EFFECTS OF RUMEN PROTECTED FAT CONTAINING OLEIC ACID SUPPLEMENTATION ON CARCASS AND BEEF QUALITY, AND BEEF FATTY ACIDS PROFILE IN WAGYU CROSSBRED CATTLE

Rattakorn Mirattanaphrai

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ผลของการเสริม ไขมันไหลผ่านที่มีองค์ประกอบของกรดไขมันโอลิอิคอยู่สูงต่อ คุณภาพซาก, คุณภาพเนื้อและองค์ประกอบของกรดไขมัน ในโคเนื้อลูกผสมวากิว

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

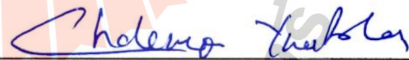
Thesis Examining Committee

(Assoc. Prof. Dr. Surintorn Boonanuntanasarn)
Chairperson

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

(Assoc. Prof. Dr. Amonrat Molee)
Member

(Asst. Prof. Dr. Pakanit Kupittayanant)
Member



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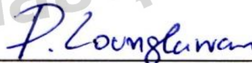

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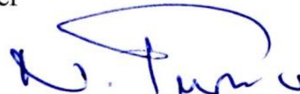

(Prof. Dr. Neung Teaumroong)
Dean of Institute of Agricultural Technology

นายรัฐกร มิรัตนไพร : ผลของการเสริมไขมันไหลผ่านที่มีองค์ประกอบของกรดไขมันโอลิอิคอยู่สูงต่อคุณภาพซาก, คุณภาพเนื้อและองค์ประกอบของกรดไขมันในโคเนื้อลูกผสมวากิว (EFFECTS OF RUMEN PROTECTED FAT CONTAINING OLEIC ACID SUPPLEMENTATION ON CARCASS AND BEEF QUALITY, AND BEEF FATTY ACIDS PROFILE IN WAGYU CROSSBRED CATTLE)
อาจารย์ที่ปรึกษา : รองศาสตราจารย์ ดร. วิศิษฐิพร สุขสมบัติ, 161 หน้า.

วัตถุประสงค์ของงานวิจัยนี้เพื่อศึกษาการเสริมไขมันไหลผ่านที่มีองค์ประกอบของกรดไขมันโอลิอิคอยู่สูงต่อ คุณภาพซาก, คุณภาพเนื้อและองค์ประกอบของกรดไขมันในโคเนื้อลูกผสมวากิว

การทดลองที่ 1 ทำการทดลองในโคเนื้อโคลูกผสมที่มีระดับสายเลือดของโควากิว 50% จำนวน 16 ตัว น้ำหนักตัวเฉลี่ย 529+28 กิโลกรัม อายุเฉลี่ย 27 เดือน วางแผนการทดลองแบบสุ่มสมบูรณ์ โดยสุ่มสัตว์ทดลองแบบแบ่งชั้นจากน้ำหนักตัว จำนวน 4 กลุ่ม โคทุกตัวได้รับอาหารข้นที่มีโปรตีน 12 % วันละ 7.5 กิโลกรัม/ตัว ได้รับฟางข้าวเป็นแหล่งอาหารหยาบแบบไม่จำกัดปริมาณ มีน้ำให้กินตลอดเวลา และถูกเลี้ยงขังในคอกเดี่ยว กลุ่มการทดลองได้แก่ 1) กลุ่มควบคุม (CON) ได้รับอาหารข้นปกติ 2) ได้รับอาหารข้นและเสริมด้วย Rumen protected palm oil (RP-PO) 200 กรัม/วัน 3) ได้รับอาหารข้นและเสริมด้วย Rumen protected rice bran oil (RP-RO) 200 กรัม/วัน และ 4) ได้รับอาหารข้นและเสริมด้วย Rumen protected corn oil (RP-CO) 200 กรัม/วัน ผลการทดลองพบว่า การเสริม Rumen protected rice bran oil สามารถเพิ่มความเข้มข้นของกรดไขมันชนิด C18:1n-9 ในเนื้อโค และสามารถเพิ่มระดับของไขมันแทรกในเนื้อสันได้อย่างมีนัยสำคัญทางสถิติเมื่อเปรียบเทียบกับกลุ่มอื่น นอกจากนี้ การเสริม Rumen protected rice bran oil และ Rumen protected corn oil สามารถเพิ่มระดับของ PUFA ในเนื้อโคได้อย่างมีนัยสำคัญทางสถิติเมื่อเปรียบเทียบกับกลุ่มทดลองอื่น

การทดลองที่ 2 ทำการทดลองในโคเนื้อโคลูกผสมที่มีระดับสายเลือดของโควากิว 50% จำนวน 12 ตัว น้ำหนักตัวเฉลี่ย 509+3.2 กิโลกรัม อายุเฉลี่ย 28 เดือน วางแผนการทดลองแบบสุ่มสมบูรณ์ โดยสุ่มสัตว์ทดลองแบบแบ่งชั้นจากน้ำหนักตัว จำนวน 3 กลุ่ม โคทุกตัวได้รับอาหารข้นที่มีโปรตีน 12 % วันละ 7.5 กิโลกรัม/ตัว ได้รับฟางข้าวเป็นแหล่งอาหารหยาบแบบไม่จำกัดปริมาณ มีน้ำให้กินตลอดเวลา และถูกเลี้ยงขังในคอกเดี่ยว กลุ่มการทดลองได้แก่ 1) กลุ่มควบคุม (CON) ได้รับอาหารข้นปกติ 2) ได้รับอาหารข้นและเสริมด้วย RP-RO 100 กรัม/วัน 3) ได้รับอาหารข้นและเสริมด้วย (RP-RO) 200 กรัม/วัน ผลการทดลองพบว่า การเสริม RP-RO 200 กรัม/วัน สามารถเพิ่ม

ความเข้มข้นของกรดไขมันชนิด C18:1n-9 ในเนื้อโคและสามารถเพิ่มระดับของไขมันแทรกในเนื้อสันได้อย่างมีนัยสำคัญทางสถิติเมื่อเปรียบเทียบกับกลุ่มทดลองอื่น

การทดลองที่ 3 ใช้โคเจาะกระเพาะ จำนวน 4 ตัว วางแผนการทดลองแบบ 4×4 Latin square โดยโคทุกตัวได้รับอาหารข้น 4 กิโลกรัม/ตัว/วัน มีโปรตีน 12% มีน้ำให้กินตลอดเวลา และถูกเลี้ยงขังในคอกเดี่ยว กลุ่มการทดลองได้แก่ 1) กลุ่มควบคุม ได้รับอาหารข้นปกติ 2) ได้รับอาหารข้นและเสริมด้วย RP-PO 200 กรัม/วัน 3) ได้รับอาหารข้นและเสริมด้วย RP-RO 200 กรัม/วัน 4) ได้รับอาหารข้นและเสริมด้วย RP-CO 200 กรัม/วัน ผลการทดลองพบว่า สัดส่วนของกรดไขมันชนิด C16:0 ในกระเพาะหมักของโคกลุ่มที่ได้รับการเสริม RP-PO มีค่าสูงกว่ากลุ่มที่เสริม RP-RO และ RP-CO อย่างมีนัยสำคัญทางสถิติ ในขณะที่โคกลุ่มที่เสริม RP-RO มีความเข้มข้นของกรดไขมันชนิด C18:1n-9 ในกระเพาะหมักสูงกว่ากลุ่มทดลองอื่นอย่างมีนัยสำคัญทางสถิติ และโคกลุ่มที่เสริม RP-CO มีความเข้มข้นของกรดไขมันชนิด C18:2n-6t ในกระเพาะหมักสูงกว่ากลุ่มทดลองอื่นอย่างมีนัยสำคัญทางสถิติ

การทดลองที่ 4 ใช้โคเจาะกระเพาะ จำนวน 3 ตัว วางแผนการทดลองแบบ 3×3 Latin square โดยโคทุกตัวได้รับอาหารข้น 4 กิโลกรัม/ตัว/วัน มีโปรตีน 12% มีน้ำให้กินตลอดเวลา และถูกเลี้ยงขังในคอกเดี่ยว กลุ่มการทดลองได้แก่ 1) กลุ่มควบคุม ได้รับอาหารข้นปกติ 2) ได้รับอาหารข้นและเสริมด้วย RP-RO 100 กรัม/วัน 3) ได้รับอาหารข้นและเสริมด้วย RP-RO 200 กรัม/วัน ผลการทดลองพบว่าโคกลุ่มที่เสริม RP-RO 200 กรัม/วัน มีความเข้มข้นของกรดไขมัน C18:1n-9 ในกระเพาะหมักสูงกว่ากลุ่มทดลองอื่นอย่างมีนัยสำคัญทางสถิติและมีความเข้มข้นของกรดไขมันชนิด C18:0 ลดลงอย่างมีนัยสำคัญทางสถิต

มหาวิทยาลัยเทคโนโลยีสุรนารี

สาขาวิชาเทคโนโลยีการผลิตสัตว์ ลายมือชื่อนักศึกษา__________

ปีการศึกษา 2560 ลายมือชื่ออาจารย์ที่ปรึกษา__________

RATTAKORN MIRATTANAPHRAI : EFFECTS OF RUMEN PROTECTED FAT CONTAINING OLEIC ACID SUPPLEMENTATION ON CARCASS AND BEEF QUALITY, AND BEEF FATTY ACIDS PROFILE IN WAGYU CROSSBRED CATTLE. THESIS ADVISOR : ASSOC. PROF. WISITIPORN SUKSOMBAT, Ph.D., 161 PP.

RUMEN PROTECTED FAT/PALM OIL/RICE BRAN OIL/CORN OIL/FATTY ACIDS/WAGYU BEEF STEERS

The objective of this research was to determine the effect of rumen protected fat containing high oleic acid including palm oil, rice bran oil, and corn oil supplementation on carcass quality, sensory evaluation and fatty acid profile of beef from crossbred Wagyu beef steers.

In Experiment I, sixteen fattening Wagyu crossbred beef steers (50% Wagyu), averaging 529±28 kg live weight (LW) and approximately 27 mo old, were stratified by their LW into 4 groups. All steers were fed 7.5 kg/d of 12% CP concentrate with *ad libitum* rice straw and had free access to clean water. The treatments were 1) control concentrate, 2) control concentrate plus 200 g/d of rumen protected palm oil (RP-PO), 3) control concentrate plus 200 g/d of rumen protected rice bran oil (RP-RO), and 4) control concentrate plus 200 g/d rumen protected corn oil (RP-CO). This present study demonstrated that supplementation of RP-RO increased C18:1n-9, however, it decreased C18:2n-6t in LD muscles. Both RP-RO and RP-CO increased PUFA in SM muscle. Beef marbling scores were increased significantly by RP-plant oil supplementation while the highest increase was found in beef of RP-RO cattle.

In Experiment II, twelve crossbred Wagyu beef steers, averaging 509±3.2 kg live weight and 28 mo old, were stratified by their LW into 3 groups. All steers were fed 7.5 kg/d of 12% CP concentrate with *ad libitum* rice straw and had free access to clean water. The treatments were 1) control concentrate, 2) supplemented with 100 g/d of RP-RO (100 RP-RO), and 3) supplemented with 200 g/d of RP-RO (200 RP-RO). This present study demonstrated that supplementation of rumen-protected rice bran oil (RP-RO) can increase C18:1n-9 and beef marbling scores.

In Experiment III, four fistulated cattle were assigned in 4 treatments in a 4×4 Latin square design. Treatments were 1) control concentrate, 2) control concentrate plus 200 g/d of RP-PO, 3) control concentrate plus 200 g/d of RP-RO, and 4) control concentrate plus 200 g/d of RP-CO. The results found that the proportion of ruminal C16: 0 in cattle fed RP-PO diet was significantly higher than those cattle fed RP-RO and RP-CO diets. The proportion of ruminal C18: 1n-9c was significantly higher in RP-RO cattle than in RP-PO and RP-CO cattle while the ruminal proportion of C18: 2n-6c was significantly higher in RP-CO cattle than RP-PO and RP-RO cattle.

In Experiment IV, three fistulated cattle were assigned in 3 treatments in a 3×3 Latin square design. The treatments were 1) control concentrate, 2) control concentrate plus 100 g/d of rumen protected rice bran oil (100 RP-RO), and 3) control concentrate plus 200 g/d of rumen protected rice bran oil (200 RP-RO). The results found that the concentration of C18:1n-9c, C18:2n-6, and *c9,t11*-C18:2 was increased while of C18:0 decreased when supplemented 200 g/d of rumen-protected rice bran oil compare to other treatments.

School of Animal Production Technology Student's Signature R. Mirattanaphrai

Academic Year 2017 Advisor's Signature [signature]

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Rattakorn Mirattanaphrai

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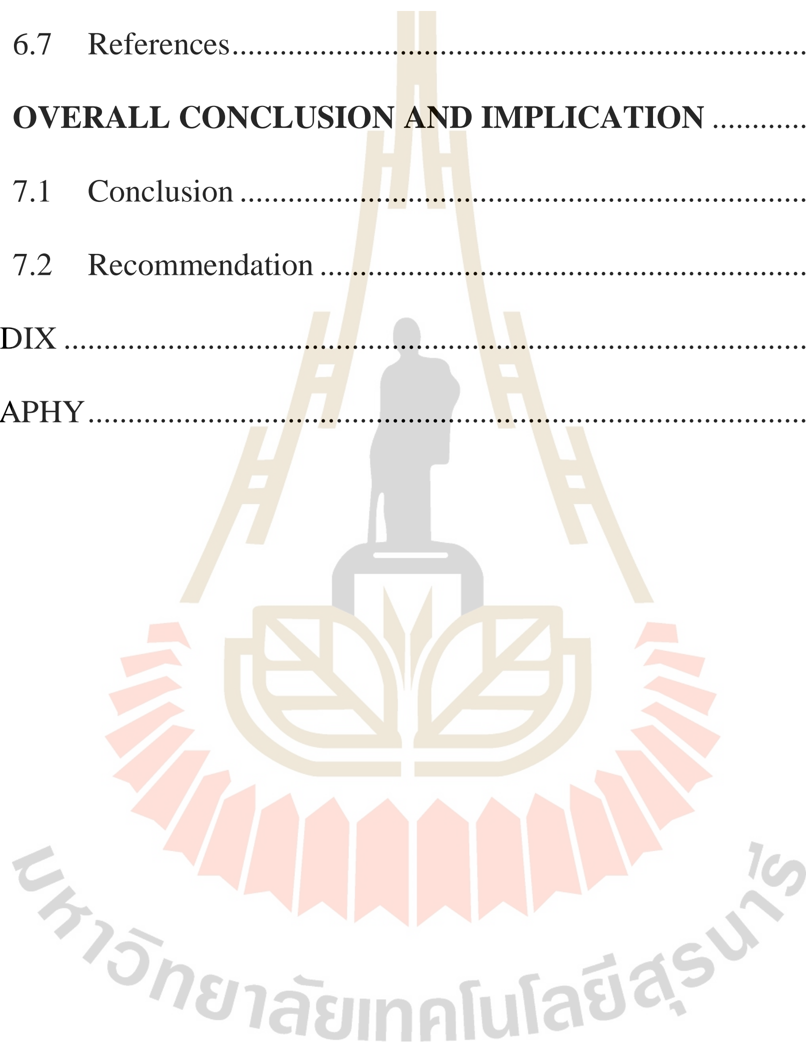

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LIST OF ABBREVIATIONS

ADF	=	Acid detergent fiber
ADICP	=	Acid detergent insoluble crude protein
ADIN	=	Acid detergent insoluble N
ADL	=	Acid detergent lignin
CLA	=	Conjugated Linoleic acids
C8:0	=	Caprylic acid
C10:0	=	Capric acid
C12:0	=	Lauric acid
C14:0	=	Myristic acid
C16:0	=	Palmitic acid
C16:1	=	Palmitoleic acid
C18:0	=	Stearic acid
C18:1n9t	=	Vaccenic acid
C18:1n9c	=	Oleic acid
C18:2n6t	=	Linolelaidic acid
C18:2n6c	=	Linoleic acid
C18:3n3	=	α -Linoleic acid
C20:1	=	Gondoic acid
C22:0	=	Behenic acid
C20:3n-6	=	dihomo-γ-linolenic acid
C20:4n-6	=	arachidonic acid

LIST OF ABBREVIATIONS (Continued)

C24:0	=	Lignoceric
HSL	=	hormone-sensitive enzymes lipase
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
PPAR	=	peroxisome proliferator-activated receptor
SCD	=	stearoyl-CoA desaturase
SREBP	=	sterol regulatory element binding protein

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CHAPTER I

INTRODUCTION

Consumers have valued highly marbled beef for nearly a century. Most consumers still prefer beef that is reasonably marbled and juicy. The amount of intramuscular fat or marbling deposited in longissimus muscle is a major determinant of carcass value and predictor of palatability. Marbling fat is comprised of over 20 individual fatty acids; however, six major fatty acids contribute over 92% of the total fatty acid content. These major fatty acids in beef marbling fat are: oleic, palmitic, stearic, linoleic, palmitoleic and myristic acids. The fatty acid primarily responsible for soft fat in cattle is oleic acid (18:1n-9). The concentration of oleic acid is also positively correlated with overall palatability of beef (Westerling and Hedrick, 1979), which may be related to fat softness. Stearic acid (18:0) is a primary determinant of fat hardness (Smith et al., 1998; Wood et al., 2004; Chung *et al*., 2006), so any dietary or production factor that enhances the conversion of stearic acid to oleic acid will increase fat softness.

Overall, the fatty acid composition of beef marbling fat is about 44% saturated fatty acids (SFA), 5% odd-chain fatty acids (OCFA), 45% monounsaturated fatty acids (MUFA), and 5% polyunsaturated fatty acids (PUFA) for beef marbling fat (Duckettet al., 1993). Human diets containing a high proportion of lipid as MUFA have been shown to be as effective as those containing high levels of PUFA at lowering serum cholesterol levels (Mattson and Grundy, 1985; Mensink and Katan, 1989; Gustafsson

et al., 1994). Stearic acid is a saturated fatty acid; however, diets high in stearic acid have been shown to lower serum cholesterol compared to other saturated fatty acids (Denke and Grundy, 1991; Bonanome and Grundy, 1988). Stearic acid is believed to be converted to oleic acid after dietary ingestion which accounts for its different effect on serum cholesterol compared to other saturated fats (Bonanome and Grundy, 1988). Research has demonstrated that high oleic acid ground beef may reduce risk factors for cardiovascular disease (Adams et al., 2010; Gilmore et al., 2011, 2013).

The beef quality is determined by FA composition of feedstuffs. Moreover, shelf-life, palatability, and nutritive value of beef are affected by FA composition in the beef. For instance, oleic acid seems to be beneficial for reducing plasma total cholesterol and total low-density lipoprotein cholesterol in humans (Bonanome and Grundy, 1988), and it contributes to better taste panel evaluations of cooked beef (Dryden and Marchello, 1970). Challenges in increasing oleic acid content of ruminant tissues and products are of interest. In addition to the issues of the effects of unsaturated fatty acids (UFAs) on the stability and sensory acceptability of products, these FAs inhibit various essential anaerobic bacteria of the rumen, especially those involved in fiber digestion, biohydrogenation of UFAs, and methanogenesis (Palmquist and Jenkins, 1980). Therefore, supplementation of palm oil (PO) and rice bran oil (RO) rich in C18:1n-9 would increase C18:1n-9 in muscle lipid.

Smith et al. (2010) demonstrated that oleic acid may have autocrine or paracrine effects in further stimulating marbling development and concluded that oleic acid is a critical factor in enhancing in marbling adipose tissue. Specific fatty acids in plasma or within the fat depots can promote marbling fat development while at the same time can cause muscle precursor cells to develop into marbling fat cells. Thus,

the objective of the present study was to examine the effect of rumen-protected plant oil supplementation on performance and beef fatty acid profile of Wagyu crossbred beef steers.

1.1 Research hypothesis

1.1.1 Supplementation of rumen protected fat containing oleic acid including palm oil,rice bran oil, and corn oil in fattening Wagyu Crossbred cattle may increase oleic acid accumulation in beef.

1.1.2 Supplementation of rumen protected fat containing oleic acid including palm oil,rice bran oil, and corn oil in fattening Wagyu Crossbred cattle may increase marbling score in beef.

1.2 Research objectives

1.2.1 To study the effects of rumen protected fat containing oleic acid including palm oil, rice bran oil, and corn oil supplementation on carcass and beef quality, and beef fatty acids profile in Wagyu Crossbred steers.

1.2.2 To study the effects of rumen protected fat containing oleic acid including palm oil,rice bran oil, and corn oil supplementation on rumen fermentation.

1.3 Scope of the study

These researches intended to examine the effect of rumen protected fat containing oleic acid including palm oil, rice bran oil, and corn oil supplementation on carcass and beef quality, beef fatty acids profile and ruminal fermentation in Wagyu Crossbred steers.

1.4 Expected results

1.4.1 Increase in oleic acid accumulation in beef may occur when supplemented with rumen protected fat containing oleic acid including palm oil, rice bran oil, and corn oil in fattening Wagyu Crossbred cattle diets.

1.4.2 Marbling score of beef may increase when supplemented with rumen protected fat containing oleic acid including palm oil, rice bran oil, and corn oil in fattening Wagyu Crossbred cattle diets.

1.5 References

Adams, T. H., Walzem, R.L., Smith, D.R., Tseng, S., and Smith,S.B. (2010). Hamburger high in total, saturated and trans-fatty acids decreases HDL cholesterol and LDL particle diameter, and increases plasma TAG, in mildly hypercholesterolaemic men. **British Journal Nutrition**. 103: 91-98.

Bonanome, A. and Grundy S.M. (1988). Effect of dietary stearic acid on plasma cholesterol and lipoprotein levels. **New England Journal of Medicein**. 318: 1244.

Chung, K. Y., Lunt, D.K., Choi, C.B., Chae, S.H., Rhoades, R.D., Adams, T.L., Booren, B., and Smith, S.B. (2006). Lipid characteristics of subcutaneous adipose tissue and M. longissimus thoracis of Angus and Wagyu steers fed to U.S. and Japanese endpoints. **Meat Science**. 73: 432-441.

Denke, M.A. and Grundy, S.M. (1991). Effects of fats high in stearic acid on lipid and lipoprotein concentrations in men. **American Journal Clinical Nutrition**. 54: 1036.

Dryden, F.D. and Marchello, J.A. (1970). Influence of total lipid and fatty acid composition upon the palatability of three bovine muscles. **Journal of Animal Science**. 31: 36.

Duckett, S. K., Wagner, D.G., Yates, L.D., Dolezal, H.G., and May, S.G. (1993). Effects of time on feed on beef nutrient composition. **Journal of Animal Science**. 71: 2079.

Gilmore, L. A., Walzem, R.L., Crouse, S.F., Smith, D.R., Adams, T.H., Vaidyananthan, V., Cao, X., and Smith, S.B. (2011). Consumption of high-oleic acid ground beef increases HDL cholesterol concentration but both high- and low-oleic acid ground beef decrease HDL particle diameter in normocholesterolemic men. **Journal of Nutrition**. 141: 1188-1194.

Gilmore, L. A., Crouse, S.F., Carbuhn, A., Klooster, J., Calles, J.A.E., Meade, T., and Smith, S.B. (2013). Exercise attenuates the increase in plasma monounsaturated fatty acids and high-density lipoprotein but not high-density lipoprotein 2b cholesterol caused by high-oleic ground beef in women. **Nutrition Research**. 33: 1003-1011.

Gustafsson, I.B., Vessby, B., Ohrvall, M., AndNydahl, M. (1994). A diet rich in monounsaturated rapeseed oil reduced the lipoprotein cholesterol concentration and increases the relative content of n-3 fatty acids in serum in hyperlipidemicsubjects. **American Journal Clinical Nutrition**. 59: 667.

Smith, S. B., Yang, A., Larsen, T.W., and Tume, R.K. (1998). Positional analysis of triacylglycerols from bovine adipose tissue lipids varying in degree of unsaturation. **Lipids**. 33: 197-207.

มหาวิทยาลัยเทคโนโลยีสุรนารี

Smith, S. B., Johnson, B., and Doumit, M. (2010). Regulation of marbling development in beef cattle by specific fatty acids. **Project Summary**, National Cattlemen's Beef Association.

Mattson, F.H. and Grundy, S.M. (1985). Comparison of effects of dietary saturated, monounsaturated, and polyunsaturated fatty acids on plasma lipids and lipoproteins in man. **Journal of Lipid Research**. 26: 194.

Mensink, R.P. and Katan, M.B. (1989). Effect of a diet enriched with monounsaturated or polyunsaturated fatty acids on levels of low-density and high-density lipoprotein cholesterol in healthy women and men. N. **Engl. J. Med**., 321: 436.

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

Westerling, D.B. and Hedrick, H.B. (1979). Fatty acid composition of bovine lipids as influenced by diet, sex and anatomical location and relationship to sensory characteristics. **J. Anim. Sci**., 48: 1343.

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

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CHAPTER II

LITERATURE REVIEW

The current situation of beef production is raising cattle to produce premium beef. The price of cattle is measured by using the level of beef marbling score. Although the consumption of beef in some consumers will decrease due to concerns about the high fat content in beef cattle, especially saturated fatty acids. However, the quality of fattening cattle has reduced the amount of SFA while the MUFA increased. The increase in marbling scores is found in cattle receiving unsaturated fatty acids from feed in the form of oilseeds, protected oil supplements or vegetable oil. Feeding unsaturated fatty acids from the diet increases the unsaturated fatty acids avoiding the bio-hydrogenation process in the rumen for increased absorption in marbling fat. Therefore, in order to increase marbling accumulation in the period of fattening cattle must improve feed to increase the flow of unsaturated fatty acids to the intestinal tract by reducing the bio-hydrogenation process in the rumen or increase the concentration of unsaturated fatty acids in feed. This research aims to study the supplementation of rumen protected fat containing high oleic acid supplementation including palm oil, rice bran oil, and corn oil on carcass quality, sensory evaluation and fatty acid profile of beef from crossbred Wagyu beef steers.

2.1 Marbling fat

Intramuscular fat or marbling is the appearance of fat that is inserted into the muscular band visible to the naked eye. Spreading within the muscle, the accumulation of marbling in different parts of the body will rise from the head to the end carcass as the body fat stores in the last order but it will be applied before the body. The amount of intramuscular fat or marbling deposited in longissimus muscle is a major determinant of carcass value and predictor of palatability. Marbling fat is comprised of over 20 individual fatty acids; however, six major fatty acids contribute over 92% of the total fatty acid content. These major fatty acids in beef marbling fat are: oleic, palmitic, stearic, linoleic, palmitoleic and myristic acids. The fatty acid primarily responsible for soft fat in cattle is oleic acid (18:1n-9). The concentration of oleic acid is also positively correlated with overall palatability of beef (Westerling and Hedrick, 1979), which may be related to fat softness. Stearic acid (18:0) is a primary determinant of fat hardness (Smith et al., 1998; Wood et al., 2004; Chung et al., 2006), so any dietary or production factor that enhances the conversion of stearic acid to oleic acid will increase fat softness. Marbling measurements will be used to measure the tenderness of the ribs between 12 and 13, with the threshold marbling being used varies according to the association or country, such as Japan. 12 levels. Level 1 is the lowest fat level, Level 12 is the level with the highest fat level. The United States is used at six levels, Thailand will use the standard fat level inserted 5 levels.

