and reduced beef color stability (a*) and beef tenderness. It had no impact on sensory perceptions of panelists.

Furthermore, beside the field feeding experiment, ruminal fluid was collected from steers by suction method. Concentration of n3 FA in ruminal fluid was negligibly detected in LSO supplemented steers. At 2, 4 post feeding of LSO treatment, ruminal fluid contained lower C18:3 and PUFA while, at 6 h post feeding C18:1 and SFA was

increased. This suggested that a large extent of BH process occurred in the rumen. However, the passage rates of C16:0, C18:0 and total C18 carbon FA linearly decreased as LSO increased. The incomplete of biohydrogenation process on LSO supplementation may result in an enhanced ruminal outflow of C18:3n3, and thus accumulated n3 FA in beef.

The second experiment was carried out to investigate the effect of LSO on n3 FA accumulation and n-6/n-3 ratio in milk. The results revealed that the milk percentage of n3 FA was increased and the milk n-6/n-3 ratio was reduced by LSO supplement. The best response of LSO supplementation on n3 FA accumulation in milk was to supplement with fresh grass which it is also high in n3 FA. In addition, LSO had no effect on DMI, milk production and milk composition. Milk fat content was not affected by LSO supplementation.

The third experiment was conducted to determine the effects of LSO supplementation on ruminal fermentation, fiber degradability and rumen digesta fatty acid profiles in fistulated cows receiving the same treatment feed of experiment II. The results demonstrated that at 2 h post feeding LSO resulted in higher C18:3 while, C18:0, C18:2 and CLA proportion in rumen digesta were unaffected. Feeding LSO inhibited BH of C18:2 to C18:0, as indicated by the increased rumen flows and proportions of BH intermediates in rumen digesta. Then, LSO supplementation showed similar response in C18:3 concentrations for incomplete BH process in rumen resulting in accumulation of n3 FA in milk fat. Furthermore, LSO did not negatively influence on ruminal fermentation, DM and NDF digestibilities and change ruminal pH, NH_3-N, protozoa and VFA concentration.

From the two present experiments, LSO supplementation increased n3 PUFA content of beef (19.35 and 41.96 mg/100 g beef; LD and SM muscle, respectively)

and milk (51.54 mg/ 100 g milk). These contents would not be sufficient for intake recommendation requirements of The European Food Safety Authority based on considerations of cardiovascular health and neurodevelopment which are about 2,000 -3,000 mg/day (EFSA, 2009). However, LSO supplementation decreased n-6/n-3 ratio of beef (6.11 and 2.89 of LD and SM muscle, respectively) and milk (3.79) which would be sufficient for improving cardiac health. Several international organizations have recommended a dietary n-6/n-3 ratio of 4:1 to 7.5:1 to decrease the risk of cardiovascular disease (CVD) (Simopoulos, 1998; Kafatos and Codrington, 1999; Fernandes, 2002)

Increasing the content of n3 FA and reducing n-6/n-3 ratio can be achieved by supplement LSO (high C18:3n3 of oil) together with fresh grass (high n3 FA of roughage) in the present study. Thus, nutrition quality is an increasingly important factor contributing to beef and milk and it is important target, along with increased understanding of the role of ruminant FA in the human diet.

6.2 Implication

The present study suggests that:

The fattening steer should be supplemented with high n3 FA source including 200 g/d LSO together with FG during late-mature period. At this stage, it was clear that the development of intramuscular fat deposition to maintain or increase fat synthesis. The supplementing period being beneficial to LSO supplemented steers is at least 70 days before slaughtering since at this stage and at this supplementing period LSO can increase n3 FA and decrease n-6/n-3 FA in beef but cause no effect on growth performance in steer. However, high n3 FA supplementation can affect beef

color. Thus, much interest has been focused on the protection beef oxidation such as vitamin E which protects cells against attacks from oxygen.

The dairy cow should be supplemented with high n3 FA source including 300 g/d LSO together with FG during early lactation period because this period obtained a larger response and the fact of milk fat composition designed by diet lipid composition. The period which is beneficial to dairy cow is at least 30 days. In early lactation and 30 d supplementation, LSO can increase n3 FA, reduce n-6/n-3 FA in milk fat without affecting milk fat percentage. Milk price in Thailand relies partly on milk fat percentage, thus LSO supplementation did not influence on famer's income. Furthermore, LSO supplementation can be used as source of energy to maintain production and BW (periods of negative energy balance) in dairy's cow.