Marbling is caused by the adipocyte lipogenesis process. Triglycerides in adipose tissue are hydrolyzed with 3 free fatty acids + glycerol. This process is caused by hormone-sensitive enzymes lipase (HSL), which is regulated by insulin and catecholamines to stimulate beta-adrenergic receptors of adipocytes, the insulin will

inhibit the lipolysis or fat burning process (García-Escobar et al., 2008). It makes fat cells by increasing the number of cells (Hyperplasia) then the pre-adipocytes will expand. Hypertrophy is a form of fat that we can see with the naked eye, also known as mature adipocytes.

The fatty acid composition of beef marbling fat is about 44% saturated fatty acids (SFA), 5% odd-chain fatty acids (OCFA), 45% monounsaturated fatty acids (MUFA), and 5% polyunsaturated fatty acids (PUFA) for beef marbling fat (Duckett et al., 1993). Human diets containing a high proportion of lipid as MUFA have been shown to be as effective as those containing high levels of PUFA at lowering serum cholesterol levels (Mattson and Grundy, 1985; Mensink and Katan, 1989; Gustafsson et al., 1994). Stearic acid is a saturated fatty acid; however, diets high in stearic acid have been shown to lower serum cholesterol compared to other saturated fatty acids (Denke and Grundy, 1991; Bonanome and Grundy, 1988). Stearic acid is believed to be converted to oleic acid after dietary ingestion which accounts for its different effect on serum cholesterol compared to other saturated fats (Bonanome and Grundy, 1988). Research has demonstrated that high oleic acid ground beef may reduce risk factors for cardiovascular disease (Adams et al., 2010; Gilmore et al., 2011, 2013).

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unsaturated fatty acids (UFAs) on the stability and sensory acceptability of products, these FAs inhibit various essential anaerobic bacteria of the rumen, especially those involved in fiber digestion, biohydrogenation of UFAs, and methanogenesis (Palmquist and Jenkins, 1980). Therefore, supplementation of palm oil (PO) and rice bran oil (RO) rich in C18:1n-9 would increase C18:1n-9 in muscle lipid.

Smith et al. (2010) demonstrated that oleic acid may have autocrine or paracrine effects in further stimulating marbling development and concluded that oleic acid is a critical factor in enhancing in marbling adipose tissue. Specific fatty acids in plasma or within the fat depots can promote marbling fat development while at the same time can cause muscle precursor cells to develop into marbling fat cells. Thus, the objective of the present study was to examine the effect of rumen-protected plant oil supplementation on performance and beef fatty acid profile of Wagyu crossbred beef steers.

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

Feeds	Fatty acids (g /100 g fatty acids)					
	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

Table 2.2 Effects of plant oil containing oleic acid supplementation on Marbling score.

References	Treatment	Oleic acid contain (% of Total FA)	Level (%Of total feed)	Period (Days)	Marbling score[*]
Archibeque et al.(2005)	WCS[*] 0%	24.2	0%	107	4.91[a]
	WCS 5%	22.9	5%	107	4.10[b]
	WCS 15%	21.2	15%	107	4.05[b]
Lee et al.(2003)	Control	-	0	45	5.52
	Oleamide	200g/d	73.1	45	4.29
	Ca-oleate	200g/d	74.5	45	3.67
	Control	-	-	90	4.75
	Oleamide	200g/d	73.1	90	5.18
	Ca-oleate	200g/d	74.5	90	5.58
Felton et al.(2004)	Control	-	0	76	4.66[b]
	Bin-run[1]	28.1	16	76	4.92[a]
	High oleic acid[2]	54.6	16	76	4.91[a]
	Choice white grease[3]	30.1	3.93	76	4.90[a]
Gillis et al.(2004)	Control	-	0	60	5.40[b]
	Corn oil[4]	27	4	60	5.80[a]
	Rumen protected CLA salt	-	2	60	5.30[b]
Andraeet al.(2001)	Tropical corn		82.2	84	5.20[b]
	High oil corn[5]	35.01	81.9	84	5.67[a]
	Isacaloric[6]	33.32	74.0	84	5.25[b]

Table 2.3 Effects of plant oil containing oleic acid supplementation on beef fatty acid profile.

References	Treatment	Fatty acid profile									
		C14:0	**C15:0**	**C16:0**	**C17:0**	**C18:0**	**C18:1**	**C18:2**	**SFA**	**MUFA**	**PUFA**
		(mg/g of fresh tissue)									
Ludden et al.(2009)	Control	1.07	0.21	10.0	0.42^{a}	4.98	15.5^{a}	1.23	16.7	18.6	1.79
	Soybean oil (60 d)	1.08	0.23	9.8	0.32^{ab}	5.02	14.6^{a}	1.48	16.4	18.3	2.05
	Soybean oil (120d)	1.02	0.17	8.9	0.29^{b}	5.04	12.3^{b}	1.46	15.5	15.7	1.97
	Soybean oil (180d)	0.90	0.14	7.6	0.23^{b}	4.47	10.7^{c}	1.56	13.4	14.1	1.95
		(% of total fatty acid)									
Felton et al.(2004)	Control	2.32	-	27.9	1.28	14.6	46.0	1.75	46.0	51.6	2.45
	Bin-run	2.48	-	26.6	1.17	15.1	45.4	3.41	45.3	50.7	4.17
	High oleic acid	2.35	-	26.6	1.05	13.5	48.7	1.63	43.5	54.3	2.28
	Choice white	2.40	-	27.5	1.24	15.3	46.8	1.37	46.3	51.9	1.67

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Table 2.3 Effects of plant oil containing oleic acid supplementation onbeef fatty acid profile (continue).

References	Treatment	Fatty acid profile (% of total fatty acid)									
		C14:0	C15:0	C16:0	C17:0	C18:0	C18:1	C18:2	SFA	MUFA	PUFA
		(mg/g of fresh tissue)									
Lee et al. (2003)	Control (45d)	-	-	-	-	-	50.81	2.24^{b}	41.93	55.25	2.81^{b}
	Oleamide(45d)	-	-	-	-	-	51.58	2.65^{ab}	40.51	56.20	3.28^{ab}
	Ca-oleate(45d)	-	-	-	-	-	48.00	3.38^{a}	57.87	53.69	4.18^{a}
	Control(90d)	-	-	-	-	-	51.16	2.20^{b}	40.66^{a}	56.25^{b}	3.09^{b}
	Oleamide(90d)	-	-	-	-	-	52.83	3.77^{a}	34.95^{b}	60.77^{a}	4.28^{a}
	Ca-oleate(90d)	-	-	-	-	-	50.90	3.96^{a}	36.94^{b}	58.46^{a}	4.59^{a}
Andrae et al.(2001)	Tropical corn	2.85	0.39^{ab}	26.94^{a}	0.97^{b}	16.90	39.99	3.78^{b}	46.41^{a}	43.35	4.69^{b}
	High oil corn	2.75	0.44^{a}	25.78^{b}	1.12^{a}	15.93	39.92	4.63^{a}	44.30^{b}	43.57	5.86^{a}
	Isacaloric	2.46	0.35^{b}	25.36^{b}	0.93^{b}	17.30	40.05	4.33^{a}	45.09^{a}	43.41	5.53^{a}

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Felton et al. (2004) found that dietary supplementation of oleic acid could significantly increase marbling in beef cattle (Table 2.2) which is similar to the results of Gillis et al., (2004) using corn oil with 27% oleic acid. Andrae at al., (2001) using high oil corn with high oleic fatty acid content can increase marbling in beef cattle. Thus, the supplementation of oleic acid can increase the marbling in cattle

Lee et al. (2003) and Andrae at al. (2001) found that the levels of PUFA and linoleic acid were significantly increased. In all experiments, stearic fatty acid was not significantly different. while Felton at al., (2004) found no significant differences in all parameters measured. However, the marbling score was significantly increased (Table 2.3).

2.2 Rumen bypass or protected fat

Rumen bypass or "protected" fats are essentially dry fats processed to be easily handled and mixed into all animal feeds. Because dry fats naturally have high melting points, they are mostly insoluble at rumen body temperature. In essence, dry fats are not as much "protected" as completely insoluble in the rumen, so they have small impacts on rumen fermentation. Today, there are only three methods of producing dry fats for animal feeds. The method that produces the least desirable product for the cow, partial hydrogenation of tallow, is seldom used for dairy rations and will not be discussed further. One acceptable method for producing a bypass fat is to hydrolyze the fatty acids from palm oil or tallow, partially hydrogenate them and then spray-chill them in a tower to form fatty acid prills.

The dominant bypass fat technology is still the reaction of vegetable fatty acids with calcium oxide to form insoluble calcium soaps. Within the feed industry, these

calcium soaps, or salts, appear on feed labels as “calcium salts of long-chain fatty acids.” Because free fatty acids are a major byproduct of producing palm oil for human food, almost all calcium salts produced worldwide come from palm oil fatty acids.

Because large amounts of dietary fat will eventually reduce feed intake of any animal species, feeding guidelines for feeding bypass fats are generally in the range of 200 to 600 grams per cow daily. Performance goals and ration cost will generally be the dominant factors in determining how much bypass fat to feed.

2.3 Type of rumen protected fat

2.3.1 Natural bypass fat

Whole oil seeds, when fed without processing except drying have natural bypass fat properties due to their hard outer seed coat, which protects the internal fatty acids from lipolysis and bio-hydrogenation in rumen (Ekeren et al., 1992). However, during mastication by animals there is physical breakdown of seed coat, which gives poor result of rumen inertness. Important whole oil seeds commonly used in the ration of dairy animals are cotton, roasted soybeans, sun flower and canola. Further, feed ingredients containing saturated fatty acids are less toxic to the ruminal microorganisms and minimize the adverse effects of the fat supplementation as they react more readily with the metal ions forming insoluble salts in rumen (Jenkins and Palmquist, 1982) and do not go for further ruminal bio-hydrogenation (Chalupa et al., 1986).

Table 2.4 Fatty acids composition Ca salts of long-chain FA, Sunflower seeds, Flaxseed, Canola seeds.

Ingredient	Ingredient			
	Ca salts of long-chain FA	**Sunflower seeds**	**Flaxseed**	**Canola seeds**
OM, %	78.3	96.5	96.5	95.3
GE, Mcal/kg	8.22	6.71	6.52	6.63
CP, %	0	17.2	26.4	30.7
NDF, %	-	24.1	25.1	14.1
ADF, %	-	19.3	11.3	12.0
NFC, %	-	22.0	8.5	14.8
NE_L, Mcal/kg (calculated)	-	-	-	-
Crude fat, %	78.3	41.8	39.6	39.7
Added fat, % (calculated)	-	-	-	-
FA, g/100 g of FA				
C14:0	1.4	0.1	0.1	0.1
C16:0	48.5	7.0	5.9	4.4
C18:0	4.0	4.1	3.9	1.8
C18:1 n-9 *cis*	35.8	17.9	16.7	56.5
C18:1 n-7 *cis*	1.0	0.74	0.8	4.0
C18:2 n-6 *cis*	9.0	70.1	19.9	22.1
C18:3 n-3	0.3	0.1	52.8	11.1

Beauchemin et al., (2009)

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2.3.2 Chemically prepared bypass fat

Chemically prepared bypass fat mainly includes crystalline or prilled fatty acids,formaldehyde treated protein encapsulated fatty acids, fatty acyl amides and calcium salts of long chain fatty acids (Ca-LCFA).

2.3.2.1 Crystalline or prilled fatty acids

Crystalline or prilled fatty acids can be made by liquifying and spraying the saturated fatty acids under pressure into cooled atmosphere, so that melting point of the fatty acids is increased and do not melt at ruminal temperature, thus resisting rumen hydrolysis and association with bacterial cells or feed particles (Chalupa et al., 1986).

2.3.2.2 Formaldehyde treated protein encapsulated fatty acids

Formaldehyde treated protein encapsulated fatty acids is also an affecting means of protecting dietary fat from rumen hydrolysis (Sutton et al., 1983). Casein-formaldehyde-coated fat has been used by the earlier workers (Bines et al., 1978). Oil seeds can be crushed and treated with formaldehyde (1.2 g per 100g protein) in plastic bags or silos and kept for about a week.

2.3.2.3 Fatty acyl amide

Fatty acyl amide can be prepared and used as a source of bypass fat. Butylsoyamideis a fatty acyl amide consisting of an amide bond between soy fatty acids and a butylamine, which increases linoleic acid content of the milk fat (Jenkins, 1998). Conversion of oleic acid to fatty acyl amide (oleamide) increases the mono-unsaturated fatty acids concentration of the milk, when fed to dairy cows (Jenkins, 1999). Amide of soybean FA is effective in enhancing the post-ruminal flow of oleic acid (Lundy et al., 2004). Fatty acyl amide of sardine oil based complete diet is

effective in protecting fat from degradation in rumen and improves the apparent and true dry matter degradability (Ambasankar and Balakrishnan, 2011).

2.3.2.4 Calcium salts of long chain fatty acids

Calcium salts of long chain fatty acids (Ca-LCFA) are insoluble soaps produced by reaction of carboxyl group of long chain fatty acids (LCFA) and calcium salts (Ca++). Degree of insolubility of the Ca soaps depends upon the rumen pH and type of fatty acids. When rumen pH is more than 5.5, Ca-LCFA is inert in rumen. As dissociation constant (pKa) of Ca-LCFA is 4 to 5, dissociation is significant, when pH decreases to 6.0 (Chalupa et al., 1986). In acidic pH of the abomasum, fatty acids is dissociated from Ca-LCFA and then absorbed efficiently from small intestine. The unsaturated soaps are less satisfactory for maintaining normal rumen function, because dissociation is relatively higher (Sukhija and Palmquist, 1990). Among all forms of bypass fat, Ca-LCFA is relatively less degradable in rumen (Elmeddah et al., 1991), has highest intestinal digestibility (Dairy Technical Service Staff, 2002) and serve as an additional source of calcium (Naik et al., 2007a; 2007b).

2.4 Lipid metabolism of ruminants

The lipid metabolism of ruminants comprises a raft of divergent processes that can roughly be classified into processes on the systemic and cellular level. The starting point of the systemic lipid metabolism is the dietary intake of feedstuffs, which is followed by an extensive ruminal de-esterification and biohydrogenation of dietary lipids as well as the formation of short-chain fatty acids from dietary fiber compounds and fermentable carbohydrates, an absorption of fatty acids and fatty acid precursors,

and further digestion/absorption processes of ruminally unaffected/protected lipids along the intestine (Bauchart 1993; Doreau and Chilliard 1997; Bauman et al. 2003; Lock et al. 2006; Nafikov and Beitz 2007; Scollan et al. 2014). Upon absorption, fatty acids and fatty acid precursors are transported to target tissues for further metabolization, syntheses, deposition and/or excretion (Hocquette and Bauchart 1999; Dodson et al. 2010a).

The cellular lipid metabolism constitutes the basis of the systemic lipid metabolism and comprises a cellular uptake of fatty acids and fatty acid precursors via transport/hydrolysis systems, a cellular de novo fatty acid/triglyceride synthesis, an intracellular lipid storage in specialised organelles (lipid droplets), a cellular lipid breakdown and fatty acid oxidation, cellular transport processes, syntheses and excretions, as well as effects of fatty acids and lipids on the cellular gene expression regulation (Hausman et al. 2009; Dodson et al. 2010a, 2013; Walther and Farese 2012; Wójcik et al. 2014).

2.4.1 Lipid metabolism and ruminant performance

Regarding performance traits, the lipid metabolism of ruminants has been reported to strongly impact meat and milk quality and quantity. In meat animals, the systemic/cellular lipid metabolism regulation shapes the development and differentiation of tissues by (1) determining the partitioning and tissue-specific deposition of lipids/fatty acids and (2) affecting the differentiation stage and number of cells of the adipogenic, myogenic and further lineages, whose numbers are, to some extent, predetermined [by genetics (Jurie et al. 2007) and prenatal nutrition (Du et al. 2013)], but whose differentiation is, to a great extent, affected by feeding and keeping conditions (Scollan et al. 2014).An envisaged ‘selective lipid accumulation, i.e. lipid

deposition in intramuscular fat depots (which is the basis for marbling development: Webb and O'Neill 2008) to the disadvantage of subcutaneous and visceral lipid stores, has proved to be challenging, since (due to an antagonism between muscle and adipose tissue growth: Bonnet et al. 2010). IMF depots develop comparatively late and, moreover, marbling adipocytes exhibit a minor potential for proliferation and adipogenic differentiation than subcutaneous adipocytes (Grant et al. 2008), display low rates of lipogenic enzyme activities, and utilise different lipogenic pathways as they prefer glucose/ lactate instead of acetate as lipogenic substrates (for review, see Hocquette et al. 2010, and Dodson et al. 2010a). Anyway, fatty acids, lipids and further PPARg agonists have been shown to exert the potential to recruit undifferentiated stromal-vascular cells in muscle tissue for lipid storage, and studies also suggest that lipids can induce a trans differentiation of muscle cells (e.g. satellite cells and myoblasts) into lipid-filled adipose-like cells (Lee et al. 2012).

Besides the direct uptake and accumulation of fatty acids that reach the tissues, most pronounced effects of fatty acids/ lipids are mediated via their effects on gene expression, preferably by their ligation with nuclear transcription factors (PPARs, SREBPs, CEBPs), among which especially PPARs have a central role (for review, see Bionaz et al. 2013). Upon ligation, activated nuclear receptors bind to specific regions of target genes encoding, for example, lipogenic enzymes, lipid storage-associated proteins, carbohydrate–lipid metabolism bridging proteins and mitochondrial/b-oxidation proteins. Unsaturated fatty acids (UFA) (especially LC-PUFA) are preferred ligands for PPARs (Bionaz et al. 2013) and induce associated downstream pathways, whereas they inhibit the expression of SREBF1 and lipogenesis-related downstream genes (Georgiadi and Kersten 2012; Nakamura et al. 2014), especially the expression

of enzymes catalysing a fatty acid de novo synthesis (acetyl-CoA carboxylase a, fatty acid synthase) and desaturation (stearoyl-CoA desaturase) (Hiller et al. 2013; Shingfield et al. 2013).The resulting lipid metabolism endpoints (i.e. fat content, fatty acid profile and fat distribution of tissues) are the major determinants of the sensory (flavor, juiciness, tenderness and consumer appeal), nutritional (health-promoting fatty acids, fat soluble vitamin and antioxidants) and techno-functional (shelflife stability, water-binding capacity, cooking loss) properties of tissue-derived food products (Webb and O'Neill 2008; Hocquette et al. 2010) as well as the meat price (Polkinghorne and Thompson 2010), linking tissue biology and economically relevant aspects.

2.4.2 Bio-hydrogenation of fatty acids in the rumen

Feeding management and rumen ecology have influenced on ruminal hydrogenation. Generally, the last step of bio–hydrogenation is to produce *t11*-C18:1. The abnormal stage in rumen particularly greater acidity in the rumen, smallest *t10* -C18:1 production could be produced when the cow received high amount of concentrate (Jenkins et al., 2008). The lower ruminal pH enhanced the growing of *Bifidobacterium*, *Propionibacterium*, *Lactococcus*, *Streptococcus* and *Lactobacillus*. The production of *t10*-C18:1 in the rumen when absorbed in lower gut can synthesized *t10,c12*-CLA or *t10* shift in hydrogenation pathways (Hinrichsen et al., 2006). Production of *t10*-C18:1 in the rumen can indicate the involute change in fatty acids in hydrogenation process.*B. fibrisolvens* and *B. proteoclasticum* have the ability to complete hydrogenation of C18:2n-6 into *c9,t11*-C18:2, *t9,t11*-C18:2 and *t11*-C18:1 as the final step (Mckain et al., 2010). However, *B. fibrisolvens* and *C. proteoclasticum* cannot convert *t11*-C18:1 into C18:0, only *P.acnes* can produce C18:0 as the final step

of bio-hydrogenation (Scollan et al., 2001; Bauman et al., 2000).The C18:2n-6 will be isomerized into *c9, t11*-C18:2 and reduced into *t11*-C18:1. Maia et al. (2010) suggested that C18:3n-3 is more toxic than C18:2n-6 especially gram-positive bacteria. The highest growing of *B.fibrisolvens* on C18:2n-6 was at 10 h, however, the highest growing of *B.fibrisolvens* on C18:3n3 was at 37 h. This is related to the toxic of two fatty acids. *B. fibrisolvens* and *B. proteoclasticus* are the main bacteria involved in bio-hydrogenation process especially *t10, c12*-C18:2 into *t10*-C18:1, and *t9,t11*-C18:2; *c9,t11*-C18:2 into *t11*-C18:1 by reductase enzyme. After producing *t11*-C18:1, *B. proteoclasticus* and *P.acnes* will add H-atom in carbon chain and change unsaturated to saturated FAs.

Bio-hydrogenation of *c9, t11*-C18:2; *t9, t11*-C18:2; *t10, c12*-C18:2 as the substrate by *B. fibrisolvens* found that *B. fibrisolvens* can convert 0.22 mg/l *c9,t11*-C18:2 into 30.64 mg/l *t11*-C18:1 (McKain et al., 2010). *B. fibrisolvens*, group A bacteria, are inefficient to convert those FAs into C18:0, only group B bacteria particular *B. proteoclasticus* and *P. acnes* can change *t10*-C18:1 and *t11*-C18:1 into C18:0.

2.4.3 Oleic acid deposition in the intramuscular fat

The major lipid class in adipose tissue (>90%) is triacylglycerol or neutral lipid. In muscle, a significant proportion is phospholipid, which has a much higher PUFA content in order to perform its function as a constituent of cellular membranes.

Oleic acid (C18:1cis-9) is the major fatty acid in meat, was much more predominant in neutral lipid. This fatty acid is formed from stearic acid (C18:0) by the enzyme stearoyl Co-A desaturase, a major lipogenic enzyme. On the other hand,

C18:2n-6 was at much higher proportions in phospholipid than neutral lipid. The proportion of C18:3n-3 was slightly higher in neutral lipid than phospholipid in pigs but in sheep and cattle the proportions were higher in phospholipid. The differences between sheep and cattle for C18:2n-6, C18:3n-3 and the long chain n-6 and n-3 PUFA. In the work with sheep, dried grass (high in 18:3n-3) formed 75% of the concentrate whereas in the cattle study the concentrate contained a high proportion of full fat soyabean meal, high in 18:2n-6. Nevertheless, we have often seen higher values for individual phospholipid PUFAs in sheep compared with cattle.

The overall fat content of the animal and muscle have an important impact on proportionate fatty acid composition because of the different fatty acid compositions of neutral lipid and phospholipid. Phospholipid is an essential component of cell membranes and its amount remains fairly constant, or increases little, as the animal increases in fatness. In young lean animals, genetically lean animals or animals fed a low energy diet, the lower 18:1cis-9 and higher 18:2n-6 content of phospholipid has a major influence on total muscle fatty acid composition. But as body fat increases, neutral lipid predominates in overall fatty acid composition.

The changes in adipose tissue fatty acid composition with age and fatness are different between pigs and cattle. Leat (1975) examined fatty acid composition in subcutaneous fat of Jersey cattle of different sexes using biopsies at different ages. Both 16:0 and 18:0 fell in proportion a sage increased from 3 to 30 months, whereas 18:1cis-9increased, similar to the observation in pigs. In a comparison of extremes, Wood (1984) found proportions of 14.7% and 2.7% for 18:0 and 41.5% and 56.4% for 18:1cis-9 in a young heifer and an old fat steer respectively.

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2.4.4 Fat supplementation and beef marbling score

Dietary lipid sources containing particular fatty acids can be used to influence meat fatty acid composition. Fat in diets will increase the amount of unsaturated fat supplied and increase the energy density of the diet. An increased dietary energy density may alter the pattern of lipid deposition in steers fed finishing diets. Although supplementing diets with tallow usually does not alter marbling scores (Haaland et al., 1981; Bartle et al., 1994; Krehbiel et al., 1995),feeding full-fat soybeans to steers has increased quality grade and marbling scores (Rule et al., 1994; Felton and Kerley, 1998).

Smith et al. (2010) demonstrated that oleic acid may have autocrine or paracrine effects in further stimulating marbling development and concluded that oleic acid is a critical factor in enhancing in marbling adipose tissue. Specific fatty acids in plasma or within the fat depots can promote marbling fat development while at the same time can cause muscle precursor cells to develop into marbling fat cells.

2.5 Energy and protein requirement of beef cattle

2.5.1 Energy requirement

2.5.1.1 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°C to 17.5°C.

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

2.5.1.2 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.

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

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.

2.5.1.4 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.

2.5.2 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).

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

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.

$$MP = 0.64\ DIP \text{ (NRC, 2000)}$$

$$MP = 0.80\ UIP \text{ (NRC, 2000)}$$

2.5.2.1 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 :

$$\text{BCP (g/d)} = 6.25\ \text{TDN (kg intake/day)} \times (8.63 + (14.6 \times \text{forage intake}) - (5.18 \times \text{forage intake})^2 + (0.59 \times \text{concentrate intake}))$$

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

$$\text{BCP (g/d)} = 6.25 \times (-31.86 + 26.12\ \text{TDN (kg intake/day)})$$

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 non protein 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.

$$RDP = BCP \text{ (NRC, 2000)}$$

2.5.2.2 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

$$0.13 \text{ TDN} = \text{BCP}$$

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

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

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

2.5.2.3 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,

$$\text{Percent efficiency of MP to NP} = 83.4 - (0.114 \times \text{EQEBW}), \text{ otherwise } 49.2,$$

$$\text{EQSBW} = \text{SBW} \times (\text{SRW}/\text{FSBW})$$

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) :

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

2.6 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. **Journal of Dairy Science.** 90: 2897-2904.

Adams, T. H., Walzem, R.L., Smith, D.R., Tseng, S., and Smith,S.B. (2010). Hamburger high in total, saturated and trans-fatty acids decreases HDL cholesterol and LDL particle diameter, and increases plasma TAG, in mildly hypercholesterolaemic men. **British Journal Nutrition**. 103: 91-98.

Ambasankar, K. and Balakrishnan, V. (2011). Influence of protected sardine oil on in vitro rumen fermentation and nutrient digestibility of complete diet. **Indian Journal of Animal Science**. 81: 84-86.

Andrae, J. G., Duckett, S.K., Hunt,C.W., Pritchard,G.T., and Owens F.N. (2001). Effect of high oil corn feeding on carcass traits, tenderness, and fatty acid composition of feedlot steers. **Journal of Animal Science.** 79:582-588.

Bartle, S. J., Preston,R.L., and Miller,M.F. (1994). Dietary energy source and density: Effects of roughage source, roughage equivalent, tallow level, and steer type on feedlot performance and carcass characteristics. J. Anim. Sci. 72:1943-1953.

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

Bauman D.E., Perfield J.W., de Veth M.J., and Lock A.L. (2003) .New perspectives on Lipiddigestion and metabolism in ruminants. **Proceedings of the Cornell Nutrition Conference.** 175-189.

Bauman, D. E., and Lock, A. L. (2006). Concepts in lipid digestion and metabolism in dairy cows. **Proceedings of Tri-State Dairy Nutrition Conference.** 1-14.

Bines, J.A., Brumby, P.E., Storry, J.E., Fulford, R.J. and Braithwaite, G.D. 1978. **Journal of Agriculture Science.** 91: 135-150.

Bonanome, A. and Grundy S.M. (1988). Effect of dietary stearic acid on plasma cholesterol and lipoprotein levels. **New England Journal of Medicein**. 318:1244.

Bionaz M, Chen S, Khan MJ,and Loor JJ (2013) Functional role of PPARs in ruminants: potential targets for fine-tuning metabolism during growth and lactation. **PPAR Research 2013**. Article ID 684159.

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

Chalupa, W., Vecchiarelli B., Elser, A.E., Kronfeld, D.S., Sklan, D., and Palmquist, D.L. (1986).Ruminal fermentation *in vivo* as influenced by long chain fatty acids. **Journal of Dairy Science.**69: 1293-1301.

Chung, K. Y., Lunt, D.K., Choi, C.B., Chae, S.H., Rhoades, R.D., Adams, T.L., Booren, B., and Smith, S.B. (2006). Lipid characteristics of subcutaneous adipose tissue and M. longissimus thoracis of Angus and Wagyu steers fed to U.S. and Japanese endpoints. **Meat Science.** 73:432-441.

Dryden, F.D. and Marchello, J.A. (1970). Influence of total lipid and fatty acid composition upon the palatability of three bovine muscles. **Journal of Animal Science.** 31:36.

Denke, M.A. and Grundy, S.M. (1991). Effects of fats high in stearic acid on lipid and lipoprotein concentrations in men. **American Journal Clinical Nutrition.**

54:1036.

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 Chilliard, Y. (1997). Digestion and metabolism of dietary fat in farm animals. **The British Journal of Nutrition .**78: 15-35.

Duckett, S. K., Wagner, D.G., Yates, L.D., Dolezal, H.G., and May, S.G. (1993). Effects of time on feed on beef nutrient composition. **Journal of Animal Science.**71:2079.

DodsonMV, Hausman GJ, Guan LL, DuM, Rasmussen TP, Poulos SP,Mir P, BergenWG, FernyhoughME,McFarland DC,RhoadsRP, SoretB, Reecy JM, Velleman SG, and Jiang Z (2010a) Lipid metabolism, adipocyte depot physiology and utilization of meat animals as experimental models for metabolic research. **International Jour of Biological Science.** 6: 691-699.

Du M, Huang Y, Das AK, Yang Q, Duarte MS, Dodson MV, and Zhu MJ (2013) Manipulating mesenchymal progenitor cell differentiation to optimize performance and carcass value of beef cattle. **Journal of Animal Science.** 91, 1419-1427.

Ekeren, P.A., Smith, D.R., Lunt, D.K. and Smith, S.B. 1992. Ruminal biohydrogenation of fatty acids from high oleate sunflower seeds. **Journal of Animal Science.**70: 2574-2580.

Elmeddah, Y., Doreau, M. and Michalet-Doreau, B. 1991. Interaction of lipid supply and carbohydrate in the diet of sheep with digestibility and ruminal digestion. **Journal of Agriculture Science.** 116: 437-445.

Felton, E. E. D., and Kerley,M.S.(1998). Effects of feeding whole soybeans on feedlot

steer performance and carcass characteristics. **Journal of Animal Science.** 76:76.

Felton E. E. D. and M. S. Kerley. 2004. Performance and carcass quality of steers fed different sources of dietary fat. **Journal of Animal Science.** 82:1794-1805.

García-Escobar, E., Soriguer,F., García-Serrano,S., Gómez-Zumaquero,JM.,Morcillo,S., Haro,J., and Rojo-Martínez,G.(2007). Dietary oleic acid and adipocyte lipolytic activity in culture. **Journal of Nutritional Biochemistiy.**19:27-731

Garrett, W.N. (1980). Energy utilization by growing cattle as determined in 72 comparative slaughter experiments**. Energy metabolism. Studies in the agricultural and food sciences.** 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. **Journal of Animal Science.**18: 528-547.

Georgiadi, A., and Kersten, S. (2012) Mechanisms of gene regulation by fatty acids. **Advances in Nutrition.**3:127-134.

Gilmore, L. A., Walzem, R.L., Crouse, S.F., Smith, D.R., Adams, T.H., Vaidyananthan, V., Cao, X., and Smith, S.B. (2011). Consumption of high-oleic acid ground beef increases HDL cholesterol concentration but both high- and low-oleic acid ground beef decrease HDL particle diameter in normocholesterolemic men. **Journal of Nutrition.** 141:1188-1194.

Gillis M. H., Duckett, S. K., Sackmann, J. R., Realini, C. E., Keisler D. H., and Pringle T. D.,(2004).Effects of supplemental rumen-protected conjugated linoleic acid or linoleic acid on feedlot performance,carcass quality, and leptin concentrations in beef cattle. **Journal of Animal Science.**82:851-859.

Grant, AC., Ortiz-Colón, G., Doumit, M.E., Tempelman, R.J., and Buskirk, D.D. (2008). Differentiation of bovine intramuscular and subcutaneous stromalvascular cells exposed to dexamethasone and troglitazone. **Journal of Animal Science**. 86: 2531-2538.

Gustafsson, I.B., Vessby, B., Ohrvall, M., and Nydahl, M. (1994). A diet rich in monounsaturated rapeseed oil reduced the lipoprotein cholesterol concentration and increases the relative content of n-3 fatty acids in serum in hyperlipidemic subjects. **American Journal Clinical Nutrition**. 59:667.

Haaland D. L., Matsushima,J.K., Johnson,D.E., and Ward,G.M.(1981). Effect of replacement of corn by protected tallow in a cattle finishing diet on animal performance and composition. **Journal of Animal Science.** 52:696-702.

Hiller, B., Angulo, J., Olivera, M., Nuernberg, G., and Nuernberg, K. (2013). How selected tissues of lactating Holstein cows respond to dietary polyunsaturated fatty acid supplementation. **Lipids.** 48: 357-367.

Hinrichsen, T., Lock, A.L., and Bauman, D.E. (2006). The relationship between trans-10 18:1 and milk fat yield in cows fed high oleic acid or high linoleic acid plant oil supplements. **Proceeding, 4th Euro-Fed Lipid Congress**, Madrid, Spain.

Hocquette, J.-F., and Bauchart, D. (1999). Intestinal abosrption, blood transport and hepatic and muscle metabolism of fatty acids in preruminant and ruminant animals. **Reproduction Nutrition Development.** 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. **International Jour of Biological Science**. 4(2): 303-319.

Hausman, GJ., Dodson, MV., Ajuwon, K., Azain, M., Barnes, K.M., Guan, L.L., Jiang, Z., Poulos, S.P., Sainz, R.D., Smith, S., Spurlock, M., Novakofski, J., Fernyhough, M.E., Bergen,and W.G. (2009). The biology and regulation of preadipocytes and adipocytes in meat animals. **Journal of Animal Science.**87: 1218-1246.

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. 1998. Fatty acid composition of milk from Holstein cows fed oleamide or canola oil. **Journal of Dairy Science.** 81: 794-800.

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.**Journal of Animal Science.**86: 397-412.

Jurie, C., Cassar-Malek, I., Bonnet, M., Leroux, C., Bauchart, D., Boulesteix, P., Pethick, D.W., and Hocquette, J.F. (2007). Adipocyte fatty acid-binding protein and mitochondrial enzyme activities in muscles as relevant indicators of marbling in cattle. **Journal of Animal Science.** 85: 2660-2669.

Krehbiel, C. R., McCoy,R.A., Stock,R.A., Klopfenstein,T.J., Shain,D.H., and Huffman,R.P. (1995). Influence of grain type, tallow level, and tallow feeding system on feedlot cattle performance. **Journal of Animal Science.** 73:2916-2921.

Leat, W. M. F. (1975). Fatty acid composition of adipose tissue of Jersey cattle during growth and development. **Journal of Agricultural Science**. 85:551-558.

Lee, E.J., Lee, H.J., Kamli, M.R., Pokharel, S., Bhat, A.R., Lee, Y.H., Choi, B.H., Chun, T., Kang, S.W., Lee, Y.S., Kim, J.W., Schnabel, R.D., Taylor, J.F.,and Choi, I. (2012). Depot-specific gene expression profiles during differentiation and transdifferentiation of bovine muscle satellite cells, and differentiation of preadipocytes. **Genomics** 100: 195-202.

Lee,H.J., Lee,S.C., Oh,Y.G., Kim,K.H., Kim1 ,H.B., Park,Y.H., Chae,H.S.,and Chung,I.B.(2003). Effects of Rumen Protected Oleic Acid in the Diet on Animal Performances, Carcass Quality and Fatty Acid Composition of Hanwoo Steers. **Asian-Australasian Journal of Animal Science.**16 (7) : 1003-1010.

Lee, S. H., D. H. Yoon, N. J. Choi, S. H. Hwang, E. Y. Cheong, S. J. Oh, I. C. Cheong, and C. S. Lee. 2005. Developmental relationship of unsaturated fatty acid composition and stearoyl-CoA desaturase mRNAlevel in Hanwoo steers' muscle. **Asian-Australasian Journal of Animal Science.** 18:562-566.

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. **Journal of Animal Science.**27 : 793-806.

Lock A.L., Harvatine K.J., Drackley J.K., and Bauman D.E. (2006). Concepts in fat and fatty acid digestion in ruminants. **Proceedings of the Intermountain Nutrition Conference.** 85-100.

Lundy, F.P., Block, E., Bridges, W.C. Jr., Bertrand, J.A. and Jenkins, T.C. 2004. Ruminalbiohydrogenation in Holstein cows fed soybean fatty acids as amides or calcium salts. **Journal of Dairy Science.**87: 1038-1046.

Mattson, F.H. and Grundy, S.M. (1985). Comparison of effects of dietary saturated, monounsaturated, and polyunsaturated fatty acids on plasma lipids and lipoproteins in man. **Journal Lipid Research.** 26: 194.

McKain, N, Shingfield, K.J., and Wallace, R.J. (2010). Metabolism of conjugated linoleic acids and 18:1 fatty acids by ruminal bacteria: products and mechanisms. **Microbiology**. 3(2): 579-588.

Mensink, R.P. and Katan, M.B. (1989). Effect of a diet enriched with monounsaturated or polyunsaturated fatty acids on levels of low-density and high-density lipoprotein cholesterol in healthy women and men. . **New England Journal of Medicein**.321:436.

Nakamura, M.T., Yudell, B.E.,and Loor, J.J. (2014). Regulation of energy metabolism by long-chain fatty acids. **Progress in lipid research**. 53: 124-144.

Naik, P.K., Saijpaul, S. and Neelam,R. (2007a). Evaluation of rumen protected fat prepared by fusion method. **Animal Nutrition and Feed Technology**.7: 95-101.

Naik, P.K., Saijpaul, S. and Neelam,R. (2007b). Preparation of rumen protected fat and its effect on nutrient utilization in buffaloes**. Indian Journal of AnimalNutrition.**24: 212-215.

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. **Journal of Animal Science.**85: 1062-1073.

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

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

Palmquist, D.L. and Jenkins, T. (1980). Fat in lactations rations: Review. **Journal of Dairy Science.**63:1-14.

Palmquist, D. L. (1991) .Influence of source and amount of dietary fat on digestibility in lactating cows. **Journal of Dairy Science.**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. **Advances in Food and Nutrition Research.**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 Science.**76(3): 444-454.

Polkinghorne, R.J.,and Thompson, J.M. (2010). Meat standards and grading: a world view. **Meat Science.**86: 227-235.

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

Rule, D. C., Busboom,J.R., and Kercher,C.J.(1994). Effect of dietary canola on fatty acid composition of bovine adipose tissue, muscle, kidney, and liver. **Journal of Animal Science**. 72:2735-2744.

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. **The British Journal of Nutrition**. 85: 115-124.

Scollan, N.D., Dannenberger, D., Nuernberg, K., Richardson, I., MacKintosh, S., Hocquette, J.F., and Moloney, A.P. (2014). Enhancing the nutritional and health value of beef lipids and their relationship with meat quality. **Meat Science.** 97: 384394.

Shingfield, K.J., Bonnet, M.,and Scollan, N.D. (2013). Recent developments in altering the fatty acid composition of ruminant-derived foods. **Animal**. 7(Suppl. 1): 132-162.

Smith, S. B., Yang, A., Larsen, T.W., and Tume, R.K. (1998). Positional analysis of triacylglycerols from bovine adipose tissue lipids varying in degree of unsaturation. **Lipids**. 33:197-207.

Smith, S. B., Johnson, B., and Doumit, M. (2010). Regulation of marbling development in beef cattle by specific fatty acids. **Project Summary, National Cattlemen's Beef Association.**

Sukhija, P.S. and Palmquist, D.L. (1990). Dissociation of calcium salts of long chain fatty acids in rumen fluid. **Journal of Dairy Science**. 73: 1784-1787.

Sutton, J.D., Knight, R., Mc Allan, A.B. and Smith, R.H. (1983). Digestion and synthesis in the rumen of sheep given diets supplemented with free and protected oils. **The British Journal of Nutrition .**49: 419-432.

มหาวิทยาลัยเทคโนโลยีสุรนารี

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

Webb, E.C., and O'Neill, H.A. (2008). The animal fat paradox and meat quality. **Meat Science**. 80: 28-36**.**

Walther, T.C., and Farese, R.V. (2012). Lipid droplets and cellular lipid metabolism. **Annual Review of Biochemistry**. 81: 687-714.

Westerling, D.B. and Hedrick, H.B. (1979). Fatty acid composition of bovine lipids as influenced by diet, sex and anatomical location and relationship to sensory characteristics. **Journal of Animal Science**. 48: 1343.

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

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. **Journal of Animal Science**. 71: 2777-2784.

Wójcik, C., Lohe ,K., Kuang, C., Xiao, Y., Jouni, Z., Poels, E. (2014). Modulation of adipocyte differentiation by omega-3 polyunsaturated fatty acids involves the ubiquitin-proteasome system. **Journal of Cellular and Molecular Medicine.** 18: 590-599.

Wood, J. D. (1984). Fat deposition and the quality of fat tissue in meatanimals. **Fats in animal nutrition**. 4074.

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CHAPTER III

EFFECTS OF RUMEN PROTECTED FAT CONTAINING OLEIC ACID SUPPLEMENTATION ON CARCASS AND BEEF QUALITY, AND BEEF FATTY ACIDS PROFILE IN WAGYU CROSSBRED CATTLE

3.1 Abstract

The objective of this study was to determine the effect of rumen protected fat containing high oleic acid supplementation on carcass quality, sensory evaluation and fatty acid profile of beef from crossbred Wagyu beef steers. Sixteen fattening Wagyu crossbred beef steers (50% Wagyu), averaging 500±18 kg live weight (LW) and approximately 27 mo old, were stratified by their LW into 4 groups and each group was randomly assigned to 4 dietary treatments.. All steers were fed approximately 7.5 kg/d of 12% CP concentrate with *ad libitum* rice straw and had free access to clean water and were individually housed in a free-stall unit. The treatments were 1) control concentrate 2) control concentrate plus 200 g/d of rumen protected palm oil (RP-PO); 3) control concentrate plus 200 g/d of rumen protected rice bran oil (RP-RO), 4) control concentrate plus 200 g/d rumen protected corn oil (RP-CO). This present study demonstrated that supplementation of plant oils rich in C18:1n-9 did not influence feed intakes, live weight changes, carcass and muscle characteristics, sensory and physical properties. RP-RO increased C18:1n-9, however, it decreased C18:2n-6t in LD

muscles. Both RP-RO and RP-CO increased PUFA in SM muscle. Beef marbling scores were increased significantly by RP-plant oil supplementation while the highest increase was found in beef of RP-RO cattle. Based on the result from the present study, it can be recommended that the addition of 200 g/d of RP-RO can increase C18:1n-9 and beef marbling score.

3.2 Introduction

Consumers have valued highly marbled beef for nearly a century. Most consumers still prefer beef that is reasonably marbled and juicy. The amount of intramuscular fat or marbling deposited in longissimus muscle is a major determinant of carcass value and predictor of palatability. Marbling fat is comprised of over 20 individual fatty acids; however, six major fatty acids contribute over 92% of the total fatty acid content. These major fatty acids in beef marbling fat are: oleic, palmitic, stearic, linoleic, palmitoleic and myristic acids. The fatty acid primarily responsible for soft fat in cattle is oleic acid (18:1n-9). The concentration of oleic acid is also positively correlated with overall palatability of beef (Westerling and Hedrick, 1979), which may be related to fat softness. Stearic acid (18:0) is a primary determinant of fat hardness (Smith et al., 1998; Wood et al., 2004; Chung et al., 2006), so any dietary or production factor that enhances the conversion of stearic acid to oleic acid will increase fat softness.

Overall, the fatty acid composition of beef marbling fat is about 44% saturated fatty acids (SFA), 5% odd-chain fatty acids (OCFA), 45% monounsaturated fatty acids (MUFA), and 5% polyunsaturated fatty acids (PUFA) for beef marbling fat (Duckett et al., 1993). Human diets containing a high proportion of lipid as MUFA have been

shown to be as effective as those containing high levels of PUFA at lowering serum cholesterol levels (Mattson and Grundy, 1985; Mensink and Katan, 1989; Gustafsson et al., 1994). Stearic acid is a saturated fatty acid; however, diets high in stearic acid have been shown to lower serum cholesterol compared to other saturated fatty acids (Denke and Grundy, 1991; Bonanome and Grundy, 1988). Stearic acid is believed to be converted to oleic acid after dietary ingestion which accounts for its different effect on serum cholesterol compared to other saturated fats (Bonanome and Grundy, 1988). Research has demonstrated that high oleic acid ground beef may reduce risk factors for cardiovascular disease (Adams et al., 2010; Gilmore et al., 2011, 2013).

The beef quality is determined by FA composition of feedstuffs. Moreover, shelf-life, palatability, and nutritive value of beef are affected by FA composition in the beef. For instance, oleic acid seems to be beneficial for reducing plasma total cholesterol and total low-density lipoprotein cholesterol in humans (Bonanome and Grundy, 1988), and it contributes to better taste panel evaluations of cooked beef (Dryden and Marchello, 1970). Challenges in increasing oleic acid content of ruminant tissues and products are of interest. In addition to the issues of the effects of unsaturated fatty acids (UFAs) on the stability and sensory acceptability of products, these FAs inhibit various essential anaerobic bacteria of the rumen, especially those involved in fiber digestion, biohydrogenation of UFAs, and methanogenesis (Palmquist and Jenkins, 1980). Therefore, supplementation of palm oil (PO) and rice bran oil (RO) rich in C18:1n-9 would increase C18:1n-9 in muscle lipid.

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Smith et al. (2010) demonstrated that oleic acid may have autocrine or paracrine effects in further stimulating marbling development and concluded that oleic acid is a critical factor in enhancing in marbling adipose tissue. Specific fatty acids in plasma or within the fat depots can promote marbling fat development while at the same time can cause muscle precursor cells to develop into marbling fat cells. Thus, the objective of the present study was to examine the effect of rumen-protected plant oil supplementation on performance and beef fatty acid profile of Wagyu crossbred beef steers.

3.3 Objectives

To study the effects of rumen protected fat containing oleic acid including palm oil, rice bran oil, and corn oil supplementation on carcass and beef quality, and beef fatty acids profile in Wagyu Crossbred cattle.

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3.4 Materials and methods

3.4.1 Animals, experimental design, and treatments

Sixteen Wagyu crossbred fattening steers (50% Wagyu), averaging 529±28 kg live weight (LW) and approximately 27 mo old, were stratified by their LW into 4 groups and each group was randomly assigned to 4 dietary treatments. All steers were fed approximately 7.5 kg/d of 12% CP concentrate with *ad libitum* rice straw. The treatments were 1) control concentrate 2) control concentrate plus 200 g/d of rumen protected palm oil (RP-PO); 3) control concentrate plus 200 g/d of rumen protected rice bran oil (RP-RO), 4) control concentrate plus 200 g/d rumen protected corn oil (RP-CO). Rumen-protected plant oils were prepared by precipitation method (Garg, 1998) with minor modification. Briefly, 1 L of water was mixed with 100 g of acid oil, stirred vigorously for 5 min., then added 200 ml of 11% NaOH. The content were heated and stirred until the fatty acids were completely dissolved. While hot, the resulting blend was slowly added with 200 ml of 20 %$CaCl_2$ solution. The calcium soap formed was separated and washed with tap water. Excess water was removed by squeezing the calcium soap through muslin cloth. Finally, the calcium soap was air dried in a dark room and stored at subzero temperature until used for feeding.

The chemical compositions of concentrate, rice straw and rumen-protected oils used in the experiment are presented in Table 1 while the fatty acid composition of feed and oils used in the present study are presented in Table 3.2.

The basal diet was formulated to meet NRC (2000) requirements. All steers were received *ad libitum* rice straw, had free access to clean water, were individually housed in a free-stall unit, and individually fed according to treatments. The

experiment lasted for 80 days with the first 10 days was the adjustment period, followed by 70 days (5 periods of 14 d), of measurement period.

3.4.2 Measurements and sample collection

At the end of feeding trial, the animals were weighed, and all animals were transported to a commercial abattoir and then slaughtered at Ibrahim slaughterhouse, Ratchaburi, 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, Royal Thai Government. Muscle samples were cut from outside *Longissimus dorsi* (LD; 6-12th rib) muscle and *Semi membranosus* (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 550°C for 4 h. The chemical analysis was expressed based on the final DM. Fatty acid compositions of concentrate and rice straw were determined by Gas chromatography.

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. In the boiled samples, shear forces were measured after cooling and drying. A steel hollow-core device with a diameter of 1.0x2.0x0.5 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 2 mm/sec and a 5kN load cell calibrated to read over a range of 0x100 N were applied.color measurements using a hunter lab (Color Quest XE, Kable, United Kingdom).

Samples of the LD and SM were minced and analyzed in duplicate for moisture, fat and protein contents according to AOAC (1995).

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, 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 100ºC for 5 min with occasional shaking and then cooled to room temperature. One ml of C17:0 internal standard (2.00 mg/mL in hexane) and 2 ml of boron trifluoride in methanol were added and heated at 100°C for 5 min with occasional shaking and 10 ml of deionized water were added. The solution was transferred to a 40 ml centrifuged tube and 5 ml of hexane were added for FAME extraction. The solution was centrifuged at 2,000 g, at 10°C for 20 min and then the hexane layer was dried over sodium sulfate and transferred into vial for analyzing by gas chromatography (GC) (7890A GC System, Agilent Technology, USA) equipped with a 100 m×0.25 mm×0.2 µm film fused silica capillary column (SP1233, SupelcoInc, 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.

Sensory evaluation for beef descriptive tenderness, juiciness, flavor and overall acceptability using 8- and 9-point scales (Texas Tech University). 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 and determined on a point scale. Samples were served subsequently in a randomized order with respect to group and animal. The 32samples (from 16 animals and two muscles) were tested by 8 persons.

3.4.4 Statistical analysis

All measured data were analysed by ANOVA for complete randomized design using the Statistical Analysis System (SAS, 2001). Significant differences among treatment were assessed by Duncan's new multiple range test. A significant level of $p<0.05$ was used (Steel and Torrie, 1980).

3.4.5 Experimental location

The experiment was conducted at Suranaree University of Technology's Cattle Farm, The Center for Scientific and Technological Equipment Building 3, 10, 11 and 14,Suranaree University of Technology.

3.4.6 Duration of the study

The study was from May to December 2016.

3.5 Result and Discussion

3.5.1 Feed Composition and performance

The chemical and fatty acid composition of the feeds are presented in Table 3.1 and 3.2, and the concentrate was formulated to meet the requirement of the steers.

Lipids from rice straw provided high proportions of C16:0 (45.30 g/100 g fat) and low proportions of C18:0 (1.01 g/100 g fat). RP-PO had the highest proportion of C16:0 (46.44 g/100 g fat) while RP-RO had the highest proportion of C18:1n9 (47.45 g/100 g fat). In the concentrate, the main SFA was C16:0 (19.58 g/100 g fat), whereas

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C18:1n-9 was the main MUFA in RP-PO, RP-RO and RP-CO (34.70, 47.46 and 36.26 g/100 g fat respectively), C18:2n-6 was the main PUFA in concentrate (14.61 g/100 g fat) (Table 3.2).

No significant difference was found for DM and CP intakes among groups (Table 3.3); however, the animals supplemented with rumen-protected plant oil had greater total fatty acid intake than that fed the control diets (P=0.001). With diets containing lower levels of added fat, Huerta-Leidenz et al. (1991) reported no influence on daily gain, intake or feed conversion ratio when dietary whole cotton seed of 15 or 30% (3.3 and 6.6% additional fat) was supplemented. In the present trial, fat contents of experimental diets were between 3.1 and 4.3%, it is unlikely that these levels of fat affected feed intake. When the consumption of individual fatty acid was calculated, cattle on RP-PO diet significantly consumed more C12:0, C14:0, C16:0, C18:0 and SFA than other cattle (Table 3) while those on RP-RO diet ate more C18:3 and on RP-CO significantly consumed more C18:1, C18:2, MUFA and PUFA than other cattle. Additionally, the cattle on control diet significantly ate less C18:1 than those cattle on dietary fat diets.

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Table 3.1 Chemical compositions of the experimental diets.

Items	Concentrate	RP-PO	RP-RO	RP-CO	Rice straw
Dry matter	92.2	83.3	83.1	83.1	90.6
	----------------% of DM---------------------------				
Ash	11.9	15.01	15.04	15.03	15.9
Crude protein	13.3				2.6
Ether extract	4.6	82.3	82.1	81.5	1.1
Neutral detergent fiber	46.5				85.1
Acid detergent fiber	30.2				57.6
Neutral detergent in soluble N	0.9				0.5
Acid detergent insoluble N	0.9				0.4
Acid detergent lignin	10.1				6.4
TDN_{1X} (%)[2]	52.60	176.0	175.4	174.0	40.73
DE_{1X} (Mcal/kg DM)[3]	2.31	7.34	7.32	7.26	1.73
ME (Mcal/kg DM)[4]	1.96	4.56	4.55	4.52	1.62
NE_M (Mcal/kg DM)[5]	1.08	4.01	4.00	3.96	0.71
NE_G (Mcal/kg DM)[6]	0.52	3.40	3.39	3.36	0.15

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

[2]Total digestible nutrients, TDN_{1X} (%) = tdNFC + tdCP + (tdFA x 2.25) + tdNDF – 7 (NRC, 2000)

[3]Digestible energy, DE_{1X} (Mcal/kg) = [(tdNFC/100)x4.2]+[(tdNDF/100) x 4.2]+[(tdCP/100) x 5.6]+[(FA/100) x 9.4] –0.3

[4]Metabolisable energy, ME = 0.82 x DE (NRC, 2000)

[5]Net energy for maintenance, NE_M = 1.37ME – 0.138ME2 + 0.0105ME3 – 1.12 (NRC, 2000)

[6]Net energy for growth, NE_G = 1.42ME – 0.174ME2 + 0.0122ME3 – 1.65 (NRC, 2000)

Table 3.2 Fatty acid compositions (g/100 g fat) of concentrate, rice straw and rumen-protected oils used in the experiment.

Fatty acids	Concentrate	Rice straw	RP-PO	RP-RO	RP-CO
C8:0	0.87	ND	ND	ND	ND
C10:0	1.10	ND	ND	ND	ND
C12:0	19.22	6.69	1.41	0.81	0.37
C14:0	6.54	9.57	3.15	1.59	1.36
C16:0	19.58	45.30	46.44	7.73	9.72
C18:0	3.44	1.01	4.69	4.54	3.86
C18:1	34.31	19.73	34.70	47.46	36.26
C18:2	14.61	12.68	5.59	33.46	43.73
C18:3	0.34	4.99	0.21	0.32	0.17
C20:0	0.42	ND	ND	ND	ND
Others	-	-	3.81	4.09	4.53
SFA[1]	50.74	62.60	59.5	14.67	19.84
MUFA[2]	34.1	19.73	34.70	47.46	36.26
PUFA[3]	14.95	17.67	5.80	33.78	43.90

[1] SFA = Sum of saturated fatty acid from C8:0-C20:0

[2] MUFA = Monounsaturated fatty acid from C18:1

[3] PUFA = Sum of polyunsaturated fatty acid from C18:2-C18:3

ND = Not detected.

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Table 3.3 DM, CP and fatty acid intake of Wagyu crossbred cattle fed rumen-protected plant oil.