Feeding LSO and grass which are n3 FA sources in ruminant diets results in beneficial responses in the decreased n-6/n-3 ratio in beef and milk, to the level that considered to be relatively low. Manipulations of feeding method (roughage and concentrate ratio), types of diets (high n3 source) and strategies to control or protect n3 FA from BH and lipolysis are required to further enhance beneficial fatty acids in beef and milk.

Although, milk and beef from LSO supplemented animals was a high quality product for healthy and made high price products. LSO is expensive (150 baht/kg) resulted in higher feed costs. Therefore, Thai farmers who would like to use LSO must be concern about the product is price in the market.

Furthermore, LSO supplementation increased n3 PUFA content of beef (19.35 and 41.96 mg/100 g beef; LD and SM muscle, respectively) and milk (51.54 mg/ 100 g milk) would not be sufficient for intake which are about 2,000 -3,000 mg/day (EFSA, 2009)

However, LSO supplementation decreased n-6/n-3 ratio of beef (6.11 and 2.89 of LD and SM muscle, respectively) and milk (3.79) which would be sufficient for improving cardiac health recommended a dietary n-6/n-3 ratio of 4:1 to 7.5:1 (Simopoulos, 1998; Kafatos and Codrington, 1999; Fernandes, 2002)

6.3 References

European Food Safety Authority. (2009). Scientific opinion: Labelling reference intake values for n−3 and n−6 polyunsaturated fatty acids. **The EFSA J.** 1176: 1-11.

Fernandes, J. (2002). **Nutrition and health-Recommendations of the Health Council of the Netherlands regarding energy, proteins, fats and carbohydrates.** Nederlands Tijdschrift voor Geneeskunde. 146: 2226-2229.

Kafatos, A., and Codrington, C. A. (1999). Nutrition and diet for healthy lifestyles in Europe: The “Eurodiet” Project. **Public Health Nutr.** 2: 327-328.

Simopoulos, A. P. (1989). Summary of the NATO advanced research workshop acids: Biological effects and nutritional essentiality. **J. Nutr.** 119: 521-528.

APPENDIX

Table 1A Standards and reference compounds of fatty acid methyl esters by gas chromatography (GC) analysis (Supelco 37 Component FAME Mix).

No.	Component	Weight (%)
1	C4:0 (Butryic)	4
2	C6:0 (Caproic)	4
3	C8:0 (Caprylic)	4
4	C10:0 (Capric)	4
5	C11:0 (Undecanoic)	2
6	C12:0 (Lauric)	4
7	C13:0 (Tridecanoic)	2
8	C14:0 (Myristic)	4
9	C14:1 (Myristoleic)	2
10	C15:0 (Pentadecanoic)	2
11	C15:1 (cis-10-Pentadecenoic)	2
12	C16:0 (Palmitic)	6
13	C16:1 (Palmitoleic)	2
14	C17:0 (Heptadecanoic)	2
15	C17:1 (cis-10-Heptadecenoic)	2
16	C18:0 (Stearic)	4
17	C18:1n9c (Oleic)	4
18	C18:1n9t (Elaidic)	2
19	C18:2n6c (Linoleic)	2
20	C18:2n6t (Linolelaidic)	2
21	C18:3n6 (g-Linolenic)	2
22	C18:3n3 (a-Linolenic)	2
23	C20:0 (Arachidic)	4
24	C20:1n9 (cis-11-Eicosenoic)	2
25	C20:2 (cis-11,14-Eicosadienoic)	2
26	C21:0 (Henicosanoic)	2
27	C22:0 (Behenic)	4
28	C20:3n6 (cis-8,11,14-Eicosatrienoic)	2
29	C22:1n9 (Erucic)	2
30	C20:3n3 (cis-11,14,17-Eicosatrienoic)	2
31	C20:4n6 (Arachidonic)	2
32	C23:0 (Tricosanoic)	2
33	C22:2 (cis-13,16-Docosadienoic)	2
34	C24:0 (Lignoceric)	4
35	C20:5n3 (cis-5,8,11,14,17-Eicosapentaenoic)	2
36	C24:1n9 (Nervonic)	2
37	C22:6n3 (cis-4,7,10,13,16,19-Docosahexaenoic)	2