Items	Control	RP-PO	RP-RO	RP-CO	SEM	P-value
DM intake, kg/d						
Concentrate	6.92	6.92	6.92	6.92	-	-
Rice straw	5.00	4.88	4.90	4.90	0.016	0.132
Protected oil	-	0.17	0.17	0.17	-	-
Total	11.92	11.97	11.99	11.99	0.015	0.142
CP intake, g/d						
Concentrate	922	922	922	922	-	-
Rice straw	128	125	126	126	0.410	0.128
Total	1050	1047	1048	1048	0.410	0.128
Fat intake, g/d						
Concentrate	321	321	321	321	-	-
Rice straw	53	51	52	52	0.168	0.128
Protected oil	0	137	136	135	-	-
Total	374[b]	509[a]	509[a]	508[a]	0.168	0.0001
NE intake, Mcal/d						
Concentrate	11.05	11.05	11.05	11.05	-	-
Rice straw	4.27	4.16	4.19	4.19	0.013	0.146
Protected oil	-	1.26	1.26	1.24	-	-
Total	15.32[b]	16.47[a]	16.50[a]	16.48[a]	0.013	0.0001

RP-PO = rumen-protected palm oil; RP-RO = rumen-protected rice bran oil; RP-CO = rumen-protected corn oil; SEM = standard error of the mean

SFA = sum of C8:0-C18:0; MUFA = C18:1; PUFA = sum of C18:2 and C18:3

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

Table 3.3 DM, CP and fatty acid intake of Wagyu crossbred cattle fed rumen-protected plant oil (continue).

Items	Control	RP-PO	RP-RO	RP-CO	SEM	P-value
Fatty acid intake, g/d						
C8:0	2.16	2.16	2.16	2.16	-	-
C10:0	2.79	2.79	2.79	2.79	-	-
C12:0	51.75[d]	53.58[a]	52.78[b]	52.18[c]	0.010	0.0001
C14:0	21.25[d]	25.40[a]	23.30[b]	22.97[c]	0.015	0.0001
C16:0	71.95[d]	134.33[a]	81.92[c]	84.49[b]	0.072	0.0001
C18:0	9.16[d]	15.50[a]	15.27[b]	14.31[c]	0.001	0.0001
C18:1n-9	96.25[d]	143.03[c]	160.01[a]	144.52[b]	0.031	0.0001
C18:2n-6	43.13[d]	50.55[c]	88.10[b]	101.46[a]	0.020	0.0001
C18:3n-3	3.34[c]	3.56[ab]	3.72[a]	3.51[b]	0.008	0.004
Total	301.79[b]	430.92[a]	430.05[a]	428.41[a]	0.160	0.001
SFA	159.06[c]	233.76[a]	178.22[b]	178.90[b]	0.032	0.0001
MUFA	96.25[d]	143.03[c]	160.01[a]	144.52[b]	0.031	0.0001
PUFA	46.47[d]	54.11[c]	91.82[b]	104.97[a]	0.016	0.0001

RP-PO = rumen-protected palm oil; RP-RO = rumen-protected rice bran oil; RP-CO = rumen-protected corn oil; SEM = standard error of the mean

SFA = sum of C8:0-C18:0; MUFA = C18:1; PUFA = sum of C18:2 and C18:3

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

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3.5.2 Animal performances and Carcass quality traits.

No remarkable changes were found for final live weight and Average daily gain among the treatments ($P>0.05$) (Table 3.4). The amount of dietary fat did not affect live weight of the steers over the course of the trial; however, mean while live weight was increased at 1.00, 1.03, 1.04 and 1.07 kg/d in the animals fed control, RP-PO, RP-RO and RP-CO, respectively (Table 3.4). Similarly, Noci et al. (2007)

reported that 150 g/d sunflower oil and 150 g/d linseed oil supplementation did not affected 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.

At slaughter, hot carcass weight, % hot carcass and dressing percentage were not significantly different among treatments (Table 3.4). Noci et al. (2007) also 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. However, beef marbling score (BMS) of cattle fed RP-RO was greater than other cattle with BMS of cattle on RP-RO > RP-CO > RP-PO > control. 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. 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.

Beef quality

No treatment effects were found on moisture cooking loss (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 storage. The moisture and protein contents in LD and SM muscles were not significantly different (P>0.05) among treatments (Table 5), however, the fat content in SM muscles of cattle fed RP-RO and RP-CO diets were greater than those fed control and RP-PO diets (P=0.011).

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The amounts of fat in the muscle typically result from a balance between dietary energy and metabolic requirements of the animal (Oliveira et al., 2012). If energy intake is higher than its metabolic demands, this excess will be storage as fat. The literature suggests that the total protein content is less variable in bovine meat, with values of approximately 20% observed in the *longissimus dorsi* muscle without the fat cover, and this is independent of food, breed, the genetic group, and the physiological condition (Marques et al., 2006).

The shear forces of LD and SM muscle were unaffected (P>0.05) by the addition of LSO in the diets (Table 3.5). Beef tenderness is a trait considered to be of great relevance for consumers while shear force is an objective measure of tenderness. Bovine meat is considered to have an acceptable tenderness if its shear strength values are below 8 N (Swan et al., 1998). 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. The present trial found shear force values between 2.65 and 2.82 kg/cm^2 in LD muscle and between 4.13 and 4.35 in SM muscle which were considered to be tender (Table 3.5). These values were closely related to the values obtained from sensory perception of tenderness by trained panelists (4.57 to 5.82 in LD muscle and 3.32 to 5.65 in SM muscle; Table 3.5). Such variations in the shear force values may be caused by differences in the thicknesses of the blades utilized in the analysis.

Beef color remained mostly unaffected by treatment with the exception of higher lightness (L*) on LD (P=0.032) and SM (P=0.007) muscles originating from the RP-RO and RP-CO supplements than other groups (Table 3.5). Values encountered in literature for L*, a* and b* were used to measure beef color 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. Values obtained in the present study were within the range given.

The sensory juiciness and beef flavor both in LD and SM muscles were unaffected by treatments (Table 3.5), however, cattle on RP-PO diet showed significantly less tender (P=0.015) in LD muscle than those on other diets and cattle on RP-CO diet showed significantly more tender (P>0.001) in SM muscle than those on other diets. The sensory overall acceptability was not significantly different in LD muscle, however, the sensory overall acceptabilities were significantly higher in SM muscle of those cattle fed RP-plant oil. 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 color stability were more closely associated with fatty acid composition and greater abnormal flavors 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 have affected taste panel assessments.

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Table 3.4 Initial weight, final weight, live weight change and beef characteristics of beef from Wagyu crossbred cattle fed rumen-protected plant oil.

Items	Control	RP-PO	RP-RO	RP-CO	SEM	P-value
Initial weight (kg)	528	530	531	531	4.87	0.994
Final weight (kg)	598	602	604	606	4.86	0.948
Average daily gain (kg/d)	1.00	1.03	1.04	1.07	0.03	0.788
Slaughter weight (kg)	581	584	586	588	4.85	0.965
Warm carcass weight (kg)	331	328	335	335	3.43	0.878
% warm carcass	56.97	56.16	57.17	56.97	0.271	0.649
Cold carcass weight (kg)	320	318	324	324	3.28	0.825
% cold carcass	55.08	54.45	55.29	55.10	0.283	0.669
Marbling score[1]	2.90^{d}	3.17^{c}	5.10^{a}	4.07^{b}	0.088	>0.001
Loin eye area (cm^2)	74.93	75.82	76.14	75.98	0.338	0.608
Back fat thickness (cm^2)	1.06	1.05	1.01	1.00	0.043	0.959

RP-PO = rumen-protected palm oil; RP-RO = rumen-protected rice bran oil; RP-CO = rumen-protected corn oil; SEM = standard error of the mean

[1] 1 = very scarce, 12 = very abundant (Japanese Meat Grading Association)

^{a,b,c,d} Mean within row which different superscripts differ ($P<0.05$); SEM = Standard error of mean

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Table 3.5 Beef chemical composition, sensory and physical evaluations of beef from Wagyu crossbred cattle fed rumen-protected plant oil.

Items	Control	RP-PO	RP-RO	RP-CO	SEM	P-value
Longissimus dorsi						
Moisture cooking loss (%)	25.27	24.37	22.91	23.59	0.477	0.384
Moisture content (%)	70.55	70.37	70.46	70.40	0.089	0.417
Crude protein (%)	21.26	21.48	21.96	21.08	0.261	0.274
Fat (%)	4.89	5.19	5.25	5.21	0.090	0.512
Shear force (kg/cm^2)	2.82	2.69	2.65	2.73	0.035	0.756
L*	37.02^{b}	37.22^{b}	40.29^{a}	38.54^{ab}	0.350	0.032
a*	5.52	6.14	6.15	6.54	0.181	0.319
b*	7.75	8.32	9.02	9.01	0.270	0.340
Tenderness	5.27^{a}	4.57^{b}	5.52^{a}	5.82^{a}	0.106	0.015
Juiciness	5.35	4.75	5.22	5.25	0.145	0.507
Beef flavor	4.20	4.82	4.75	4.32	0.082	0.067
Overall acceptability	5.55	5.40	6.10	5.92	0.111	0.172
Semimembranosus						
Moisture cooking loss (%)	24.25	24.26	24.82	24.09	0.214	0.653
Moisture content (%)	72.17	71.63	71.04	71.02	0.075	0.289
Crude protein (%)	22.31	21.45	21.75	21.44	0.271	0.658
Fat (%)	4.14^{b}	4.34^{b}	5.13^{a}	5.17^{a}	0.010	0.011
Shear force (kg/cm^2)	4.29	4.35	4.13	4.13	0.042	0.087
L*	38.31^{b}	38.92^{b}	42.37^{a}	41.41^{a}	0.350	0.007
a*	6.79	7.07	7.65	8.06	0.176	0.110
b*	8.75	9.11	9.69	9.27	0.208	0.489
Tenderness	3.32^{c}	4.35^{b}	4.75^{b}	5.65^{a}	0.106	>0.001
Juiciness	4.80	5.45	5.35	5.42	0.111	0.209
Beef flavor	3.60	4.20	3.95	4.80	0.106	0.077
Overall acceptability	4.75^{b}	5.80^{a}	5.47^{a}	5.92^{a}	0.079	0.002

RP-PO = rumen-protected palm oil; RP-RO = rumen-protected rice bran oil; RP-CO = rumen-protected corn oil; SEM = standard error of the mean.
Tenderness, Juiciness, Beef flavor and overall acceptability: 1 = extremely though, dry, bland, and less accept respectively; 8 = extremely tender, juicy, intense and more accept respectively.
[a,b,] Mean within row which different superscripts differ ($P<0.05$); SEM = Standard error of mean

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3.5.3 Fatty acid composition of beef

In the current study, RP-RO-containing diets resulted in marked alternations in both LD and SM beef C18:1 composition relative to the diet added none or other RP-plant oils (Table 3.6). To compare with control diet, RP-plant oil diets had no effect on C10:0-C24:0 SFAs in LD and SM muscles. However, in LD muscle, RP-PO diet induced to significantly increase C16:1 (P=0.046) and RP-RO significantly increased C18:1n-9c (P=0.023) but decreased C18:2n-6 (P=0.027). In SM muscle, RP-CO diet significantly increased C20:4n-6 (P=0.002) and PUFAs (P=0.043).

The increase in concentrations of C18:1n-9 in LD muscle by RP-RO supplementation may be explained by a high intake of C18:1n-9 (144.52 g/d). The concentration of C18:1n-9 was respectively 48.26 and 49.45% of total fatty acid in LD and SM muscles. The lack of diet effect on C18:1n-9 in SM muscles relates to microbial bio-hydrogenation of C81:1n-9 to not only C18:0 but also *trans*C18:1 positional isomers (Mosley et al., 2002). Jenkins (2000) fed high oleic canola oil (78% oleic acid) to lactating Jersey cows and found a significant increase in the *trans*C18:1 concentration in milk fat. The increase in *trans*C18:1 content in milk fat could possibly be that the isomers were formed during the bio-hydrogenation of oleic acid in the rumen.

SFA, UFA, and MUFA in LD and SM muscles were unaffected by dietary treatments (P >0.05). However, PUFA in SM muscle of cattle on RP-CO diet was significantly higher than those cattle on control and RP-PO diets. The PUFA: SFA ratio is used to evaluate the nutritional value of fat for human consumption. Increasing the PUFA content of the diet, by including sources rich in PUFA, generally improves the PUFA: SFA ratio, this was observed in the present trial, and in all diets where PUFA: SFA ratio was always lowered than 0.29 (Table 3.6 and Table 3.7), which the

minimum value recommended for the human diet is 0.45 (BDH, 1994). The C18:2n-6 was the most concentrated PUFA across treatment. The lack of dietary effects on total PUFA in LD muscles indicates that RP-plant oil addition had no effect on rates of lipolysis in the rumen. However, the higher total PUFA found in SM when feeding 200 g/d RP-CO may indicate that either the rate of lipolysis and/or the initial step in C18:1n-9 bio-hydrogenation was reduced. 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, and this is likely due to extensive bio-hydrogenation of PUFA to C18:0, and potentially to reduced delta-9 desaturase activity when feeding PUFA rich oils (Waters, et al., 2009).

Treatments had no effect on total or individual SFA in LD and SM ($P>0.01$, Table 6 and 7). 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 C18:1n-9 and its bio-hydrogenation intermediates were less effective at down-regulating SCD activity than C18:2n-6 and C18:3n-3. SFA relates to changes in endogenous FA synthesis that may not have been differentially affected by diet (Mapiye et al., 2013). Oliveira et al. (2012) with feeding different oils reported lower percentages (about 45%) of SFA. 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).

Table 3.6 Fatty acid composition (g/100 g fat) of *Longissimus dorsi* muscle from Wagyu crossbred cattle fed rumen-protected plant oil.

Items	Control	RP-PO	RP-RO	RP-CO	SEM	P-value
No. of cattle	**4**	**4**	**4**	**4**		
Longissimus dorsi						
C10:0	0.018	0.022	0.028	0.034	0.002	0.106
C12:0	0.087	0.065	0.070	0.086	0.006	0.532
C14:0	2.58	2.59	2.29	2.31	0.082	0.484
C14:1	0.45	0.57	0.54	0.43	0.066	0.854
C15:0	0.38	0.30	0.31	0.33	0.011	0.141
C16:0	26.13	25.59	25.01	25.31	0.273	0.548
C16:1	3.18[ab]	4.16[a]	2.85[b]	2.55[b]	0.173	0.046
C18:0	16.38	14.06	14.47	16.07	0.711	0.601
C18:1n9t	3.65	3.45	3.93	3.84	0.200	0.840
C18:1n9c	41.15[b]	42.72[ab]	44.33[a]	42.01[b]	0.180	0.023
C18:2n6t	0.24[a]	0.23[a]	0.16[b]	0.27[a]	0.007	0.027
C18:2n6c	3.38	3.86	3.82	4.11	0.108	0.211

RP-PO = rumen-protected palm oil; RP-RO = rumen-protected rice bran oil; RP-CO = rumen-protected corn oil; SEM = standard error of the mean

[1] Sum of saturated fatty acid from C10:0 – C24:0

[2] Sum of unsaturated fatty acid from MUFA, and PUFA

[3] Sum of monounsaturated fatty acid from C16:1 – C20:1

[4] Sum of polyunsaturated fatty acid

[5] Sum of CLA from CLA c9,t11 and CLA t10,c12

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

Table 3.6 Fatty acid composition (g/100 g fat) of *Longissimus dorsi* muscle from Wagyu crossbred cattle fed rumen-protected plant oil (continue).

Items	Control	RP-PO	RP-RO	RP-CO	SEM	P-value

No. of cattle	4	4	4	4		
C18:3n6	0.25	0.19	0.22	0.28	0.007	0.054
C20:1	0.14	0.12	0.14	0.14	0.006	0.543
C18:3n3	0.33	0.32	0.33	0.42	0.050	0.871
CLA c9,t11	0.38	0.37	0.37	0.36	0.036	0.846
C20:4n6	0.77	0.88	0.74	0.91	0.061	0.748
C24:0	0.08	0.08	0.06	0.09	0.003	0.132
SFA[1]	45.67	42.72	42.26	45.67	0.969	0.607
UFA[2]	54.31	57.27	57.74	55.73	0.969	0.607
MUFA[3]	48.58	51.04	51.82	48.98	0.897	0.543
PUFA[4]	5.73	6.23	5.92	6.75	0.162	0.208
Total CLA[5]	0.386	0.376	0.376	0.367	0.038	0.794
UFA:SFA	1.189	1.341	1.366	1.220	0.073	0.326
PUFA:SFA	0.125	0.146	0.140	0.148	0.021	0.249

RP-PO = rumen-protected palm oil; RP-RO = rumen-protected rice bran oil; RP-CO = rumen-protected corn oil; SEM = standard error of the mean

[1] Sum of saturated fatty acid from C10:0 – C24:0

[2] Sum of unsaturated fatty acid from MUFA, and PUFA

[3] Sum of monounsaturated fatty acid from C16:1 – C20:1

[4] Sum of polyunsaturated fatty acid

[5] Sum of CLA from CLA c9,t11 and CLA t10,c12

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

Table 3.7 Fatty acid composition (g/100 g fat) of *Semimembranosus* muscle from Wagyu crossbred cattle fed rumen-protected plant oil.

Items	Control	RP-PO	RP-RO	RP-CO	SEM	P-value

No. of cattle	4	4	4	4		
Semimembranosus						
C10:0	0.020	0.025	0.018	0.020	0.025	0.842
C12:0	0.083	0.064	0.073	0.067	0.004	0.524
C14:0	2.29	2.37	2.16	1.95	0.055	0.132
C14:1	0.77	0.75	0.71	0.71	0.050	0.971
C15:0	0.32	0.30	0.29	0.29	0.011	0.381
C16:0	24.81	24.36	23.23	23.29	0.337	0.315
C16:1	4.56	4.58	3.98	4.13	0.196	0.630
C18:0	12.24	12.42	11.54	12.13	0.407	0.881
C18:1n9t	3.36	3.16	3.04	3.23	0.108	0.167
C18:1n9c	43.52	43.87	46.41	43.18	0.699	0.374
C18:2n6t	0.34	0.25	0.24	0.23	0.023	0.492
C18:2n6c	4.23	4.60	4.94	6.04	0.226	0.057
C18:3n6	0.23	0.25	0.21	0.23	0.011	0.221
C20:1	0.14	0.15	0.14	0.17	0.007	0.443
C18:3n3	0.35	0.36	0.35	0.35	0.007	0.347
CLA c9,t11	0.35	0.36	0.35	0.35	0.028	0.844

RP-PO = rumen-protected palm oil; RP-RO = rumen-protected rice bran oil; RP-CO = rumen-protected corn oil; SEM = standard error of the mean

[1] Sum of saturated fatty acid from C10:0 – C18:0

[2] Sum of unsaturated fatty acid from MUFA, and PUFA

[3] Sum of monounsaturated fatty acid from C16:1 – C18:1

[4] Sum of polyunsaturated fatty acid

[5] Sum of CLA from CLA c9,t11 and CLA t10,c12

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

Table 3.7 Fatty acid composition (g/100 g fat) of *Semimembranosus* muscle from Wagyu crossbred cattle fed rumen-protected plant oil (continue).

Items	Control	RP-PO	RP-RO	RP-CO	SEM	P-value
No. of cattle	**4**	**4**	**4**	**4**		
CLA t10,c12	0.007	0.006	0.007	0.006	0.003	0.603
C20:3n6	0.64	0.56	0.60	1.08	0.070	0.127
C20:4n6	1.45[b]	1.38[b]	1.49[b]	2.29[a]	0.070	0.002
C24:0	0.15	0.15	0.13	0.21	0.013	0.162
SFA[1]	39.93	39.71	37.45	37.86	0.725	0.569
UFA[2]	60.05	60.28	62.53	62.12	0.725	0.569
MUFA[3]	52.36	52.53	54.30	51.43	0.735	0.577
PUFA[4]	7.69[b]	7.75[b]	8.23[ab]	10.69[a]	0.162	0.043
Total CLA[5]	0.357	0.366	0.357	0.356	0.031	0.724
UFA:SFA	1.504	1.518	1.670	1.641	0.064	0.314
PUFA:SFA	0.193	0.195	0.220	0.282	0.023	0.263

RP-PO = rumen-protected palm oil; RP-RO = rumen-protected rice bran oil; RP-CO = rumen-protected corn oil; SEM = standard error of the mean

[1] Sum of saturated fatty acid from C10:0 – C18:0

[2] Sum of unsaturated fatty acid from MUFA, and PUFA

[3] Sum of monounsaturated fatty acid from C16:1 – C18:1

[4] Sum of polyunsaturated fatty acid

[5] Sum of CLA from CLA c9,t11 and CLA t10,c12

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

3.6 Conclusion

Feeding dietary treatment that inclusion of 200 g/d of rumen-protected plant oils (palm oil, rice bran oil and corn oil) did not negatively affect any of performance and carcass quality of steers. The overall feed consumption of the steers was unaffected when dietary oil was provided. RP-plant oils supplement did also not influence muscle sensory and physical characteristics. RP-RO increased the percentage of C18:1n-9) in the LD fat and lowered the C18:2n-6t in beef. RP-RO and RP-CO increased beef marbling score. Thus, it can be concluded that 200 g/d RP-RO can be safety supplemented to diets of steers to enrich beef with potential health beneficial FA.

3.7 References

Adams, T. H.,Walzem, R.L., Smith, D.R., Tseng, S., and Smith,S.B. (2010). Hamburger high in total, saturated and trans-fatty acids decreases HDL cholesterol and LDL particle diameter, and increases plasma TAG, in mildly hypercholesterolaemic men. **British Journal Nutrition**. 103: 91-98.

Association of Official Analytical Chemists. (1995). **Official Method of Analysis**. Washington DC, USA.

Bonanome, A., and Grundy, S.M. (1988). Effect of dietary stearic acid on plasma cholesterol and lipoprotein levels. **New England Journal of Medicein**. 318: 1244.

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

Chung, K. Y., Lunt, D. K., Choi, C. B., Chae, S. H., Rhoades, R. D., Adams, T. L., Booren, B., and Smith, S. B. (2006). Lipid characteristics of subcutaneous

adipose tissue and M. longissimus thoracis of Angus and Wagyu steers fed to U.S. and Japanese endpoints. **Meat Science**. 73: 432-441.

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.

Denke, M.A., and Grundy, S. M. (1991). Effects of fats high in stearic acid on lipid and lipoprotein concentrations in men. **American Journal Clinical Nutrition**. 54: 1036.

Dryden, F.D., and Marchello, J. A. (1970). Influence of total lipid and fatty acid composition upon the palatability of three bovine muscles. **Journal of Animal Science**. 31: 36.

Duckett, S. K., Wagner, D. G., Yates, L. D., Dolezal, H. G., and May, S. G. (1993). Effects of time on feed on beef nutrient composition. **Journal of Animal Science**. 71: 2079.

Folch, J., Lees, M., and Sloane-Stanley, G.H. (1957). A simple method for the isolation and purification of total lipids from animal tissues .**Journal of Biological Chemistry**.226 (1): 495-509.

Garg, M.. R. (1998). Effect of feeding bypass fat on rumenfermentation, DM digestibility and N balance in sheep. **IndianVetarinary Journal**.75: 800-802.

Gilmore, L. A., Walzem, R. L., Crouse, S. F., Smith, D. R., Adams, T. H., Vaidyananthan, V., Cao, X., and Smith, S. B. (2011). Consumption of high-oleic acid ground beef increases HDL cholesterol concentration but both high- and low-oleic acid ground beef decrease HDL particle diameter in normocholesterolemic men. **Journal of Nutrition**. 141: 1188-1194.

มหาวิทยาลัยเทคโนโลยีสุรนารี

Gilmore, L. A., Crouse, S. F., Carbuhn, A., Klooster, J., Calles, J. A. E., Meade, T., and Smith, S.B. (2013). Exercise attenuates the increase in plasma monounsaturated fatty acids and high-density lipoprotein but not high-density lipoprotein 2b cholesterol caused by high-oleic ground beef in women. **Nutrition Research**. 33: 1003-1011.

Gustafsson, I.B., Vessby, B., Ohrvall, M., and Nydahl, M. (1994). A diet rich in monounsaturated rapeseed oil reduced the lipoprotein cholesterol concentration and increases the relative content of n-3 fatty acids in serum in hyperlipidemicsubjects.**American Journal Clinical Nutrition**. 59: 667.

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. **Journal of Dairy Science**. 94: 2481-2491.

Huerta-Leidenz, N. O., Cross, H. R., Lunt, D. K., Pelton, L. S., Savell, J. W., and Smith, S. B. (1991). Growth, carcass traits and fatty acid profiles of adipose tissues from steers fed whole cottonseed. **Journal of Animal Science**. 69: 3665-3672.

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

Jenkins, T. C. (2000). Feeding oleamide to lactating Jersey cows. I. Effects on lactation performance and milk fatty acid composition. **Journal of Dairy Science**. 83: 332-337.

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 Science**. 95: 98-109.

Marques, J. A., Prado, I. N., Moletta, J. L., Prado, I. M., Prado, J. M., Macedo, L. M. A., Souza, N. A., and Matsushita, M. (2006). Característicasfísicoquímicas da carcaça e da carne de novilhassubmetidasaoanestrocirúrgicooumecânicoterminadasemconfinamento. **RevistaBrasileira de Zootecnia**. 35: 1514-1522.

Mattson, F.H., and Grundy, S. M. (1985). Comparison of effects of dietary saturated, monounsaturated, and polyunsaturated fatty acids on plasma lipids and lipoproteins in man. **Journal of Lipid Research**. 26: 194.

Mensink, R.P., and Katan, M. B. (1989). Effect of a diet enriched with monounsaturated or polyunsaturated fatty acids on levels of low-density and high-density lipoprotein cholesterol in healthy women and men. **New England Journal of Medicein**. 321: 436.

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

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. **Journal of Animal Science**. 85: 1062-1073.

NRC (National Research Council). (2000). Nutrient Requirements of Beef Cattle, 7th Rev. Ed. Update. **National Academy Press**, Washington DC, USA.

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 Science**. 90: 28-35.

มหาวิทยาลัยเทคโนโลยีสุรนารี

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., and Jenkins, T. (1980). Fat in lactations rations: Review. **Journal of Dairy Science**.63: 1-14.

SAS (Statistical Analysis System). (2001). **SAS User' Guide: Statistics**. SAS Institute Inc., 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 receiving different forms of soybean oil in the rumen. **Animal Feed Science and Technology**. 194: 35-43.

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 Science**. 58: 321-328.

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 Science**. 74: 17-33.

Smith, S. B., Yang, A., Larsen, T.W., and Tume, R.K. (1998). Positional analysis of triacylglycerols from bovine adipose tissue lipids varying in degree of unsaturation. **Lipids**. 33: 197-207.

Smith, S. B., Johnson, B., and Doumit, M. (2010). Regulation of marbling development in beef cattle by specific fatty acids. Project Summary, **National Cattlemen's Beef Association**.

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

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 Ruminant Research**. 28: 273-280.

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. **Journal of Dairy Science**. 74(10): 3583-3597.

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. **Journal of Animal Science**. 87(1): 244-252.

Westerling, D.B. and Hedrick, H.B. (1979). Fatty acid composition of bovine lipids as influenced by diet, sex and anatomical location and relationship to sensory characteristics. **Journal of Animal Science**. 48: 1343.

Williams, C.(2000). Dietary fatty acids and human health. **Annales de Zootechnie**. 49(3): 165-180.

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

Zinn, R. A., Gulati, S.K., Plascencia, A., and Salinas, J. (2000). Influence of ruminal biohydrogenation on the feeding value of fat in finishing diets for feedlot cattle. **Journal of Animal Science**.78: 1738.

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CHAPTER IV

EFFECTS OF RUMEN PROTECTED RICE BRAN OILSUPPLEMENTATION ON CARCASS AND BEEF QUALITY, AND BEEF FATTY ACIDS PROFILE IN WAGYU CROSSBRED CATTLE

4.1 Abstract

The objective of this study was to determine the effect of rumen-protected rice bran oil supplementation on carcass quality, sensory evaluation and fatty acid profile of beef from crossbred Wagyu beef steers. Twelve fattening Wagyu crossbred beef steers (50% Wagyu), averaging 509±3.2 kg live weight (LW) and approximately 28 mold, were stratified by their LW into 3 groups and each group was randomly assigned to 3 dietary treatments.. All steers were fed approximately 7.5 kg/d of 12% CP concentrate with *ad libitum* rice straw and had free access to clean water and were individually housed in a free-stall unit. The treatments were 1) control concentrate 2) control concentrate plus 100 g/d of rumen protected rice bran oil (100 RP-RO); 3) control concentrate plus 200 g/d of rumen protected rice bran oil (200 RP-RO). This present study demonstrated that supplementation of rumen-protected rice bran oil rich in C18:1n-9 did not influence DM and CP intakes, live weight changes, carcass and muscle characteristics, sensory and physical properties. RP-RO increased C18:1n-9, however, it decreased SFA but increased MUFA in LD and SM muscles. Beef

marbling scores were increased significantly by RP-RO supplementation while the highest increase was found in beef of 200 g/d RP-RO cattle. Based on the result from the present study, it can be recommended that the addition of RP-RO can increase C18:1n-9 and beef marbling score.

4.2 Introduction

Most consumers prefer beef that is reasonably marbled and juicy. The amount of intramuscular fat or marbling deposited in longissimus muscle is a major determinant of carcass value and predictor of palatability. Overall, the fatty acid composition of beef marbling fat is about 44% saturated fatty acids (SFA), 5% odd-chain fatty acids (OCFA), 45% monounsaturated fatty acids (MUFA), and 5% polyunsaturated fatty acids (PUFA) for beef marbling fat (Duckett, Wagner, Yates, Dolezal, & May,1993). Human diets containing a high proportion of lipid as MUFA have been shown to be as effective as those containing high levels of PUFA at lowering serum cholesterol levels (Mattson and Grundy, 1985; Mensink and Katan, 1989; Gustafsson, Vessby, Ohrvall, &Nydahl, 1994). The concentration of oleic acid is also positively correlated with overall palatability of beef (Westerling & Hedrick, 1979), which may be related to fat softness. Stearic acid (18:0) is a primary determinant of fat hardness (Smith, Yang, Larsen, &Tume, 1998; Wood et al., 2004; Chung et al., 2006), so any dietary or production factor that enhances the conversion of stearic acid to oleic acid will increase fat softness. Stearic acid is a saturated fatty acid; however, diets high in stearic acid have been shown to lower serum cholesterol compared to other saturated fatty acids (Denke & Grundy, 1991; Bonanome & Grundy, 1988). Stearic acid is believed to be converted to oleic acid after dietary ingestion

which accounts for its different effect on serum cholesterol compared to other saturated fats (Bonanome & Grundy, 1988). Research has demonstrated that high oleic acid ground beef may reduce risk factors for cardiovascular disease (Adams, Walzem, Smith, Tseng, & Smith, 2010; Gilmore et al., 2011, 2013).

The beef quality is determined by FA composition of feedstuffs. Moreover, shelf-life, palatability, and nutritive value of beef are affected by FA composition in the beef. For instance, oleic acid seems to be beneficial for reducing plasma total cholesterol and total low-density lipoprotein cholesterol in humans (Bonanome & Grundy, 1988), and it contributes to better taste panel evaluations of cooked beef (Dryden & Marchello, 1970). Challenges in increasing oleic acid content of ruminant tissues and products are of interest. In addition to the issues of the effects of unsaturated fatty acids (UFAs) on the stability and sensory acceptability of products, these FAs inhibit various essential anaerobic bacteria of the rumen, especially those involved in fiber digestion, biohydrogenation of UFAs, and methanogenesis (Palmquist & Jenkins, 1980). Therefore, supplementation of rice bran oil (RO) rich in C18:1n-9 would increase C18:1n-9 in muscle lipid.

Smith, Johnson, and Doumit (2010) demonstrated that oleic acid may have autocrine or paracrine effects in further stimulating marbling development and concluded that oleic acid is a critical factor in enhancing in marbling adipose tissue. Specific fatty acids in plasma or within the fat depots can promote marbling fat development while at the same time can cause muscle precursor cells to develop into marbling fat cells. Recently, Mirattanaphrai and Suksombat (2018) demonstrated an increase in C18:1n-9 in beef fat from crossbred Wagyu steers receiving 200 g/d RP-RO compared with those feeding control diet. Thus, the objective of the present study was to examine the effect of different level of rumen-protected rice bran oil

supplementation on performance and beef fatty acid profile of Wagyu crossbred beef steers.

4.3 Objectives

The objective of the present study was to examine the effect of different level of rumen-protected rice bran oil supplementation on performance and beef fatty acid profile of Wagyu crossbred beef steers.

4.4 Materials and methods

4.4.1 Animals and feeding

Twelve Wagyu crossbred fattening steers (50% Wagyu), averaging 509$\pm$3.2 kg live weight (LW) and approximately 28 mo old, were stratified by their LW into 3 groups and each group was randomly assigned to 3 dietary treatments. All steers were fed approximately 7.5 kg/d of 12% CP concentrate with *ad libitum* rice straw. The treatments were 1) control concentrate 2) control concentrate plus 100 g/d of rumen protected rice bran oil (100 RP-RO); 3) control concentrate plus 200 g/d of rumen protected rice bran oil (200 RP-RO). Rumen-protected plant oils were prepared by precipitation method (Garg, 1998) with minor modification. Briefly, 1 L of water was mixed with 100 g of acid oil, stirred vigorously for 5 min., then added 200 ml of 11% NaOH. The content were heated and stirred until the fatty acids were completely dissolved. While hot, the resulting blend was slowly added with 200 ml of 20% $CaCl_2$solution. The calcium soap formed was separated and washed with tap water. Excess water was removed by squeezing the calcium soap through muslin cloth.

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

The chemical compositions of concentrate, rice straw and rumen-protected rice bran oil used in the experiment are presented in Table 1 while the fatty acid composition of feeds and rumen-protected rice bran oil used in the present study are presented in Table 2. The basal diet was formulated to meet NRC (2000) requirements. All steers received *ad libitum* rice straw, had free access to clean water, were individually housed in a free-stall unit, and individually fed according to treatments. The experiment lasted for 80 days with the first 10 days was the adjustment period, followed by 70 days (5 periods of 14 d), of measurement period.

4.4.2 Fattening steers and slaughter procedures

At the end of feeding trial the animals were weighed, and all animals were transported to a commercial abattoir and then slaughtered at Ratchaburi slaughterhouse, Ratchaburi, 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**.**

4.4.3 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 550°C for 4 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.

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. In the boiled samples, shear forces were measured after cooling and drying. A steel hollow-core device with a diameter of 1.0x2.0x0.5 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 2 mm/sec and a 5kN load cell calibrated to read over a range of 0x100 N were applied.color measurements using a hunter lab (Color Quest XE, Kable, United Kingdom).

Samples of the LD and SM were minced and analyzed in duplicate for moisture, fat and protein contents according to AOAC (1995).

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, 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 100ºC for 5 min with occasional shaking and then cooled to room temperature. One ml of C17:0 internal standard (2.00 mg/mL in hexane) and 2 ml of boron trifluoride in methanol were added and heated at 100°C for 5 min with occasional shaking and 10 ml of deionized water were added. The solution was transferred to a 40 ml centrifuged tube and 5 ml of hexane were added for FAME extraction. The solution was centrifuged at 2,000 g, at 10°C for 20 min and then the hexane layer was dried over sodium sulfate and transferred into vial for analyzing by gas chromatography (GC) (7890A GC System, Agilent Technology, USA) equipped with a 100 m×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℃ 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.

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. Grilled 2.5-cm slices of LD and SM were cut into pieces of 1.3x 1.3 x 1.9 cm and served warm. Panelists were asked to grade samples for tenderness, juiciness, flavor and overall acceptability and assessments were given individually. 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.

4.4.5 Statistical analysis

All data were statistically analyzed as Completely Randomized Design using ANOVA procedure of SAS (SAS, 2001).

4.4.6 Experimental location

The experiment was conducted at Suranaree University of Technology's Cattle Farm, The Center for Scientific and Technological Equipment Building 3, 10, 11 and 14, Suranaree University of Technology.

4.4.7 Duration of the study

The study was from January to August 2017

4.5 Results

4.5.1 Feed Composition and performance

The concentrate was formulated to meet the requirement of the steers. In the concentrate, the main SFAs were C12:0 and C16:0 (19.38 and 19.06 g/100 g fat

respectively), whereas C18:1n-9 was the main MUFA (32.34 g/100 g fat) and C18:2n-6 was the main PUFA (16.89 g/100 g fat). Lipids from rice straw provided high proportions of C16:0 (45.30 g/100 g fat) and low proportions of C18:0 (1.01 g/100 g fat). RP-RO had the highest proportion of C18:1n-9 (47.46 g/100 g fat) and C18:2n-6 (33.46 g/100 g fat) (Table 4.2).

DM and CP intakes were not statistically altered by dietary treatments (Table 4.3); however, the animals supplemented with RP-RO had greater total fatty acid intake than that fed the control diets (P=0.001). With diets containing lower levels of added fat, Huerta-Leidenz et al. (1991) reported no influence on daily gain, intake or feed conversion ratio when dietary whole cotton seed of 15 or 30% (3.3 and 6.6% additional fat) was supplemented. In the present trial, fat contents of experimental diets were between 3.1 and 4.3%, it is unlikely that these levels of fat affected feed intake. When the consumption of individual fatty acid was calculated, the intakes of individual FA from C12:0 to C18:2n-6 increased with increasing RP-RO addition as well as SFA, MUFA and PUFA (Table 4.3). Cattle on 200 RP-RO diet ate more C18:3n-3 than those cattle on control and 100 RP-RO diets. The differences in individual FA intake reflected differences in FA composition of RP-RO added.

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Table 4.1 Chemical compositions of the experimental diets.

Items	Concentrate	RP-RO	Rice straw
Dry matter	**92.2**	**83.1**	**90.6**
	-----------------------% of DM------------------------		
Ash	10.9	15.04	15.9
Crude protein	13.7	-	2.6
Ether extract	4.8	82.1	1.1
Neutral detergent fiber	43.2	-	85.1
Acid detergent fiber	18.0	-	57.6
Neutral detergent in soluble N	1.0	-	0.5
Acid detergent insoluble N	0.9	-	0.4
Acid detergent lignin	9.9	-	6.4
TDN_{1X} (%)[2]	58.83	175.4	40.73
DE_{1X} (Mcal/kg DM)[3]	2.62	7.32	1.73
ME (Mcal/kg DM)[4]	2.12	4.55	1.62
NE_M (Mcal/kg DM)[5]	1.28	4.00	0.71
NE_G (Mcal/kg DM)[6]	0.71	3.39	0.15

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

[2]Total digestible nutrients, TDN_{1X} (%) = tdNFC + tdCP + (tdFA x 2.25) + tdNDF-7 (NRC, 2000)

[3]Digestible energy, DE_{1X} (Mcal/kg) = [(tdNFC/100)x4.2]+[(tdNDF/100) x 4.2]+[(tdCP/100) x 5.6]+[(FA/100) x 9.4] –0.3

[4]Metabolisable energy, ME = 0.82 x DE (NRC, 2000)

[5]Net energy for maintenance, $NE_M = 1.37ME - 0.138ME^2 + 0.0105ME^3 - 1.12$ (NRC, 2000)

[6]Net energy for growth, $NE_G = 1.42ME - 0.174ME^2 + 0.0122ME^3 - 1.65$ (NRC, 2000)

Table 4.2 Fatty acid compositions (g/100 g fat) of concentrate, rice straw and rumen-protected rice bran oil used in the experiment.

Fatty acids	Concentrate	Rice straw	RP-RO
C8:0	0.75	ND	ND
C10:0	1.08	ND	ND
C12:0	19.38	6.69	0.81
C14:0	6.39	9.57	1.59
C16:0	19.06	45.30	7.73
C18:0	3.49	1.01	4.54
C18:1	32.34	19.73	47.46
C18:2	16.89	12.68	33.46
C18:3	0.38	4.99	0.32
C20:0	0.21	ND	ND
Others	-	-	4.09
SFA[1]	50.38	62.60	14.67
MUFA[2]	32.34	19.73	47.46
PUFA[3]	17.27	17.67	33.78

[1] SFA = Sum of saturated fatty acid from C8:0-C20:0

[2] MUFA = Monounsaturated fatty acid from C18:1

[3] PUFA = Sum of polyunsaturated fatty acid from C18:2-C18:3

ND = Not detected.

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Table 4.3 DM, CP and fatty acid intake of Wagyu crossbred cattle fed rumen-protected rice bran oil.

Items	Control	100 RP-RO	200 RP-RO	SEM	P-value
DM intake, kg/d					
Concentrate	6.92	6.92	6.92	-	-
Rice straw	4.90	4.86	4.96	0.020	0.892
Protected oil	0	0.083	0.17		
Total	11.82	11.77	11.89	0.020	0.887
CP intake, g/d					
Concentrate	951	951	951	-	-
Rice straw	126	125	127	0.514	0.887
Total	1077	1076	1078	0.514	0.887
Fat intake, g/d					
Concentrate	335	335	335	-	-
Rice straw	51	51	52	0.210	0.887
Protected oil	0	68	137	-	-
Total	366[c]	434[b]	514[a]	0.210	0.001
NE intake, Mcal/d					
Concentrate	13.74	13.74	13.74	-	-
Rice straw	4.18	4.15	4.24	0.014	0.885
Protected oil	-	0.63	1.25	-	-
Total	17.93[c]	18.52[b]	19.25[a]	0.016	0.001

RP-RO = rumen-protected rice bran oil; SEM = standard error of the mean

SFA = sum of C8:0-C18:0; MUFA = C18:1; PUFA = sum of C18:2 and C18:3

[a, b,c] Mean within row which different superscripts differ ($P<0.05$), SEM is standard error of mean

Table 4.3 DM, CP and fatty acid intake of Wagyu crossbred cattle fed rumen-protected rice bran oil (continue).

Items	Control	100 RP-RO	200 RP-RO	SEM	P-value
Fatty acid intake, g/d					
C8:0	2.00	2.00	2.00	-	-
C10:0	2.87	2.87	2.87	-	-
C12:0	54.89[c]	55.41[b]	56.03[a]	0.013	0.001
C14:0	21.72[c]	22.75[b]	23.94[a]	0.019	0.0002
C16:0	72.96[c]	77.96[b]	83.71[a]	0.090	0.0002
C18:0	9.79[c]	12.85[b]	15.93[a]	0.001	0.0001
C18:1n-9	95.78[c]	127.66[b]	160.01[a]	0.039	0.0001
C18:2n-6	51.19[c]	73.68[b]	96.47[a]	0.025	0.0001
C18:3n-3	3.46[b]	3.65[b]	3.93[a]	0.010	0.023
Total	314.68[c]	378.86[b]	444.89[a]	0.200	0.0001
SFA	164.24[c]	173.85[b]	184.47[a]	0.125	0.0001
MUFA	95.78[c]	127.67[b]	160.01[a]	0.039	0.0001
PUFA	54.66[c]	77.34[b]	100.40[a]	0.035	0.0001

RP-RO = rumen-protected rice bran oil; SEM = standard error of the mean

SFA = sum of C8:0–C18:0; MUFA = C18:1; PUFA = sum of C18:2 and C18:3

[a, b,c] Mean within row which different superscripts differ ($P<0.05$), SEM is standard error of mean

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4.5.2 Carcass quality traits.

Slaughter weight, warm carcass weight, % warm carcass, cold carcass weight and % cold carcass were not statistically significantly different among treatments (Table 4). Mirattanaphrai and Suksombat (2018) also reported similar result when cattle were fed different RP-plant oils. No remarkable changes were found for loin eye area (LEA) and back fat thickness (Table 4.4). The eye muscle area can be

used as a representative measure of the quantity, quality, and distribution of the muscle mass. Similarly, Zinn, Gulati, Plascencia, and Salinas (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. However, beef marbling score (BMS) of cattle fed 200 RP-RO was greater than other cattle with BMS of cattle on 200 RP-RO > 100 RP-RO > control. Beef Marbling score was the best single trait predictor of beef tenderness. The increases in BMS of LD and SM muscle fats was related to lowered shear force values (Table 5). Although sensory tenderness of both muscles were not statistically significantly different, there was a tendency towards higher sensory tenderness score for SM muscle (P=0.05; Table 4.5).

Beef quality

The cooking loss corresponds to the loss of water plus a small portion of fat, protein and minerals. No treatment effects were found on moisture cooking loss (Table 4.5) in the present study. Cooking loss values are related to several factors, such as pH, slow post-mortem glycolysis, rapid cooling of the carcass before storage. The moisture and protein contents in LD and SM muscles were not significantly different (P>0.05) among treatments (Table 4.5), however, the fat content in LD muscles of cattle fed 200 RP-RO diets were greater than those fed control and 100 RP-RO diets (P=0.026). The amounts of fat in the muscle typically result from a balance between dietary energy and metabolic requirements of the animal (Oliveira et al., 2012). If energy intake is higher than its metabolic demands, this excess will be storage as fat. The literature suggests that the total protein content is less variable in bovine meat, with values of approximately 20% observed in the *longissimus dorsi* muscle without the fat cover, and this is independent of food, breed, the genetic group, and the physiological condition (Marques et al., 2006).

Beef tenderness is a trait considered to be of great relevance for consumers while shear force is an objective measure of tenderness. The present study revealed that shear forces of both LD and SM muscles were lower in beef from 200 RP-RO and 100 RP-RO cattle (P=0.018 and P=0.001, respectively; Table 4.5). Bovine meat is considered to have an acceptable tenderness if its shear strength values are below 8 N (Swan, Esguerra, & Farouk, 1998). 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. The present trial found shear force values between 2.15 and 2.75 kg/cm^2 in LD muscle and between 3.60 and 4.32 in SM muscle which were considered to be tender (Table 4.5). These values were closely related to the values obtained from sensory perception of tenderness by trained panelists (4.57 to 5.82 in LD muscle and 3.32 to 5.65 in SM muscle; Table 4.5). Such variations in the shear force values may be caused by differences in the thicknesses of the blades utilized in the analysis.

Beef color remained mostly unaffected by treatment with the exception of higher lightness (L*) on LD (P=0.028) muscle originating from the 200 RP-RO supplement than other groups (Table 5). Values encountered in literature for L*, a* and b* were used to measure beef color 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. Values obtained in the present study were within the range given.

The sensory tenderness and beef flavor both in LD and SM muscles were unaffected by treatments (Table 4.5), however, cattle on 200 RP-RO diet showed significantly more juicy (P=0.035) in SM muscle and tended to have more juicy (P=0.094) in LD muscle than those on control diet. The sensory overall acceptability

was not significantly different in both LD and SM muscles. 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 color stability were more closely associated with fatty acid composition and greater abnormal flavors and rancidity scores (Scollan et al., 2006).

Table 4.4 Initial weight, final weight, live weight change and beef characteristics of beef from Wagyu crossbred cattle fed rumen-protected rice bran oil.

Items	Control	100 RP-RO	200 RP-RO	SEM	P-value
Initial weight (kg)	510	505	511	2.612	0.642
Final weight (kg)	593	590	597	3.218	0.723
Average daily gain (kg/d)	1.19	1.21	1.23	0.021	0.537
Slaughter weight (kg)	559	557	563	3.854	0.684
Warm carcass weight (kg)	315	315	311	3.389	0.931
% warm carcass	56.35	56.55	55.24	0.243	0.684
Cold carcass weight (kg)	304	301	304	3.03	0.861
% cold carcass	54.33	53.98	54.06	0.261	0.700
Marbling score[1]	3.27[c]	4.02[b]	4.65[a]	0.026	0.0001
Loin eye area (cm^2)	73.80	73.73	73.86	0.466	0.927
Back fat thickness (cm)	0.79	0.82	0.84	0.046	0.535

RP-RO = rumen-protected rice bran oil; SEM = standard error of the mean

[1] 1 = very scarce, 12 = very abundant (Japanese Meat Grading Association)

[a, b,c] Mean within row which different superscripts differ ($P<0.05$), SEM is standard error of mean

Table 4.5 Beef chemical composition, sensory and physical evaluations of beef from Wagyu crossbred cattle fed rumen-protected rice bran oil.

Items	Control	100 RP-RO	200 RP-RO	SEM	P-value
Longissimus dorsi					
Moisture cooking loss (%)	24.50	24.69	23.90	0.376	0.320
Moisture content (%)	71.46	71.59	71.12	0.164	0.556
Crude protein (%)	21.60	21.05	21.67	0.273	0.770
Fat (%)	4.64^{b}	4.86^{b}	5.39^{a}	0.066	0.026
Shear force (kg/cm^2)	2.75^{a}	2.42^{b}	2.15^{b}	0.029	0.018
L*	37.01^{b}	36.62^{b}	39.62^{a}	0.307	0.028
a*	8.95	8.30	9.22	0.527	0.843
b*	5.93	6.49	6.55	0.352	0.930
Tenderness	4.87	5.60	5.27	0.111	0.149
Juiciness	4.97	5.72	5.77	0.118	0.094
Beef flavor	4.95	5.02	4.77	0.173	0.323
Overall acceptability	5.45	5.97	5.55	0.127	0.365
Semimembranosus					
Moisture cooking loss (%)	25.19	24.43	25.83	0.304	0.069
Moisture content (%)	70.78	71.56	70.70	0.128	0.807
Crude protein (%)	21.28	20.80	21.05	0.171	0.232
Fat (%)	4.82	4.96	5.26	0.081	0.164
Shear force (kg/cm^2)	4.32^{a}	3.63^{b}	3.60^{b}	0.036	0.001
L*	38.78	38.71	42.22	0.586	0.119
a*	8.67	9.31	9.48	0.189	0.186
b*	6.65	7.26	7.69	0.165	0.101
Tenderness	4.65	4.75	5.50	0.104	0.055
Juiciness	3.50^{b}	3.71^{b}	4.65^{a}	0.123	0.035
Beef flavor	4.71	4.84	5.12	0.098	0.385
Overall acceptability	5.09	5.18	5.87	0.100	0.064

RP-RO = rumen-protected rice bran oil; SEM = standard error of the mean.
Tenderness, Juiciness, Beef flavor and overall acceptability: 1 = extremely though, dry, bland, and less accept respectively; 8 = extremely tender, juicy, intense and more accept respectively.
[a, b,] Mean within row which different superscripts differ ($P<0.05$), SEM is standard error of mean

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4.5.3 Fatty acid composition of beef

In the current study, 200 RP-RO-containing diets resulted in marked alternations in both LD and SM beef C18:1, SFA, MUFA and PUFA composition relative to the diet added none RP-RO (Table 4.6). To compare with control diet, RP-RO diets had no effect on C10:0 – C24:0 SFAs in LD and SM muscles with the exception for reduced C16:0 in LD muscle. However, in LD muscle, RP-PO diets induced to significantly decrease C20:3n-6 (P=0.004). The increase in concentrations of C18:1n-9 in LD muscle by 200 RP-RO supplementation may be explained by a high intake of C18:1n-9 (160.01 g/d). The concentration of C18:1n-9 was respectively 43.38 and 46.01% of total fatty acid in LD and SM muscles.

In LD muscle fat, 200 RP-RO diet showed a marked increase in MUFA (P=0.029) but significantly reduced SFA and PUFA (P=0.041 and 0.046 respectively; Table 4.6). Both RP-RO diets significantly increased MUFA (0.024) but decreased SFA (0.017) in SM muscle fat. Typically, SFA, MUFA and PUFA levels in intramuscular fat range from 45 to 48, 35 to 45 and up to 5.0 g/100 g, respectively (Scollan et al., 2006). However, dietary inclusion of supplemental fat as RP-RO altered the pattern toward more UFA. This leads to slightly higher UFA: SFA ratio in RP-RO-supplemented diet as compared to control. The PUFA: SFA ratio is used to evaluate the nutritional value of fat for human consumption. Increasing the PUFA content of the diet, by including sources rich in PUFA, generally improves the PUFA: SFA ratio and in all diets where PUFA: SFA ratio was always lowered than 0.29 (Table 4.6 and Table 4.7), which the minimum value recommended for the human diet is 0.45 (BDH, 1994). The C18:2n-6 was the most concentrated PUFA across treatment. The lack of dietary effects on total PUFA in SM muscle indicates that RP-RO addition had no effect on rates of lipolysis in the rumen. However, the higher total PUFA found in LD when

feeding 200 g/d RP-RO may indicate that either the rate of lipolysis and/or the initial step in C18:1n-9 bio-hydrogenation was reduced. 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, and this is likely due to extensive bio-hydrogenation of PUFA to C18:0, and potentially to reduced delta-9 desaturase activity when feeding PUFA rich oils (Waters, Kelly, O'Boyle, Moloney, & Kenny, 2009). The predominant SFA across all diets in LD and SM muscles was C16:0, followed by C18:0 and C14:0. These results could again suggest that C18:1n-9 and its bio-hydrogenation intermediates were less effective at down-regulating SCD activity than C18:2n-6. SFA relates to changes in endogenous FA synthesis that may not have been differentially affected by diet (Mapiye et al., 2013). Oliveira et al. (2012) with feeding different oils reported lower percentages (about 45%) of SFA. 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).

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Table 4.6 Fatty acid composition (g/100 g fat) of *Longissimus dorsi* muscle from Wagyu crossbred cattle fed rumen-protected rice bran oil.

Items	Control	100 RP-RO	200 RP-RO	SEM	P-value
No. of cattle	**4**	**4**	**4**		
Longissimus dorsi					
C10:0	0.11	0.10	0.09	0.011	0.875
C12:0	0.07	0.06	0.06	0.007	0.969
C14:0	2.63	2.46	2.28	0.100	0.207
C14:1	0.81	0.58	0.28	0.086	0.394
C15:0	0.21	0.19	0.16	0.022	0.937
C16:0	27.71[a]	27.08[a]	24.39[b]	0.235	0.008
C16:1	2.28	2.41	2.27	0.266	0.982
C18:0	19.20	19.54	17.89	0.970	0.715
C18:1n9t	3.02	2.78	2.71	0.253	0.809
C18:1n9c	37.10	37.99	43.88	0.858	0.061
C18:2n6t	1.06	0.98	0.74	0.065	0.465
C18:2n6c	3.23	3.32	3.40	0.163	0.415
C18:3n6	0.21	0.22	0.23	0.007	0.685
C20:1	0.25	0.28	0.23	0.022	0.721
C18:3n3	0.33	0.36	0.29	0.038	0.648
CLA c9,t11	0.37	0.38	0.35	0.002	0.416
CLA t10,c12	0.005	0.005	0.005	0.090	0.249
C20:3n6	0.45[a]	0.37[b]	0.27[c]	0.001	0.004
C20:4n6	0.78	0.68	0.35	0.024	0.376
C24:0	0.11	0.13	0.08	0.002	0.222

RP-RO = rumen-protected rice bran oil; SEM = standard error of the mean
[1] Sum of saturated fatty acid from C10:0 – C24:0
[2] Sum of unsaturated fatty acid from MUFA, and PUFA
[3] Sum of monounsaturated fatty acid from C16:1 – C20:1
[4] Sum of polyunsaturated fatty acid
[5] Sum of CLA from CLA c9,t11 and CLA t10,c12
[a,b,c] Mean within row which different superscripts differ (P<0.05); SEM = Standard error of mean

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Table 4.6 Fatty acid composition (g/100 g fat) of *Longissimus dorsi* muscle from Wagyu crossbred cattle fed rumen-protected rice bran oil (continue).