Table 2A Percentage of fatty acids intake of beef steers (g/d) (Chapter III)

FA Intake (g/d)	Treatments				SEM	Pr<F
	HC	200 g/d PO	200 g/d MO	200 g/d LSO		
C8:0	0.06	0.12	0.09	0.13	0.005	<0.01
C10:0	0.09	0.09	0.05	0.05	0.002	<0.01
C12:0	1.38	1.15	0.97	0.79	0.003	<0.01
C14:0	0.49	2.11	1.21	0.39	0.001	<0.01
C16:0	1.49	74.04	41.19	10.22	0.007	<0.01
C18:0	0.23	8.58	7.70	6.75	0.003	<0.01
C18:1n9c	2.50	79.06	57.35	35.60	0.006	<0.01
C18:2n6c	1.62	27.07	31.06	32.93	0.005	<0.01
C18:3n3	0.04	0.65	53.41	106.98	0.008	<0.01
SFA[1]	3.75	86.35	51.69	18.74	0.012	<0.01
MUFA[2]	2.50	79.94	58.03	35.75	0.007	<0.01
PUFA[3]	1.71	29.59	86.06	141.36	0.012	<0.01
total n-3[4]	0.04	0.97	53.84	107.60	0.008	<0.01
total n-6[5]	1.67	28.62	32.11	33.56	0.005	<0.01
PUFA:SFA	0.46	0.34	1.67	7.54	0.001	<0.01
n-6:n-3	40.02	29.40	0.60	0.31	0.400	<0.01

SEM = Standard error of mean

[1] SFA = Sum of saturated fatty acid from C4:0 – C20:0

[2] MUFA = Sum of monounsaturated fatty acid from C14:1 – C22:1

[3] PUFA = Sum of polyunsaturated fatty acids from C18:2 – C22:6

[5] Sum of n3 fatty acids C18:3n-3 – C22:6n-3

[4] Sum of n6 fatty acids C18:2n-6 – C22:4n-6

Table 3A Fatty acid intake of dairy cows (Chapter IV).

FA Intake (g/d)	Treatment				SEM	Pr<F
	CS		FG			
	300 g/d	300 g/d	300 g/d	300 g/d		
C12:0	1.09	0.56	1.09	0.55	0.021	<0.01
C14:0	2.96	0.38	2.95	0.37	0.008	<0.01
C16:0	110.49	14.76	110.43	14.69	0.022	<0.01
C18:0	12.82	10.07	12.79	10.04	0.004	<0.01
C18:1n9c	117.53	52.34	117.53	52.34	0.127	<0.01
C18:2n6c	40.15	48.95	40.11	48.90	0.109	<0.01
C20:0	0.15	0.43	0.13	0.41	0.003	<0.01
C18:3n3	0.80	160.29	0.90	160.36	0.008	<0.01
C9,T11	0.25	0.26	0.05	0.05	0.004	0.358
SFA[1]	128.01	26.62	127.90	26.49	0.057	<0.01
MUFA[2]	118.84	52.57	118.85	52.57	0.129	<0.01
PUFA[3]	44.04	211.71	43.83	211.45	0.126	<0.01
total n-3[4]	1.29	161.23	1.39	161.30	0.008	<0.01
tatal n-6[5]	42.75	50.18	42.44	49.85	0.035	<0.01
PUFA:SFA	0.34	7.95	0.34	7.98	0.011	<0.01
n-6:n-3	33.09	0.31	30.65	0.31	0.025	<0.01

SEM = Standard error of mean

[1] SFA = Sum of saturated fatty acid from C4:0 – C20:0

[2] MUFA = Sum of monounsaturated fatty acid from C14:1 – C22:1

[3] PUFA = Sum of polyunsaturated fatty acids from C18:2 – C22:6

[4] Sum of n3 fatty acids C18:3n-3 – C22:6n-3;[5] Sum of n6 fatty acids C18:2n-6 – C22:4n-6

BIOGRAPHY

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