Items	Control	100 RP-RO	200 RP-RO	SEM	P-value
No. of cattle	**4**	**4**	**4**		
SFA[1]	50.05[a]	49.60[a]	44.94[b]	0.305	0.041
UFA[2]	49.95[b]	50.40[b]	55.06[a]	0.305	0.042
MUFA[3]	43.48[b]	44.06[b]	49.40[a]	0.301	0.029
PUFA[4]	6.46[a]	6.33[a]	5.66[b]	0.064	0.046
Total CLA[5]	0.375	0.385	0.355	0.013	0.194
UFA:SFA	0.998	1.016	1.225	0.088	0.234
PUFA:SFA	0.129	0.128	0.126	0.006	0.438

RP-RO = rumen-protected rice bran oil; SEM = standard error of the mean

[1] Sum of saturated fatty acid from C10:0 – C24:0

[2] Sum of unsaturated fatty acid from MUFA, and PUFA

[3] Sum of monounsaturated fatty acid from C16:1 – C20:1

[4] Sum of polyunsaturated fatty acid

[5] Sum of CLA from CLA c9,t11 and CLA t10,c12

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

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Table 4.7 Fatty acid composition (g/100 g fat) of *Semimembranosus* muscle from Wagyu crossbred cattle fed rumen-protected rice bran oil.

Items	Control	100 RP-RO	200 RP-RO	SEM	P-value
No. of cattle	**4**	**4**	**4**		
Semimembranosus					
C10:0	0.095	0.090	0.094	0.011	0.922
C12:0	0.050	0.053	0.052	0.006	0.720
C14:0	2.59	2.49	2.31	0.075	0.318
C14:1	0.61	0.51	0.52	0.118	0.926
C15:0	0.17	0.18	0.19	0.017	0.841
C16:0	26.85	25.26	24.71	0.293	0.289
C16:1	3.63	3.33	3.43	0.196	0.784
C18:0	14.58	14.71	12.31	0.681	0.509
C18:1n9t	2.52	2.22	2.59	0.162	0.427
C18:1n9c	41.93[b]	44.06[ab]	46.01[a]	0.373	0.039
C18:2n6t	1.48	1.53	1.43	0.111	0.842
C18:2n6c	2.73	2.90	3.48	0.176	0.567
C18:3n6	0.27	0.28	0.30	0.007	0.877
C20:1	0.19	0.22	0.23	0.015	0.847
C18:3n3	0.45	0.43	0.46	0.033	0.312
CLA c9,t11	0.37	0.36	0.37	0.009	0.543
CLA t10,c12	0.005	0.007	0.005	0.003	0.472
C20:3n6	0.47	0.45	0.47	0.006	0.873
C20:4n6	0.82	0.75	0.88	0.032	0.949
C24:0	0.13	0.12	0.14	0.003	0.738
SFA[1]	44.49[a]	42.92[b]	39.82[c]	0.197	0.017
UFA[2]	55.51[c]	57.08[b]	60.18[a]	0.116	0.027
MUFA[3]	48.90[c]	50.35[b]	52.79[a]	0.138	0.024
PUFA[4]	6.61	6.73	7.39	0.298	0.732
Total CLA[5]	0.375	0.367	0.375	0.004	0.683
UFA:SFA	1.248	1.330	1.511	0.092	0.203
PUFA:SFA	0.149	0.157	0.186	0.020	0.391

RP-RO = rumen-protected rice bran oil; SEM = standard error of the mean

[1] Sum of saturated fatty acid from C10:0–C24:0

[2] Sum of unsaturated fatty acid from MUFA, and PUFA

[3] Sum of monounsaturated fatty acid from C16:1–C20:1

[4] Sum of polyunsaturated fatty acid

[5] Sum of CLA from CLA c9,t11 and CLA t10,c12

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

4.6 Conclusion

Feeding dietary treatment that inclusion of 100 or 200 g/d of rumen-protected rice bran oils did not negatively affect any of performance and carcass quality of steers. The overall feed consumption of the steers was unaffected when dietary rumen-protected rice bran oil was provided. RP-RO supplement did also not influence muscle sensory and physical characteristics with the exception of an increase in beef tenderness score of both LD and SM fat. RP-RO increased the percentage of C18:1n-9 in SM fat and increased beef marbling score. Thus, it can be concluded that RP-RO addition can be safety supplemented to diets of steers to enrich beef with potential health beneficial FA.

4.7 References

Adams, T. H., Walzem, R. L., Smith, D. R., Tseng, S., and Smith, S. B. (2010). Hamburger high in total, saturated and trans-fatty acids decreases HDL cholesterol and LDL particle diameter, and increases plasma TAG, in mildly hypercholesterolaemic men. **British Journal Nutrition**. 103: 91-98.

Association of Official Analytical Chemists. (1995). **Official Method of Analysis**. Washington DC, USA.

Bonanome, A., and Grundy, S. M. (1988). Effect of dietary stearic acid on plasma cholesterol and lipoprotein levels. **New England Journal of Medicein**. 318: 1244.

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

มหาวิทยาลัยเทคโนโลยีสุรนารี

Chung, K. Y., Lunt, D. K., Choi, C. B., Chae, S. H., Rhoades, R. D., Adams, T. L., Booren, B., and Smith, S. B. (2006). Lipid characteristics of subcutaneous adipose tissue and M. longissimus thoracis of Angus and Wagyu steers fed to U.S. and Japanese endpoints. **Meat Science**. 73: 432-441.

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.

Denke, M.A., and Grundy, S. M. (1991). Effects of fats high in stearic acid on lipid and lipoprotein concentrations in men. **American Journal Clinical Nutrition**. 54:1036.

Dryden, F.D., and Marchello, J. A. (1970). Influence of total lipid and fatty acid composition upon the palatability of three bovine muscles. **Journal of Animal Science**. 31: 36.

Duckett, S. K., Wagner, D. G., Yates, L. D., Dolezal, H. G., and May, S. G. (1993). Effects of time on feed on beef nutrient composition. **Journal of Animal Science**. 71: 2079.

Folch, J., Lees, M., and Sloane-Stanley, G.H) .1957 .(A simple method for the isolation and purification of total lipids from animal tissues .**Journal of Biological Chemistry**.226 (1): 495-509.

Garg, M. R. (1998). Effect of feeding bypass fat on rumenfermentation, DM digestibility and N balance in sheep. **IndianVetarinary Journal**.75: 800-802.

Gilmore, L. A., Walzem, R. L., Crouse, S. F., Smith, D. R., Adams, T. H., Vaidyananthan, V., Cao, X., and Smith, S. B. (2011). Consumption of high-oleic acid ground beef increases HDL cholesterol concentration but both high-

and low-oleic acid ground beef decrease HDL particle diameter in normocholesterolemic men. **Journal of Nutrition**. 141: 1188-1194.

Gilmore, L. A., Crouse, S. F., Carbuhn, A., Klooster, J., Calles, J. A. E., Meade, T., and Smith, S.B. (2013). Exercise attenuates the increase in plasma monounsaturated fatty acids and high-density lipoprotein but not high-density lipoprotein 2b cholesterol caused by high-oleic ground beef in women. **Nutrition Research**. 33: 1003-1011.

Gustafsson, I.B., Vessby, B., Ohrvall, M., and Nydahl, M. (1994). A diet rich in monounsaturated rapeseed oil reduced the lipoprotein cholesterol concentration and increases the relative content of n-3 fatty acids in serum in hyperlipidemicsubjects.**American Journal Clinical Nutrition**. 59: 667.

Huerta-Leidenz, N. O., Cross, H. R., Lunt, D. K., Pelton, L. S., Savell, J. W., and Smith, S. B. (1991). Growth, carcass traits and fatty acid profiles of adipose tissues from steers fed whole cottonseed. **Journal of Animal Science**. 69: 3665-3672.

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

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 Science**. 95: 98-109.

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

Mattson, F.H., and Grundy, S. M. (1985). Comparison of effects of dietary saturated, monounsaturated, and polyunsaturated fatty acids on plasma lipids and lipoproteins in man. **Journal of Lipid Research**. 26: 194.

Mensink, R.P., and Katan, M. B. (1989). Effect of a diet enriched with monounsaturated or polyunsaturated fatty acids on levels of low-density and high-density lipoprotein cholesterol in healthy women and men. **New England Journal of Medicein**. 321: 436.

Mirattanaphrai, R. and Suksombat, W. (2018). Fatty acid profile of beef from crossbred Wagyusteers fed high oleic acid rumen-protected fat. **Suranaree Journal of Science and Technology.** (in press).

NRC (National Research Council). (2000). Nutrient Requirements of Beef Cattle, 7th Rev. Ed. Update. **National AcademyPress**. Washington DC, USA.

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 Science**. 90: 28-35.

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., and Jenkins, T. (1980). Fat in lactations rations: Review. **Journal of Dairy Science**. 63: 1-14.

SAS (Statistical Analysis System). (2001). **SAS User' Guide: Statistics**. SAS Institute Inc., 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 receiving different forms of soybean oil in the rumen.**Animal Feed Science and Technology**. 194: 35-43.

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 Science**. 74: 17-33.

Smith, S. B., Yang, A., Larsen, T. W., and Tume, R. K. (1998). Positional analysis of triacylglycerols from bovine adipose tissue lipids varying in degree of unsaturation. **Lipids**. 33: 197-207.

Smith, S. B., Johnson, B., and Doumit, M. (2010). Regulation of marbling development in beef cattle by specific fatty acids. **Project Summary**, National Cattlemen's Beef Association.

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

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 Ruminant Research**. 28: 273-280.

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 nutrition. **Journal of Dairy Science**. 74(10): 3583-3597.

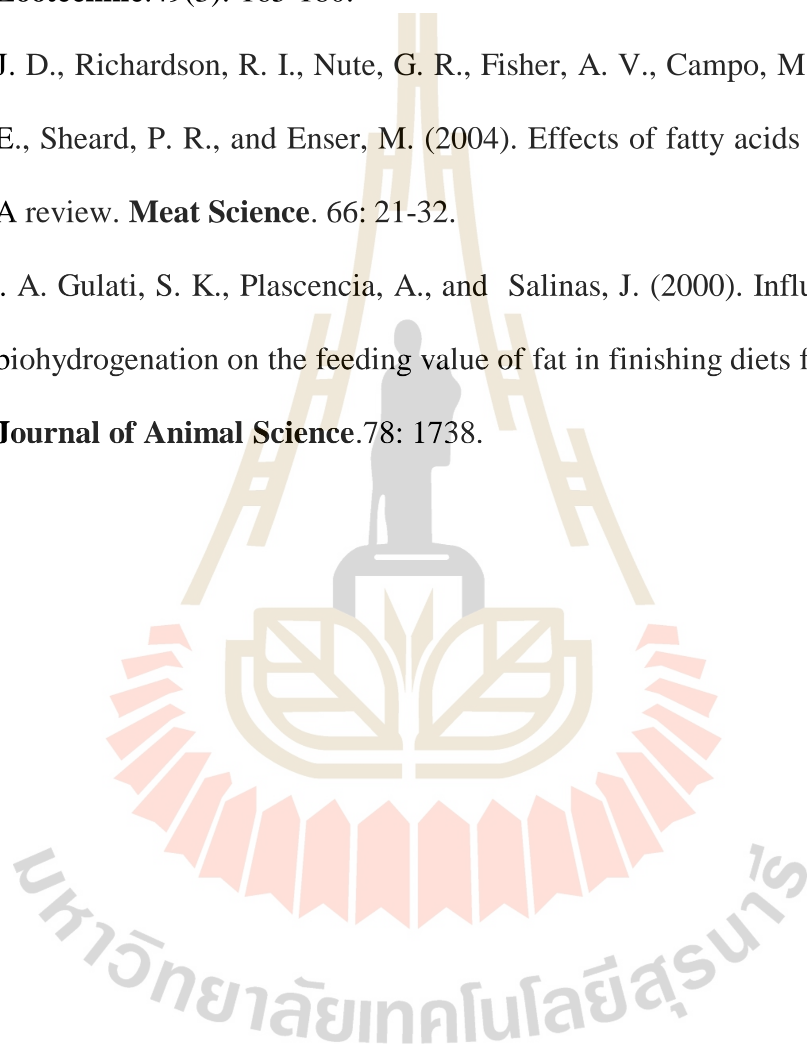
Waters, S. M., Kelly, J. P., O'Boyle, P., Moloney, A. P., and Kenny, D. A. (2009). Effect of level and duration of dietary n-3 polyunsaturated fatty acid supplementation on the transcriptional regulation of Δ9-desaturase in muscle of beef cattle. **Journal of Animal Science**. 87(1): 244-252.

Westerling, D.B., and Hedrick, H. B. (1979). Fatty acid composition of bovine lipids as influenced by diet, sex and anatomical location and relationship to sensory characteristics. **Journal of Animal Science**. 48: 1343.

Williams, C. (2000). Dietary fatty acids and human health. **Annales de Zootechnie**.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 Science**. 66: 21-32.

Zinn, R. A. Gulati, S. K., Plascencia, A., and Salinas, J. (2000). Influence of ruminal biohydrogenation on the feeding value of fat in finishing diets for feedlot cattle. **Journal of Animal Science**.78: 1738.



CHAPTER V

EFFECTS OF RUMEN PROTECTED FAT CONTAINING OLEIC ACID SUPPLEMENTATION ON RUMEN FERMENTATION IN FISTULATEDCATTLES

5.1 Abstract

The aim of this experiment was to study the effects of rumen protected fat containing oleic acid including palm oil, rice bran oil, and corn oil supplementation on ruminal fermentation in fistulated cattle. The treatments were: 1) control concentrate 2) control concentrate plus 200 g/d of rumen protected palm oil (RP-PO); 3) control concentrate plus 200 g/d of rumen protected rice bran oil (RP-RO), 4) control concentrate plus 200 g/d rumen protected corn oil (RP-CO). Each period in the Latin square design lasted 21 d, with the first 7 d for adaptation. The results found that supplementation of rumen-protected containing oleic acid including palm oil, rice bran oil, and corn oil had no effects on pH, ammonia nitrogen, VFA, DMD, and CPD when compare to non-supplemented control. However, at 4 and 6 hours after feeding, there was a change in the proportion of fatty acids in the rumen. It was found that the proportion of ruminal C16:0 in cattle fed RP-PO diet was significantly higher than those cattle fed RP-RO and RP-CO diets. The proportion of ruminal C18:1n-9c was significantly higher in RP-RO cattle than in RP-PO and RP-CO cattle while the ruminal proportion of C18:2n-6c was significantly higher in RP-CO cattle than RP-PO and RP-RO cattle.

5.2 Introduction

Bio-hydrogenation process occurs in the rumen due to microorganisms that prevent their toxicity by converting unsaturated fatty acids into saturated fatty acids which final product is stearic acid (Stephen et al., 2006). Nowadays, various researches related to the prevention of bio-hydrogenation process. In addition, research in beef cattle found that supplementation of rumen-protected fat could increase the C18:1n-9c concentration in the meat, which resulted in increased marbling in the cattle (Felton et al., 2004; Gillis et al., 2004; Andrae et al., 2001). Fat supplementation in ruminants must be in the form of protected fat that fat is not digested in the rumen but is digested in the digestive tract. The process of making bypass fat is the process of saponification which is to make fat or fatty acids in the form of Ca-soap of long chain fatty acids. This product is less digestible at normal pH in the rumen, so it does not interfere with the microbial activity, but when it comes to the low pH, this soap is broken down into free fatty acids that are further digested in the small intestine (McDonald et al., 1995; Pond et al., 2005).

Conversion of FAs in the rumen by various ruminal microbes is rarely completed (Harfoot and Hazelwood, 1988). It is recognized that a species of bacteria cannot process activity for all step of bio-hydrogenation. Kemp and Lander (1984) reported that groups of bacteria should be balanced to complete conversion of UFAs. Ruminal bacteria can be classified as group A and group B bacteria (Kemp and Lander, 1984). Group A bacteria are those bacteria that can convert linoleic and α-linolenic to *trans*-11-octadecenoic acid but cannot hydrogenate octadecenoic acids whereas group B convert oleic, *trans*-11 C18:1 and linoleic acids to stearic acid. In the studies using mixed ruminal microbes, oleic acid may hydrate, hydrogenate or

isomerize (Kemp et al., 1975; Harzlewood et al., 1976; Hudson et al., 1995). *Fusocillu sbabrahamensis*P2/2, *Fusocillus*T344 and R8/5 Gram-negative rod are only 3 species that converted oleic acid to stearic acid. While *F. Babrahamensis* P2/2 converted oleic acid to hydroxyl stearic acid (Kemp et al., 1975; Harzlewood et al., 1976) *Fusocillus*T344 converted oleic acid to *trans*-11 C18:1 (Kemp et al., 1975; Harzlewood et al., 1976). *Butyrivibrio fibrisolvens* could not converted oleic acid (Kepler et al., 1966). However, when added purified oleic acid to culture containing mixed ruminal microbes, oleic acid is converted to stearic acid and small amount to *trans* C18:1 (Ward et al., 1964) Hudson et al. (1995) indicated that *Selenomonas ruminatium* and *Enterococcus faecalis* can also convert oleic acid to10-hydroxy stearic acid.

CLA contains *cis*-9,*trans*-11 CLA 75-90%which is synthesized from linoleic acid and α-linolenic acid (Bauman et al., 2003). Linoleic acid (*cis*-9,*cis*-12 C18:2) is isomerized to *cis*-9,*trans*-11 CLA by *cis*-12,*trans*-11 isomerase in the first step, then is hydrogenated by *B. Fibrisolvens* to vaccenic acid (VA, *trans*-11 C18:1) in the rumen (Kepler and Tove, 1967). Loor et al. (2002) reported that hydrogenation of VA to stearic acid involved many group of bacteria and rate of hydrogenation was very low (Harfoot and Hazelwood, 1997). As a result, VA therefore accumulated in the rumen. This *trans* FA is used to synthesize *cis*-9,*trans*-11 CLA, which is the function of Δ9-desaturase that desaturateVA from the rumen in animal tissues (Griinari et al., 2000; Piperova et al., 2000). The occurrence of hydrogenation involved the protection of toxic which influenced on *B. fibrisolvens*. The toxicity from PUFAs occurred in different level depending on type of FAs, for example, linoleic acid has more toxic than linoleic or CLA (Maia et al., 2006). After hydrogenation by *B. fibrisolvens*, *cis*-

9,*trans*-11CLA accumulated because microbes lack of ability to hydrogenate CLA to vaccenic acid (*trans*-1118:1) (Jenkins et al., 2008). Thus, the objective of the present study was to examine the effect of RP-PO, RP-RO and RP-CO supplementation on ruminal fermentation in fistulated cattle.

5.3 Objectives

To study the effects of rumen protected fat containing oleic acid including palm oil,rice bran oil and corn oil supplementation on ruminal fermentation in fistulated cattle.

5.4 Materials and methods

5.4.1 Animals and feeding

Four fistulated cattle were assigned in 4 treatments in a 4×4 Latin square design. All cattle were fed approximately 4 kg/d of 12% CP concentrate and 4 kg/d of rice straw. Treatments were: 1) control concentrate 2) control concentrate plus 200 g/d of RP-PO 3) control concentrate plus 200 g/d of RP-RO 4) control concentrate plus 200 g/d of RP-CO. Ingredients of concentrate and chemical composition of concentrate and rice straw used in the experiment are presented in Table 5.1 while the fatty acid composition of feed and oils used in the present study are presented in Table 5.2. All cattle also had free access to clean water and were individually housed in a free-stall unit and individually fed according to treatments. The experiment lasted for 84 days (4 periods) with 21 d in each period, the first 7 d of each period for adaptation to diets followed by 14 d for ruminal sample collection and *in sacco* disappearance trial.

5.4.2 Sample Collection

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

5.4.3 Laboratory Analyses

5.4.3.1 Feed chemical composition analysis

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

furnace at 600°C for 3 h. The chemical analysis was expressed on the basis of the final DM.

5.4.3.2 Ruminal fermentation

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. The pH of rumen fluid were immediately determined at the time of sampling by pH meter. For VFAs and ammonia N determination, 36 ml of rumen fluid was put into 50 ml centrifuge tube containing 4 ml of 1M H_2SO_4, then centrifuged at1895 rpm for 15 min. Supernatant was collected and put into 25 ml test tube, then capped and stored at -20°C until analysis. Analysis of acetic, propionic and butyric acids used GC (Hewlett Packard GC system HP6890, USA, 19091N-113 INNOWAX , Length (meters) 30, I.D. (mm) 0.32 WIDEBORE, Film (um) 0.25). Ammonia N concentrations was determined by Kjeldahl analysis (AOAC, 1995).

5.4.3.3 Analysis of fatty acids in ruminal content

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

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

5.4.3.4 Degradability Determination of DM, CP

Concentrate and rice straw were ground through a 2 mm screen for *in sacco* ruminal disappearance determination. Approximately 5 g of 2 mm ground samples were placed into 8 x 11 cm nylon bags with 47 µm pore size and suspended in the rumen of each fistulated cattle for 0 (pre feeding), 2, 4, 6, 12, 24, 48 (concentrate) and 72 h (rice straw), and were then removed and washed in water and then dried at 65°C for 48 h. After weighing each bag individually, the residues were

then subjected to DM determination. The contents of the bags were then assayed for CP content (CPD).

5.4.4 Statistical Analysis

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

5.4.5 Experimental location

The experiment was conducted at Suranaree University of Technology's Cattle Farm, The Center for Scientific and Technological Equipment Building 10,and 14,Suranaree University of Technology.

5.4.6 Duration

The duration of the present experiment was from October to December 2017.

5.5 Results and Discussion

5.5.1 Feed Composition and performance

The chemical and fatty acid composition of the feeds are presented in Table 5.1 and 5.2, and the concentrate was formulated to meet the requirement of the steers.

Lipids from rice straw provided high proportions of C16:0 (45.30 g/100 g fat) and low proportions of C18:0 (1.01 g/100 g fat). Ca-PO had the highest proportion of C16:0 (46.44 g/100 g fat) while Ca-RO had the highest proportion of C18:1n9 (47.45 g/100 g fat). In the concentrate, the main SFA was C16:0 (19.58 g/100 g fat), whereas

C18:1n-9 was the main MUFA in Ca-PO, Ca-RO and Ca-CO (34.70, 47.46 and 36.26 g/100 g fat respectively), C18:2n-6 was the main PUFA in concentrate (14.61 g/100 g fat) (Table 5.2).

No significant difference was found for CP intakes among groups (Table 5.3); however, the animals supplemented with rumen-protected plant oil had greater DM intakes and total fatty acid intake than that fed the control diets (P=0.0001). With diets containing lower levels of added fat, Huerta-Leidenz et al. (1991) reported no influence on daily gain, intake or feed conversion ratio when dietary whole cotton seed of 15 or 30% (3.3 and 6.6% additional fat) was supplemented. In the present trial, fat contents of experimental diets were between 3.1 and 4.3%, it is unlikely that these levels of fat affected feed intake. When the consumption of individual fatty acid was calculated, cattle on RP-PO diet significantly consumed more C12:0, C14:0, C16:0, and C18:0 than other cattle (Table 5.2) while those on RP-RO diet ate more C18:3 and on RP-CO significantly consumed more C18:1, and C18:2 than other cattle. Additionally, the cattle on control diet significantly ate less C18:1 than those cattle on dietary fat diets.

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Table 5.1 Chemical compositions of the experimental diets.

Items	Concentrate	RP-PO	RP-RO	RP-CO	Rice straw
Dry matter	92.2	83.3	83.1	83.1	90.6
		--------------------% of DM------------------			
Ash	11.9	15.01	15.04	15.03	15.9
Crude protein	13.3	-	-	-	2.6
Ether extract	4.6	82.3	82.1	81.5	1.1
Neutral detergent fiber	46.5	-	-	-	85.1
Acid detergent fiber	30.2	-	-	-	57.6
Neutral detergent in soluble N	0.9	-	-	-	0.5
Acid detergent insoluble N	0.9	-	-	-	0.4
Acid detergent lignin	10.1	-	-	-	6.4
TDN_{1X} (%)[2]	52.60	176.0	175.4	174.0	40.73
DE_{1X} (Mcal/kg DM)[3]	2.31	7.34	7.32	7.26	1.73
ME (Mcal/kg DM)[4]	1.96	4.56	4.55	4.52	1.62
NE_M (Mcal/kg DM)[5]	1.08	4.01	4.00	3.96	0.71
NE_G (Mcal/kg DM)[6]	0.52	3.40	3.39	3.36	0.15

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

[2]Total digestible nutrients, TDN_{1X} (%) = tdNFC + tdCP + (tdFA x 2.25) + tdNDF – 7 (NRC, 2000)

[3]Digestible energy, DE_{1X} (Mcal/kg) = [(tdNFC/100)x4.2]+[(tdNDF/100)x 4.2]+[(tdCP/100) x 5.6]+[(FA/100) x 9.4] –0.3

[4]Metabolisable energy, ME = 0.82 x DE (NRC, 2000)

[5]Net energy for maintenance, NE_M = 1.37ME – 0.138ME2 + 0.0105ME3 – 1.12 (NRC, 2000)

[6]Net energy for growth, NE_G = 1.42ME – 0.174ME2 + 0.0122ME3 – 1.65 (NRC, 2000)

Table 5.2 Fatty acid compositions (g/100 g fat) of concentrate, rice straw and rumen-protected oils used in the experiment.

Fatty acids	Concentrate	Rice straw	RP-PO	RP-RO	RP-CO
C8:0	0.87	ND	ND	ND	ND
C10:0	1.10	ND	ND	ND	ND
C12:0	19.22	6.69	1.41	0.81	0.37
C14:0	6.54	9.57	3.15	1.59	1.36
C16:0	19.58	45.30	46.44	7.73	9.72
C18:0	3.44	1.01	4.69	4.54	3.86
C18:1	34.31	19.73	34.70	47.46	36.26
C18:2	14.61	12.68	5.59	33.46	43.73
C18:3	0.34	4.99	0.21	0.32	0.17
C20:0	0.42	ND	ND	ND	ND
Others	-	-	3.81	4.09	4.53
SFA[1]	50.74	62.60	59.5	14.67	19.84
MUFA[2]	34.1	19.73	34.70	47.46	36.26
PUFA[3]	14.95	17.67	5.80	33.78	43.90

[1] SFA = Sum of saturated fatty acid from C8:0-C20:0

[2] MUFA = Monounsaturated fatty acid from C18:1

[3] PUFA = Sum of polyunsaturated fatty acid from C18:2-C18:3

ND = Not detected.

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Table 5.3 DM, CP and fatty acid intake of fistulated cattle fed rumen-protected plant oils.

Items	Control	RP-PO	RP-RO	RP-CO	SEM	P-value
DM intake, kg/d						
Concentrate	3.69	3.69	3.69	3.69	-	-
Rice straw	3.63	3.63	3.63	3.63	-	-
Protected oil	0	0.17	0.17	0.17	-	-
Total	7.31^{b}	7.48^{a}	7.48^{a}	7.48^{a}	0.016	0.0001
CP intake, g/d						
Concentrate	491.63	491.63	491.63	491.63	-	-
Rice straw	92.97	92.97	92.97	92.97	-	-
Total	584.60	584.60	584.60	584.60	-	-
Fat intake, g/d						
Concentrate	171.2	171.2	171.2	171.2	-	-
Rice straw	38.12	38.12	38.12	38.12	-	-
Protected oil	0	137.2	136.4	135.3	-	-
Total	209.3^{b}	346.5^{a}	345.8^{a}	344.7^{a}	0.013	0.0001
NE intake, Mcal/d						
Concentrate	5.90	5.90	5.90	5.90	-	-
Rice straw	3.31	3.31	3.31	3.31	-	-
Protected oil	0	1.26	1.26	1.24	-	-
Total	9.01	10.27	10.27	10.25	0.013	0.0001
Fatty acid intake, g/d						
C8:0	1.15	1.15	1.15	1.15	-	-
C10:0	1.48	1.48	1.48	1.48	-	-
C12:0	28.25^{d}	28.75^{c}	29.35^{b}	30.16^{a}	0.010	0.0001
C14:0	12.26^{d}	16.53^{a}	14.40^{b}	14.08^{c}	0.015	0.0001
C16:0	42.76^{d}	105.71^{a}	53.22^{c}	55.70^{b}	0.072	0.0001
C18:0	4.98^{c}	11.33^{a}	11.10^{b}	11.14^{b}	0.001	0.0001
C18:1n-9	53.20^{d}	100.32^{c}	117.27^{a}	101.74^{b}	0.016	0.0001
C18:2n-6	24.23^{d}	31.81^{c}	69.32^{b}	82.68^{a}	0.020	0.0001
C18:3n-3	2.26^{d}	2.54^{b}	2.69^{a}	2.49^{c}	0.008	0.0001
Total	170.61^{b}	300.95^{a}	299.86^{a}	298.22^{a}	0.160	0.0001

RP-PO = rumen-protected palm oil; RP-RO = rumen-protected rice bran oil; RP-CO = rumen-protected corn oil; SEM = standard error of the mean

^{a,b,c,d} Mean within row which different superscripts differ (P<0.05); SEM = Standard error of mean

5.5.2 Fatty acid profile in rumen content

In the current study, rumen-protected plant oil supplementation had no effects on fatty acid composition of rumen at pre-feeding and 2 h after feeding (Table 5.4). However, at 4 and 6 h after feeding, there were a change in the proportion of fatty acids in the rumen. It was found that the proportion of C16: 0 in cattle fed RP-PO diet significantly higher than those cattle fed RP-RO and RP-CO diets ($p<0.05$).RP-RO supplementation resulted in significantly higher ruminal proportion of C18:1n-9c than RP-PO and RP-CO supplementation ($p<0.001$), while RP-CO supplementation resulted in significantly higher ruminal proportion of C18: 2n-6c than RP-PO and RP-RO supplementation. The proportions of *c9,t11*-C18:2 in cattle on rumen-protected plant oil diets were significantly higher than cattle on control diet.

In the current study, rumen-protected plant oil supplementation had no effects on ruminal fatty acid composition at pre-feeding and 2 h after feeding. This was due to the level of pH in the rumen of this study ranged from 6.74 to 6.88 at pre-feeding and 2 h after feeding. Chalupa (1986) reported that calcium salts of long chain fatty acids (Ca-LCFA) are insoluble in the rumen. The ability to prevent the dissolution of calcium soaps depends on the acid in the rumen and the type of fatty acid. Shelke et al. (2012) recommends that at pH 6.5 the flow rate of Ca salt of plant oil is 96.9% when the pH level drops to 6.0, the flow rate decreases to 90.1%, therefore at pre-feeding and 2 h after feeding rumen-protected plant oil is less solubility. Thus the fatty acid dissolved in the rumen was not significantly different. However, at 4 and 6 h after feeding, the higher proportion of C16:0 in cattle on RP-PO reflecting higher intake of C16:0 from RP-PO which some of C16:0 was soluble. Similarly, the higher proportions of C18:1n-9c and C18:2n-6c in respective cattle on RP-RO and RP-CO

reflecting respective higher intakes of C18:1n-9 and C18:2n-6 and some of these FAs were soluble in the rumen. The higher proportion of *c9,t11*-C18:2 in cattle fed rumen-protected plant oils reflecting ruminal bio-hydrogenation of C18 PUFAs in plant oils.

RP-PO, RP-RO and RP-CO contained C18:1n-9 while RP-RO and RP-CO also contained C18:2n-6. Although small amount of these FAs were soluble in the rumen, they subjected to ruminal bio-hydrogenation by ruminal bacteria to yield *trans*-11 C18:1 and *c9,t11*-C18:2. The increases in ruminal proportions of *t11*-C18:1 and *c9,t11* - C18:2 in cattle fed CO and RO can be attributable to the bio-hydrogenation of C18 UFAs. It is well known that in the bio-hydrogenation process, there are 2 groups of bacteria involved, one group can hydrogenate C18 UFAs including C18:1n-9, C18:2n-6 and C18:3n-3 to C18:0 (Jenkins et al., 2008). Bacteria involved in bio-hydrogenation process can be classified as Group A and Group B bacteria according to metabolic pathway involved (Kemp and Lander, 1984). Both groups of bacteria are needed to complete bio-hydrogenation of PUFA. Group A bacteria that can hydrogenate PUFA to *t11*-C18:1is *Butyrivibrio fibrisolvens, Micrococcus* sp.and *Ruminococcus albus while Group B bacteria, Fucocillus* sp., involve in hydrogenation of C18:1 and C18:1 isomers to C18:0. Thus the major intermediates of bio-hydrogenation process are*t11-C18:1 and C18:0* (Abughazaleh et al., 2002). The increases in *c9,t11*-C18:2 and C18:1tare the results of ruminal bio-hydrogenation of C18:1n-9 and C18:2n-6 in the rumen. Bergman et al. (1990) found the conversion of oleic acid to stearic acid by ruminal micro-organisms. Additionally, the result also indicated that oleic acid was also converted to *trans*C18:1. The *trans*C18:1 isomers were formed during bio-hydrogenation of oleic acid in the rumen (Chow et al., 2004; Côrtes et al., 2010). Another possibility was that oleic acid might

disturbed bio-hydrogenation process of linoleic acid resulting in the accumulation of t*rans*C18:1. There are various possible processes to produce *trans*C18:1 isomers from oleic acid. Firstly, bacteria in the rumen can form numerous *cis*/*trans* isomerases. It is accepted that there is one isomerase converting *cis*-12 bond of linoleic and linolenic acids to *trans*-11 bond (Harfoot and Hazelwood, 1997). In addition, *c9,t11*-C18:2 is bio-hydrogenation intermediate of C18:2. Linoleic acid (*cis*-9,*cis*-12 C18:2) is isomerized to *cis*-9,*trans*-11 CLA by*cis*-12,*trans*-11 isomerase in the first step. It is then hydrogenated by *B.fibrisolvens* to vaccenic acid (VA, *trans*-11 C18:1) in the rumen (Kepler and Tove, 1967). However, after bio-hydrogenation process by *B. fibrisolvens*, there is *cis*-9,*trans*-11 CLA accumulation because bacteria lack of ability to hydrogenate CLA to *trans*-11 C18:1 (Jenkins et al., 2008).

Table 5.4 Effect of rumen-protected plant oil supplementation on fatty acid profile in fistulated cattle (g/100g fatty acids).

Fatty acid (g/100g)	Control	RP-PO	RP-RO	RP-CO	SEM	p-value
Pre -feeding						
C12:0	10.40	10.37	10.51	10.68	0.014	0.145
C14:0	8.47	8.20	8.41	8.45	0.018	0.648
C16:0	33.36	33.18	33.06	33.05	0.029	0.578
C18:0	37.41	37.60	37.36	37.24	0.041	0.923
C18:1n-9t	2.78	3.17	3.12	3.00	0.029	0.672
C18:1n-9c	4.51	4.38	4.41	4.45	0.024	0.659
C18:2n-6c	2.56	2.55	2.57	2.59	0.006	0.468
C18:3n-3	0.49	0.52	0.51	0.54	0.005	0.896

RP-PO = rumen-protected palm oil; RP-RO = rumen-protected rice bran oil; RP-CO = rumen-protected corn oil; SEM = standard error of the mean

Table 5.4 Effect of rumen-protected plant oil supplementation on fatty acid profile in fistulated cattle (g/100g fatty acids) (Continue).

Fatty acid (g/100g)	Control	RP-PO	RP-RO	RP-CO	SEM	p-value
2 h after feeding						
C12:0	13.48	13.42	13.38	13.50	0.023	0.422
C14:0	8.86	8.30	8.24	8.59	0.023	0.103
C16:0	23.22	23.55	23.68	23.50	0.028	0.404
C18:0	27.47	27.95	26.50	26.46	0.053	0.820
C18:1n-9t	6.96	6.56	7.52	7.21	0.018	0.782
C18:1n-9c	12.74	13.37	13.60	13.59	0.038	0.948
C18:2n-6c	2.69	3.31	3.08	3.52	0.004	0.376
C18:3n-3	1.23	1.16	1.77	1.56	0.006	0.103
c9,t11-C18:2	2.31	2.35	2.20	2.04	0.003	0.649
4 h after feeding						
C12:0	15.48	14.50	14.08	14.74	0.027	0.281
C14:0	8.16	8.19	8.00	8.44	0.016	0.238
C16:0	26.53[b]	27.90[a]	23.43[c]	22.37[c]	0.035	0.001
C18:0	26.93[a]	24.55[b]	24.14[b]	24.45[b]	0.050	0.015
C18:1n-9t	7.15[c]	7.70[bc]	8.52[a]	7.99[ab]	0.035	0.051
C18:1n-9c	9.43[b]	9.87[b]	13.30[a]	9.94[b]	0.025	0.001
C18:2n-6c	2.49[c]	2.65[c]	3.35[b]	7.13[a]	0.017	0.001
C18:3n-3	1.26[c]	1.27[c]	2.00[a]	1.74[b]	0.004	0.001
c9,t11-C18:2	2.54[b]	3.35[a]	3.12[a]	3.18[a]	0.011	0.005

RP-PO = rumen-protected palm oil; RP-RO = rumen-protected rice bran oil; RP-CO = rumen-protected corn oil; SEM = standard error of the mean

Table 5.4 Effect of rumen-protected plant oil supplementation on fatty acid profile in fistulated cattle (g/100g fatty acids) (Continue).

Fatty acid (g/100g)	Control	RP-PO	RP-RO	RP-CO	SEM	p-value
6 h after feeding						
C12:0	11.75	11.63	11.59	11.52	0.038	0.899
C14:0	6.28	5.64	5.63	5.42	0.021	0.164
C16:0	26.88^{b}	28.14^{a}	25.01^{c}	25.42^{c}	0.038	0.002
C18:0	34.88^{a}	32.03^{b}	30.70^{c}	30.99^{c}	0.026	0.0001
C18:1n-9t	6.98	7.26	8.38	7.68	0.035	0.069
C18:1n-9c	6.89^{c}	7.68^{b}	10.44^{a}	7.24^{bc}	0.025	0.0001
C18:2n-6c	1.85^{c}	1.93^{c}	2.60^{b}	5.90^{a}	0.013	0.0001
C18:3n-3	1.28^{c}	2.06^{b}	2.09^{a}	1.79^{b}	0.002	0.0001
c9,t11-C18:2	3.17^{b}	3.61^{a}	3.52^{a}	3.59^{a}	0.015	0.050

RP-PO = rumen-protected palm oil; RP-RO = rumen-protected rice bran oil; RP-CO = rumen-protected corn oil; SEM = standard error of the mean

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

5.5.3 Ruminal fermentation

In the current study, at 2, 4 and 6 h after feeding, pH, NH3-N, acetate, propionate, butyrate and acetate: propionate ratio were unaffected by dietary treatments (Table 5.5 and 5.6). Chalupa., (1986) reported that calcium salts of long chain fatty acids (Ca-LCFA),produced by the carboxyl group reaction of long chain fatty acids (LCFA) and calcium salts (Ca ++), are insoluble soaps in the rumen. The ability to prevent the dissolution of calcium soaps depends on the acid in the rumen and the type of fatty acid. When rumen pH is higher than 6.5Ca-LCFA is not digested in the rumen, the breakdown is significant when the pH is lowered to 6.0 and the different pH is also related to the breakdown of rumen-protected plant oils. At a pH in

the abomasum, fatty acid in Ca-LCFA breaks down and absorbs efficiently in the small intestine. Among the various bypass fat, Ca-LCFA is less digested in the rumen (Elmeddah et al., 1991), however it is markedly digested in the lower gut. In this study the pH level in the rumen was between 6.31-6.90 and rumen-protected plant oil supplementation did not affect pH.

Ammonia nitrogen has been reported to vary due to many factors such as the level of feeding, degradability of protein in the rumen and feeding frequency (Neveu et al.,2014). Ammonia nitrogen uses for the efficiency of amino acid synthesis and microbial growth, and was not affected by oil supplementation. The present study found that rumen-protected plant oil supplementation had no effect on ammonia nitrogen, because the protein intake of each group in the experiment was not significantly different. Satter and Slyter (1974) recommended that the appropriate concentration of ammonia nitrogen in the rumen should be at the level that the microbes in the rumen grow best and the digestibility of the dry matter is highest in the range of 50-80 mg/L.

VFA was the product produced from fermentation of bacteria in the rumen. Bergman (1990) founded that VFA was used for cattle energy up to 80%. VFA are acidic if the amount of VFA in the rumen is too high, the pH of the rumen is reduced and may cause rumen acidosis. The present study found that rumen-protected plant oil supplementation had no effect on acetate, propionate, butyrate and acetate: propionate ratio. Schauff and Clark (1992) reported that when supplemented with Ca-LCFA, the acetic acid concentration was between 69.79 and 73.95 (mol/100mol). The concentration of propionic acid was between 20.20 and 21.65 (mol/100mol). The

concentration of butyric acid was between 5.82-9.50 (mol/100mol) and the acetate: propionate ratio was between 3.25-3.66.

Table 5.5 Effect of rumen-protected plant oil supplementation on pH, ammonia nitrogen (mg/100 ml) in fistulated cattle.

Item	Control	RP-PO	RP-RO	RP-CO	SEM	P-Value
pH						
Hour 0	6.90	6.89	6.90	6.86	0.004	0.410
Hour 2	6.75	6.76	6.78	6.78	0.003	0.960
Hour 4	6.31	6.34	6.32	6.33	0.001	0.389
Hour 6	6.51	6.50	6.49	6.52	0.003	0.338
NH_3-N	……………….mg/l…………………					
Hour 0	26.51	26.40	26.36	26.49	0.054	0.774
Hour 2	42.19	42.29	42.42	42.33	0.039	0.201
Hour 4	34.25	33.83	33.42	31.43	0.208	0.673
Hour 6	24.25	24.23	24.27	24.46	0.051	0.646

RP-PO = rumen-protected palm oil; RP-RO = rumen-protected rice bran oil; RP-CO = rumen-protected corn oil; SEM = standard error of the mean

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Table 5.6 Effect of rumen-protected plant oil supplementation onvolatile fatty acids (mol/100 mol) in fistulated cattle.

Item	Control	RP-PO	RP-RO	RP-CO	SEM	P-Value
Acetate (mol/100mol)						
Hour 0	72.22	73.02	71.83	71.90	0.052	0.237
Hour 2	72.29	72.33	72.08	72.70	0.040	0.447
Hour 4	72.26	71.98	72.20	72.03	0.063	0.920
Hour 6	72.46	72.52	72.24	72.06	0.072	0.936
Propionate (mol/100mol)						
Hour 0	18.26	17.75	18.70	18.52	0.054	0.535
Hour 2	18.47	18.52	18.59	18.80	0.033	0.488
Hour 4	18.28	18.76	18.55	18.63	0.041	0.262
Hour 6	18.17	17.92	18.26	1851	0.061	0.843
Butyrate (mol/100mol)						
Hour 0	9.51	9.22	9.46	9.57	0.028	0.121
Hour 2	9.11	9.13	9.31	9.48	0.038	0.946
Hour 4	9.46	9.25	9.23	9.33	0.024	0.470
Hour 6	9.36	9.34	9.49	9.22	0.020	0.456
Acetate : Propionate						
Hour 0	3.96	4.11	3.84	3.88	0.014	0.465
Hour 2	3.91	3.90	3.87	4.08	0.011	0.301
Hour 4	3.95	3.84	3.90	3.86	0.011	0.418
Hour 6	3.98	4.06	3.96	3.85	0.017	0.890

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5.5.4 Degradability of DM, and CP

Rumen-protected plant oil supplements had no effect on dry matter degradability (DMD) of concentrate and rice straw (Table 5.7 and Table 5.8). For crude protein degradability (CPD) of concentrate, rumen-protected plant oil supplements had no effects on crude protein degradability (CPD) (Table 5.9).

In this study, rumen-protected plant oil supplementation with very low solubility in the rumen resulted in higher pH than 6.0,therefore, the lipid supplement did not affect DM and CP degradation of concentrate and rice straw at 0, 3, 6, 12, 24, 48 and 72 h after feeding.

Table 5.7 Effect of rumen-protected plant oil supplementation on dry matter degradability (DMD) of concentrate in fistulated cattle.

Item	Control	RP-PO	RP-RO	RP-CO	SEM	P-Value
0	18.43	18.43	18.43	18.43	-	-
2	31.62	31.75	31.57	31.59	0.021	0.749
4	35.48	35.29	35.43	35.37	0.045	0.244
6	38.28	38.39	38.79	38.64	0.040	0.161
12	42.98	42.93	42.83	42.74	0.053	0.567
24	51.15	50.76	50.59	51.35	0.108	0.902
48	67.60	67.84	67.88	67.78	0.061	0.339

RP-PO = rumen-protected palm oil; RP-RO = rumen-protected rice bran oil; RP-CO = rumen-protected corn oil; SEM = standard error of the mean

Table 5.8 Effect of rumen-protected plant oil supplementation on dry matter degradability (DMD) of rice straw in fistulated cattle.

Item	Control	RP-PO	RP-RO	RP-CO	SEM	P-Value
0	9.92	9.92	9.92	9.92	-	-
2	15.66	15.79	15.54	15.51	0.055	0.987
4	20.53	20.82	20.55	20.73	0.058	0.625
6	24.31	24.48	24.16	24.39	0.064	0.630
12	29.55	29.59	29.73	29.47	0.041	0.556
24	42.36	42.01	42.45	42.53	0.094	0.826
48	52.17	52.32	52.08	52.24	0.041	0.136
72	55.59	55.64	55.87	55.65	0.082	0.834

RP-PO = rumen-protected palm oil; RP-RO = rumen-protected rice bran oil; RP-CO = rumen-protected corn oil; SEM = standard error of the mean

Table 5.9 Effect of rumen-protected plant oil supplementation on crude protein degradability (CPD) of concentrate infistulated cattle.

Item	Control	RP-PO	RP-RO	RP-CO	SEM	P-Value
0	12.33	12.33	12.33	12.33	-	-
2	56.92	56.87	56.98	56.86	0.027	0.088
4	62.23	62.04	62.43	61.76	0.037	0.131
6	67.93	68.21	67.97	67.99	0.029	0.198
12	72.04	72.18	72.41	72.26	0.024	0.409
24	79.14	78.82	78.67	78.85	0.038	0.067
48	87.36	87.50	87.17	87.01	0.042	0.487

RP-PO = rumen-protected palm oil; RP-RO = rumen-protected rice bran oil; RP-CO = rumen-protected corn oil; SEM = standard error of the mean

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5.6 Conclusion

Supplementation of rumen-protected plant oils had no effects on pH, ammonia nitrogen, VFA, DMD, and CPD when compare to non-supplemented control. However, at 4 and 6 hours after feeding, there were a change in the proportion of fatty acids in the rumen. It was found that the proportion of ruminal C16:0 in cattle fed RP-PO diet was significantly higher than those cattle fed RP-RO and RP-CO diets ($p<0.05$). The proportion of ruminal C18:1n-9c was significantly higher in RP-RO cattle than in RP-PO and RP-CO cattle ($p<0.001$) while the ruminal proportion of C18: 2n-6cwas significantly higher in RP-CO cattle than RP-PO and RP-RO cattle.

5.6 References

AbuGhazaleh, A.A., Schingoethe, D.J., Hippen, A.R., Kalscheur, K.F., and Whitlock, L.A. (2002). Fatty acid profiles of milk and rumen digesta from cows fed fish oil, extruded soybeans or their blend. **Journal of Dairy Science**. 85(9): 2266-2276.

Andrae, J.G., Duckett, S.K., Hunt, C.W., Pritchard, G.T., and Owens, F.N. (2001). Effects of feeding high-oil corn to beef steers on carcass characteristics and meat quality. **Journal of Animal Science.** 79(3): 582-588.

Bergman, E.N. (1990). Energy contribution of volatile fatty acids from the gastrointestine tract in various species. **Physiological reviews.** 70(2): 567-590

Chalupa, W. (1986). Ruminal fermentation in vivo as influenced by long-chain fatty acids. **Journal of Dairy Science**. 69(5): 1293-1301.

Chow, T.T., Fievez, V., Moloney, A.P., Raes K., Demeyer D., and Smet, S. D. (2004). Effect of fish oil on *in vitro* rumen lipolysis, apparent bio-

hydrogenation of linoleic and linolenic acid and accumulation of bio-hydrogenation intermediates. **Animal Feed Science and Technology**. 117(1): 1-12.

Cortes, C., da Silva-Kazama, D. C., Kazama,R., Gagnon,R., Benchear,C., GSantos,G.T.D., Zeoula,L.M., and Petit,H.V. (2010). Milk composition, milk fatty acid profile, digestion, and ruminal fermentation in dairy cows fed whole flaxseed and calcium salts of flaxseed oil. **Journal of Dairy Science**. 93 (7-8): 3146-3157.

Dairy Records Management Systems. 2014. DHI Glossary, Accessed April 2014. http://www.drms.org/PDF/materials/glossary.pdf.

Elmeddah, Y., Doreau, M., and Michalet-Doreau, B. (1991). Interaction of lipid supply and carbohydratein the diet of sheep with digestibility and ruminal digestion. **Journal of Agricultural Science**. 116(3): 437-445.

Griinari, J.M., Corl, B.A., Lacy, S.H., Chouinard, P.Y., Nurmela, K.V.V., and Bauman, D.E. (2000). Conjugated linolenic acid is synthesized endogenously in lactatind dairy cows by Δ9-desaturase. **Journal of Nutrition**. 130(9): 2285-2291.

Hazlewood, G.P ., Kemp, P ., Lander, D .,and Dawson, R.M.(1976).C18 unsaturated fatty acid hydrogenation patterns of some rumen bacteria and their ability to hydrolyse exogenous phospholipid.**The British Journal of Nutrition** . 35(2): 293-297

Harfoot, C.G., and Hazlewood G.P. (1997). Lipid metabolism in the rumen. In: P.N. Hobson, C.S. Stewart (Editors). **The Rumen Microbial Ecosystem**. Stewart Published by Blackie Academic and Professional.London, pp. 382-419.

Hawke, J.C., and Silcock, W.R. (1969). Lipolysis and hydrogenation in the rumen. **Biochemical Journal.** 112(1): 131.

Huerta-Leidenz, N. O., Cross, H.R., Lunt, D.K., Pelton, L.S., Savell, J.W., and Smith,S.B. (1991). Growth, carcass traits and fatty acid profiles of adipose tissues from steers fed whole cottonseed. **J. Anim. Sci**., 69: 3665-3672.

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. **Journal of Animal Science**. 86: 397-412.

Kemp,P., White,R.W., and Lander,D.J.,(1975). The Hydrogenation of Unsaturated Fatty Acids by Five Bacterial Isolates from the Sheep Rumen, Including a New Species. **Microbiology**. 90: 100-114

Kemp, P., and Lander, D.J. (1984). Hydrogenation in vitro of a-linolenic acid to stearic acid by mixed cultures of pure strains of rumen bacteria. **Journal of general microbiology**. 130(3): 527-533.

Kepler, C.R., Hirons, K.P., McNeil,l J.J., and Tove, S.B. (1966). Intermediates and products of the biohydrogenation of linoleic acid by *Butyrinvibrio fibrisolvens*. **Journal of Biological Chemistry**. 241(6): 1350-1354.

Kepler,C.R. and Tove,S.B. (1967). Purification and properties of a linoleate Δ^{12}-*cis*,Δ^{11}-*trans*-isomerase from *butyrivibrio fibrisolvens*. Journal of Biological Chemistry. 242: 5686-5692.

Maia,M.R.G., Chaudhary,L.C., Figueres,L., and Wallace,R.J.(2007).Metabolism of polyunsaturated fatty acids and their toxicity to the microflora of therumen. Springer Science.91(4): 303-314.

McDonald, P., Edwards, R.A., Greenhalgh, J. F. D., Morgan, C. A. (1995). Animal Nutrition. **5th Ed. Longman Scientific & Technical**. New York. 607 pp.

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. **Journal of Dairy Science**. 97(3): 1543-1557.

Peters, J.P., Shen, R.Y., Robinson, J.A., and Chester, S.T. (1990). Disappearance and passage of propionic acid from the rumen of the beef steer. **Journal of animal science.** 68(10): 3337-3349.

Piperova,L.S., Teter,B.B., Bruckental, I., Joseph Sampugna, J., Mills,S.E., Yurawecz, M.P., Fritsche,J., Ku,K., and Erdman,R.A.(2000). Mammary Lipogenic Enzyme Activity, *trans* Fatty Acids and Conjugated Linoleic Acids Are Altered in Lactating Dairy Cows Fed a Milk Fat–Depressing Diet. The Journal of **Nutrition**. 130(10): 2568-2574.

Pond, W.G., Church, D.C., Pond, K.R.,and Schokecht, P.A.(2005). **Basic Animal Nutrition and Feeding**. Inc. 580 pp.

SAS. (2002). **SAS software User’s Guide, release 9.0**. SAS Institute. Inc., Cary, NC

Satter, L. D., and Slyter, L. L. (1974). Effect of ammonia concentration on rumen microbial production in vitro. **British Journal of Nutrition**. 32: 199.

Schauff, D.J., and Clark, J.H. (1992). Effects of Feeding Diets Containing Calcium Salts of Long-Chain Fatty Acids to Lactating Dairy Cows1. **Journal of Dairy Science**. 75(11): 2990-3002.

Shelke, S. K., Thakur, S. S. and Shete, S. M. (2012). Productive and reproductive performance of Murrah buffaloes (Bubalusbubalis) supplemented with rumen protected fat and protein**. Indian Journal of Animal Nutrition**, 29: 317-323.

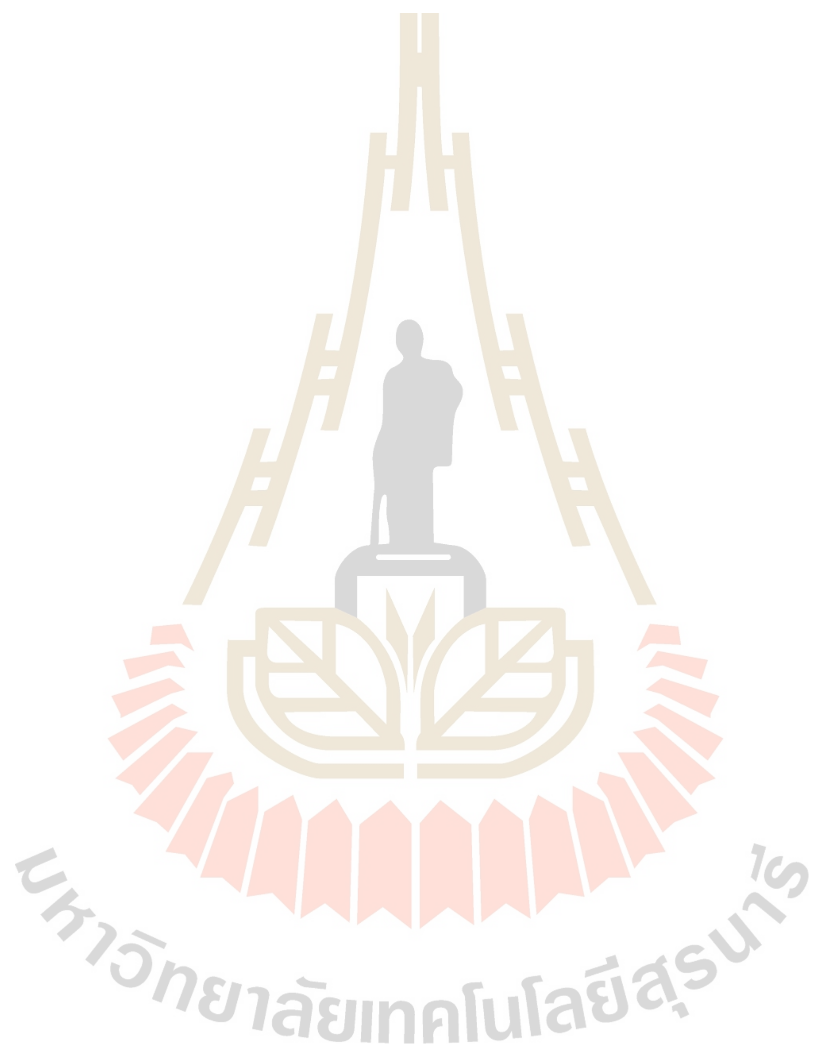
มหาวิทยาลัยเทคโนโลยีสุรนารี

Steel, R. G. D., and Torrie, J. H. (1980). **Principles and Procedures of Statistics: a biometric approach (2nd Ed)**, New York: McGrowhill.

Smith, S.B., Lunt, D.K., Chung, K.Y., Choi, C.B., Tume, R.K., and Zembayashi, M. (2006). Adiposity, fatty acid composition, and delta-9 desaturase activity during growth in beef cattle. **Animal Science Journal.** 77(5): 478-486.

Ward,P.F.V., Scott,T.W., and Dawson,R.M.C. (1964). The hydrogenation of unsaturated fatty acids in the ovine digestive tract. **Biochemical Journal.** 92(1): 60-68.

Wu, Z., and Palmquist, D.L. (1991). Synthesis and Biohydrogenation of Fatty Acids by Ruminal Microorganisms In Vitro1. **Journal of Dairy Science.** 74(9): 3035-3046.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CHAPTER VI

EFFECTS OF RUMEN PROTECTED RICE BRAN OILSUPPLEMENTATION ON RUMEN FERMENTATION INFISTULATEDCATTLES

6.1 Abstract

The aim of this experiment was to investigate the effect of rumen-protected rice bran oil supplementation on ruminal bio-hydrogenation and fermentation in fistulated cattle. Three fistulated cattle were assigned into 3 dietary treatments in a 3×3 Latin square design. All cattle were fed approximately 4 kg/d of 12% CP concentrate and 4 kg/d of rice straw. The treatments were 1) control concentrate 2) control concentrate plus 100 g/d of rumen protected rice bran oil (100 RP-RO); 3) control concentrate plus 200 g/d of rumen protected rice bran oil (200 RP-RO).Each period in the Latin square design lasted 21 d, with the first 7 d for adaptation. The results found that supplementation of rumen-protected rice bran oil had no effects on pH, Ammonia nitrogen, VFA, DMD, and CPD when compare to non-supplemented control. However, the concentration of C18:1n-9c, C18:2n-6, and *c9,t11*-C18:2 was increased while of C18:0 decreased when supplemented 200 g/d of rumen-protected rice bran oil compare to other treatments.

6.2 Introduction

Bio-hydrogenation process in rumen occurs in the rumen due to microorganisms that prevent their toxicity by converting unsaturated fatty acids into saturated fatty acids which final product is stearic acid (Stephen et al., 2006). Nowadays, various researches related to the prevention of bio-hydrogenation process. In addition, research in beef cattle found that supplementation rumen-protected fat can increased the C18:1n-9c concentration in the meat, which resulted in increased marbling in the cattle (Felton et al., 2004; Gillis et al., 2004; Andrae et al., 2001). Fat supplementation in ruminants must be in the form of protected fat that fat is not digested in the rumen but bypassed to digest in the digestive tract. The process of making bypass fat the process of saponification which is to make fat or fatty acids in the form of Ca-soap of long chain fatty acids. This product is less digestible in the typical pH of the rumen, so it does not interfere with the microbial activity, but when it comes to the low pH, this soap is broken down into free fatty acids that are further digested in the small intestine (McDonald et al., 1995; Pond et al., 2005). Thus, the objective of the present study was to examine the effect of rumen-protected rice bran oil supplementation on ruminal bio-hydrogenation and fermentation in fistulated cattle.

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6.3 Objective

To study the effects of rumen protected rice bran oil supplementation on ruminal fermentation in fistulated cattle.

6.4 Materials and methods

6.4.1 Animals and feeding

Three fistulated cattle were assigned in 3 treatments in a 3×3 Latin square design. All cattle were fed approximately 4 kg/d of 12% CP concentrate and 4 kg/d of rice straw. The treatments were 1) control concentrate 2) control concentrate plus 100 g/d of rumen protected rice bran oil (100 RP-RO); 3) control concentrate plus 200 g/d of rumen protected rice bran oil (200 RP-RO). Ingredients of concentrate and chemical composition of concentrate and rice straw used in the experiment are presented in Table 6.1 while the fatty acid composition of feed and oils used in the present study are presented in Table 6.2. All cattle also had free access to clean water and were individually housed in a free-stall unit and individually fed according to treatments. The experiment lasted for 63 days (3 periods) with 21 d in each period, the first 7 d of each period for adaptation to diets followed by 14 d for ruminal sample collection and *in sacco* disappearance trial.

6.4.2 Sample Collection

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

in the laboratory. Every sample was mixed one more time by hand, sub sampled (approximately 200 g), and frozen (-20°C).

6.4.3 Laboratory Analyses

6.4.3.1 Feed chemical composition analysis

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

6.4.3.2 Ruminal fermentation

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. The pH of rumen fluid were immediately determined at the time of sampling by pH meter. For VFAs and ammonia N determination, 36 ml of rumen fluid was put into 50 ml centrifuge tube containing 4 ml of 1M H_2SO_4, then centrifuged at 1895 rpm for 15 min. Supernatant was collected and put into 25 ml test tube, then capped and stored at -20°C until analysis. Analysis of acetic, propionic and butyric acids used GC (Hewlett

Packard GC system HP6890, USA, 19091N-113 INNOWAX, Length (meters) 30, I.D. (mm) 0.32 WIDEBORE, Film (um) 0.25). Ammonia N concentrations was determined by Kjeldahl analysis (AOAC, 1995)

6.4.3.3 Analysis of fatty acids in the rumen

Rumen fluid of each period was extracted for fatty acid using a modified method used by Romeu-Nadal et al. (2004). From a well-mixed aliquot of rumen fluid, 20 ml was placed in 50 ml centrifuge tubes. Then added 27 ml of dichloromethane-methanol solution (2:1, v/v) to each tube. The mixture was shaken mechanically for 15 min and centrifuged at 2500 rpm for 8 min at 4°C. Approximately 8 ml of distilled water was pipette into each tube and, after shaking for a further 15 min, the sample was again centrifuged at 2500 rpm for 8 min at 4°C. As much of the upper aqueous fraction as possible was carefully removed with a pipette. The organic layer was washed with 8 ml of a saturated solution of the sodium chloride, and finally mixed mechanically for 15 min and centrifuged for 8 min at 2500 rpm at 4°C. Again, the upper aqueous fraction was carefully removed with a pipette. The organic fraction was carefully transferred to a separating funnel and filtered through 1PS paper (Whatman, Maidstone, UK) containing anhydrous sodium sulfate, and 3-5 ml of dichloromethane was passed through the filter. The fat solution was taken in pre-weighed conical flask. Finally the extract was concentrated by removing dichloromethane in a rotatory evaporator and dried under a gentle stream of nitrogen. The weight difference of the conical flask before/after was assumed to be fat. The fat was stored at -20°C and re-dissolved in dichloromethane (3%, w/v) immediately analyzing by gas chromatography (GC) (7890A GC System, Agilent Technology, USA), equipped with a 100 m x 0.25 mm x 0.2 µm film fused silica capillary column (SP1233, Supelco Inc, Bellefonte, PA, USA). Injector and detector temperatures were

250°C. The column temperature was kept at 70°C for 4 min, then increased at 13 °C/min to 175°C and held at 175°C for 27 min, then increased at 4°C/min to 215°C and held at 215°C for 17 min, then increased at 4°C/min to 240°C and held at 240°C for 10 min

6.4.3.4 Degradability determination of DM, CP

Concentrate and rice straw were ground through a 2 mm screen for *in sacco* ruminal disappearance determination. Approximately 5 g of 2 mm ground samples were placed into 8x11 cm nylon bags with 47 µm pore size and then suspended in the rumen of each fistulated cattle for 0 (pre feeding), 2, 4, 6, 12, 24, 48 (concentrate) and 72 h (rice straw), and were then removed and washed in water and then dried at 65°C for 48 h. After weighing each bag individually, the residues were then dried at 65°Cand subjected to DM determination. The contents of the bags were then assayed for CP content (CPD). The degradability value was obtained by subjecting nutrient losses at arbitrary of time using NEWAY EXCEL (Chen, 1996).

6.4.4 Statistical analysis

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

6.4.5 Experimental location

The experiment was conducted at Suranaree University of Technology's Cattle Farm, The Center for Scientific and Technological Equipment Building 10, and 14,Suranaree University of Technology.

6.4.6 Duration

The duration of the present experiment was from October to December 2017.

6.5 Result and Discussion

6.5.1 Feed Composition and performance

The concentrate was formulated to meet the requirement of the steers. In the concentrate, the main SFAs were C12:0 and C16:0 (19.38 and 19.06 g/100 g fat respectively), whereas C18:1n-9 was the main MUFA (32.34 g/100 g fat) and C18:2n-6 was the main PUFA (16.89 g/100 g fat). Lipids from rice straw provided high proportions of C16:0 (45.30 g/100 g fat) and low proportions of C18:0 (1.01 g/100 g fat). RP-RO had the highest proportion of C18:1n-9 (47.46 g/100 g fat) and C18:2n-6 (33.46 g/100 g fat) (Table 6.2).

DM and CP intakes were not statistically altered by dietary treatments (Table 6.3), however, the animals supplemented with RP-RO had greater total fatty acid intake than that fed the control diet (P=0.0001). With diets containing lower levels of added fat, Huerta-Leidenz et al. (1991) reported no influence on daily gain, intake or feed conversion ratio when dietary whole cotton seed of 15 or 30% (3.3 and 6.6% additional fat) was supplemented. In the present trial, fat contents of experimental diets were between 3.1 and 4.3%, it is unlikely that these levels of fat affected feed intake. When the consumption of individual fatty acid was calculated, the intakes of individual FA from C12:0 to C18:2n-6 increased with increasing RP-RO addition (Table 6.3). Cattle on 200 RP-RO diet ate more C18:3n-3 than those cattle on control and 100 RP-RO diets. The differences in individual FA intake reflected differences in FA composition of RP-RO added.

Table 6.1 Chemical compositions of the experimental diets.

Items	Concentrate	RP-RO	Rice straw
Dry matter	**92.2**	**83.1**	**90.6**
	----------------------% of DM------------------------		
Ash	10.9	15.04	15.9
Crude protein	13.7	-	2.6
Ether extract	4.8	82.1	1.1
Neutral detergent fiber	43.2	-	85.1
Acid detergent fiber	18.0	-	57.6
Neutral detergent in soluble N	1.0	--	0.5
Acid detergent insoluble N	0.9	-	0.4
Acid detergent lignin	9.9	-	6.4
TDN_{1X} (%)[2]	58.83	175.4	40.73
DE_{1X} (Mcal/kg DM)[3]	2.62	7.32	1.73
ME (Mcal/kg DM)[4]	2.12	4.55	1.62
NE_M (Mcal/kg DM)[5]	1.28	4.00	0.71
NE_G (Mcal/kg DM)[6]	0.71	3.39	0.15

[1]kg/100 kg concentrate: 30 dried cassava chip, 4 ground corn, 10 rice bran, 25 palm meal, 15 coconut meal, 6 dried distillers grains with solubles, 0.5 sodium bicarbonate, 6 molasses, 1 dicalciumphosphate (16%P), 1.5 urea, 0.5 salt and 0.5 premix. Premix: provided per kg of concentrate including vitamin A, 5,000 IU; vitamin D3, 2,200 IU; vitamin E, 15 IU; Ca, 8.5 g; P, 6 g; K, 9.5 g; Mg, 2.4 g; Na, 2.1 g; Cl, 3.4 g; S, 3.2 g; Co, 0.16 mg; Cu, 100 mg; I, 1.3 mg; Mn, 64 mg; Zn, 64 mg; Fe, 64 mg; Se, 0.45 mg.
[2]Total digestible nutrients, TDN_{1X} (%) = tdNFC + tdCP + (tdFA x 2.25) + tdNDF – 7 (NRC, 2000)
[3]Digestible energy, DE_{1X} (Mcal/kg) = [(tdNFC/100)x4.2]+[(tdNDF/100) x 4.2]+[(tdCP/100) x 5.6]+[(FA/100) x 9.4] –0.3
[4]Metabolisable energy, ME = 0.82 x DE (NRC, 2000)
[5]Net energy for maintenance, $NE_M = 1.37ME – 0.138ME^2 + 0.0105ME^3 – 1.12$ (NRC, 2000)
[6]Net energy for growth, $NE_G = 1.42ME – 0.174ME^2 + 0.0122ME^3 – 1.65$ (NRC, 2000)

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Table 6.2 Fatty acid compositions (g/100 g fat) of concentrate, rice straw and rumen-protected rice bran oil used in the experiment.

Fatty acids	Concentrate	Rice straw	RP-RO
C8:0	0.75	ND	ND
C10:0	1.08	ND	ND
C12:0	19.38	6.69	0.81
C14:0	6.39	9.57	1.59
C16:0	19.06	45.30	7.73
C18:0	3.49	1.01	4.54
C18:1	32.34	19.73	47.46
C18:2	16.89	12.68	33.46
C18:3	0.38	4.99	0.32
C20:0	0.21	ND	ND
Others	-	-	4.09
SFA[1]	50.38	62.60	14.67
MUFA[2]	32.34	19.73	47.46
PUFA[3]	17.27	17.67	33.78

[1] SFA = Sum of saturated fatty acid from C8:0 – C20:0

[2] MUFA = Monounsaturated fatty acid from C18:1

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

ND = Not detected.

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Table 6.3 DM, CP and fatty acid intake of fistulateleled cattle fed rumen-protected rice bran oil.

Items	Control	100 RP-RO	200 RP-RO	SEM	P-value
DM intake, kg/d					
Concentrate	3.69	3.69	3.69	-	-
Rice straw	3.63	3.63	3.63	-	-
Protected oil	0	0.083	0.17	-	-
Total	7.31[c]	7.40[b]	7.48[a]	0.016	0.0001
CP intake, g/d					
Concentrate	507.36	507.36	507.36	-	-
Rice straw	92.97	92.97	92.97	-	-
Total	600.34	600.34	600.34	-	-
Fat intake, g/d					
Concentrate	179.0	179.0	179.0	-	-
Rice straw	38.12	38.12	38.12	-	-
Protected oil	0	68.21	136.73	-	-
Total	217.1	285.3	353.8		
NE intake, Mcal/d					
Concentrate	7.34	7.34	7.34	-	-
Rice straw	3.11	3.11	3.11	-	-
Protected oil	0	0.63	1.26	-	-
Total	10.45	11.08	11.71	0.217	0.0001
Fatty acid intake, g/d					
C8:0	1.067	1.067	1.067	-	-
C10:0	1.53	1.53	1.53	-	-
C12:0	29.97[c]	30.51[b]	31.06[a]	0.013	0.0001
C14:0	12.56[c]	13.63[b]	14.70[a]	0.217	0.0001
C16:0	43.53[c]	48.74[b]	53.97[a]	0.470	0.0001
C18:0	5.32[c]	8.38[b]	11.46[a]	0.198	0.0001
C18:1n-9	53.10[c]	85.08[b]	117.20[a]	0.123	0.0001
C18:2n-6	28.60[c]	51.14[b]	73.79[a]	0.224	0.0001
C18:3n-3	2.35[c]	2.57[b]	2.78[a]	0.04	0.0001
Total	178.1[c]	242.7[b]	307.6[a]	0.25	0.0001

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

6.5.2 Fatty acid profile in rumen content

In the current study, rumen-protected rice bran oil (RP-RO) supplementation had no effects on fatty acid composition of rumen at pre feeding and 2 h after feeding (Table 6.4). However, at 4 and 6 h after feeding, there were a change

in the proportion of fatty acids in rumen. 200 RP-RO supplementation resulted in significantly higher ruminal proportion of C18:1n-9c, C18:2n-6,and *c9,t11*-C18:2 compare with control diet ($p<0.05$). while The proportion of ruminal C18:0 decreased when supplemented 200 g/d of rumen-protected rice bran oil compare to other treatments.

In the current study, rumen-protected rice bran oil supplementation had no effects on fatty acid composition of rumen at pre feeding and 2 hour after feeding. This was due to the level of pH in the rumen of this study ranged from 6.74 to 6.88 at pre-feeding and 2 hours after feeding. Chalupa (1986) reported that calcium salts of long chain fatty acids (Ca-LCFA)are insoluble in the rumen. The ability to prevent the dissolution of calcium soaps depends on the acid in the rumen and the type of fatty acid. Shelke et al. (2012) recommends that at pH 6.5 the flow rate of Ca salt of plant oil is 96.9% when the pH level drops to 6.0, the flow rate decreases to 90.1%, Therefore at pre feeding and 2 h after feeding rumen-protected rice bran oil is less solubility. Thus the fatty acid dissolved in the rumen was not significantly different. However, at 4 and 6 hours after feeding, the higher proportions of C18:1n-9c and C18:2n-6c in respective cattle on RP-RO reflecting respective higher intakes of C18:1n-9 and C18:2n-6 and some of these FAs were soluble in the rumen. The higher proportion of *c9,t11*-C18:2 in cattle fed rumen-protected rice bran oils reflecting ruminal bio-hydrogenation of C18 PUFAs in rice bran oils.

RP-RO contained C18:1n-9 and C18:2n-6. Although small amount of these FAs were soluble in the rumen, they subjected to ruminal bio-hydrogenation by ruminal bacteria to yield *trans*-11 C18:1 and *c9,t11*-C18:2. The increases in ruminal proportions of *t11*-C18:1 and *c9,t11*-C18:2 in cattle fed CO and RO can be attributable to the bio-hydrogenation of C18 UFAs. It is well known that in the bio-

hydrogenation process, there are 2 groups of bacteria involved, one group can hydrogenate C18 UFAs including C18:1n-9, C18:2n-6 and C18:3n-3 to C18:0 (Jenkins et al., 2008). Bacteria involved in bio-hydrogenation process can be classified as Group A and Group B bacteria according to metabolic pathway involved (Kemp and Lander, 1984). Both groups of bacteria are needed to complete bio-hydrogenation of PUFA. Group A bacteria that can hydrogenate PUFA to *t11*-C18:1is *Butyrivibrio fibrisolvens, Micrococcus* sp.and *Ruminococcus albus while Group B bacteria, Fucocillus* sp., involve in hydrogenation of C18:1 and C18:1 isomers to C18:0. Thus the major intermediates of bio-hydrogenation process are *t11-C18:1 and C18:0* (Abughazaleh et al., 2002). The increases in *c9,t11*-C18:2 and C18:1tare the results of ruminal bio-hydrogenation of C18:1n-9 and C18:2n-6 in the rumen. Bergman et al. (1990) found the conversion of oleic acid to stearic acid by ruminal micro-organisms. Additionally, the result also indicated that oleic acid was also converted to*trans*C18:1. The *trans*C18:1isomers were formed during bio-hydrogenation of oleic acid in the rumen (Chow et al., 2004; Côrtes et al., 2010). Another possibility was that oleic acid might disturbed bio-hydrogenation process of linoleic acid resulting in the accumulation of t*rans*C18:1. There are various possible processes to produce *trans*C18:1 isomers from oleic acid. Firstly, bacteria in the rumen can form numerous *cis*/*trans* isomerases. It is accepted that there is one isomerase converting *cis*-12 bond of linoleic and linolenic acids to *trans*-11 bond (Harfoot and Hazelwood, 1997). In addition, *c9,t11*-C18:2 is bio-hydrogenation intermediate of C18:2. Linoleic acid (*cis*-9,*cis*-12 C18:2) is isomerized to *cis*-9,*trans*-11 CLA by *cis*-12,*trans*-11 isomerase in the first step. It is then hydrogenated by *B.fibrisolvens* to vaccenic acid (VA, *trans*-11 C18:1) in the rumen (Kepler and Tove, 1967). However, after bio-hydrogenation process by *B. fibrisolvens*, there is *cis*-

9,*trans*-11 CLA accumulation because bacteria lack of ability to hydrogenate CLA to *trans*-11 C18:1 (Jenkins et al., 2008).

Table 6.4 Effect of rumen-protected rice bran oil supplementation on fatty acid profile in fistulated cattle (g/100g fatty acids).

Fatty acid (g/100g)	Control	100 RP-RO	200 RP-RO	SEM	p-value
Pre -feeding					
C12:0	8.49	8.39	8.64	0.045	0.477
C14:0	7.67	8.02	7.93	0.022	0.214
C16:0	33.93	34.03	33.83	0.024	0.366
C18:0	39.33	38.84	38.86	0.037	0.360
C18:1n-9t	2.47	2.55	2.54	0.005	0.221
C18:1n-9c	4.82	4.63	4.75	0.014	0.793
C18:2n-6c	2.46	2.71	2.55	0.030	0.909
C18:3n-3	0.50	0.51	0.56	0.002	0.181

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Table 6.4 Effect of rumen-protected rice bran oil supplementation on fatty acid profile in fistulated cattle (g/100g fatty acids) (Continue).

Fatty acid (g/100g)	**Control**	**100 RP-RO**	**200 RP-RO**	**SEM**	**p-value**
2 h after feeding					
C12:0	13.16	13.06	13.03	0.014	0.153
C14:0	8.01	8.06	8.05	0.013	0.173
C16:0	28.32	27.50	27.62	0.053	0.477
C18:0	31.16	29.83	29.93	0.016	0.177
C18:1n-9t	5.23	6.26	5.84	0.042	0.496
C18:1n-9c	8.01	8.84	8.82	0.046	0.577
C18:2n-6c	2.63	3.11	3.35	0.006	0.085
C18:3n-3	1.28	1.15	1.14	0.006	0.422
c9,t11-C18:2	2.16	2.15	2.19	0.006	0.918
4 h after feeding					
C12:0	13.76	13.12	12.75	0.029	0.337
C14:0	7.58	7.32	6.95	0.021	0.245
C16:0	31.58	30.02	29.63	0.046	0.236
C18:0	25.61[a]	24.14[b]	23.75[b]	0.020	0.053
C18:1n-9t	6.31	6.60	7.04	0.017	0.156
C18:1n-9c	9.23[b]	11.60[a]	12.09[a]	0.054	0.056
C18:2n-6c	2.34[c]	3.24[b]	3.59[a]	0.005	0.009
C18:3n-3	1.17	1.16	1.12	0.001	0.066
c9,t11-C18:2	2.38[c]	2.75[b]	3.04[a]	0.005	0.031

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

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Table 6.4 Effect of rumen-protected rice bran oil supplementation on fatty acid profile in fistulated cattle (g/100g fatty acids) (Continue).

Fatty acid (g/100g)	Control	100 RP-RO	200 RP-RO	SEM	p-value
6 h after feeding					
C12:0	10.42	10.45	10.32	0.037	0.787
C14:0	6.43	6.17	6.17	0.023	0.847
C16:0	31.74	29.96	29.82	0.029	0.085
C18:0	31.41[a]	29.94[b]	29.43[b]	0.020	0.048
C18:1n-9t	5.65	5.68	5.86	0.005	0.08
C18:1n-9c	8.20[b]	10.64[a]	10.50[a]	0.025	0.043
C18:2n-6c	1.94[b]	2.59[a]	2.95[a]	0.011	0.021
C18:3n-3	1.27	1.18	1.20	0.008	0.516
c9,t11-C18:2	2.90	3.36	3.71	0.008	0.063

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

6.5.3 Ruminal fermentation

In the current study, At 2 h after feeding, pH, NH3-N, acetate, propionate, butyrate and acetate: propionate ratio were unaffected by dietary treatments (Table 6.5 and 6.6). Ruminal pH, acetate, propionate, and butyrate were similar among treatments at 4 h post-feeding. At 6 h post-feeding, dietary treatments did not affect ruminal pH, NH3-N, acetate, propionate, and butyrate concentrations.

Chalupa., (1986) reported that calcium salts of long chain fatty acids (Ca-LCFA),produced by the carboxyl group reaction of long chain fatty acids (LCFA) and calcium salts (Ca ++), are insoluble soaps in the rumen. The ability to prevent the dissolution of calcium soaps depends on the acid in the rumen and the type of fatty

acid. When rumen pH is higher than 6.5Ca-LCFA is not digested in the rumen, the breakdown is significant when the pH is lowered to 6.0 and the different pH is also related to the breakdown of rumen-protected plant oils. At a pH in the abomasum, fatty acid in Ca-LCFA breaks down and absorbs efficiently in the small intestine. Among the various bypass fat, Ca-LCFA is less digested in the rumen (Elmeddah et al., 1991), however it is markedly digested in the lower gut. In this study the pH level in the rumen was between 6.30-6.88 and rumen-protected rice bran oil supplementation did not affect pH.

Ammonia nitrogen has been reported to vary due to many factors such as the level of feeding, degradability of protein in the rumen and feeding frequency (Neveu et al., 2014). Ammonia nitrogen uses for the efficiency of amino acid synthesis and microbial growth, and was not affected by oil supplementation. The present study found that rumen-protected plant oil supplementation had no effect on ammonia nitrogen, because the protein intake of each group in the experiment was not significantly different. Satter and Slyter. (1974) recommended that the appropriate concentration of ammonia nitrogen in the rumen should be at the level that the microbes in the rumen grow best and the digestibility of the dry matter is highest in the range of 50-80 mg/L.

VFA was the product produced from fermentation of bacteria in the rumen. Bergman (1990) founded that VFA was used for cattle energy up to 80%. VFA are acidic if the amount of VFA in the rumen is too high, the pH of the rumen is reduced and may cause rumen acidosis. The present study found that rumen-protected plant oil supplementation had no effect on acetate, propionate, butyrate and acetate: propionate ratio. Schauff and Clark (1992) reported that when supplemented with Ca-LCFA, the acetic acid concentration was between 69.79 and 73.95 (mol/100mol). The

concentration of propionic acid was between 20.20 and 21.65 (mol/100mol). The concentration of butyric acid was between 5.82-9.50 (mol/100mol) and the acetate: propionate ratio was between 3.25-3.66.

Table 6.5 Effect of rumen-protected rice bran oil supplementation on pH, ammonia nitrogen (mg/100 ml) in fistulated cattle.

Item	Control	100 RP-RO	200 RP-RO	SEM	P-Value
pH					
Hour 0	6.88	6.83	6.86	0.009	0.91
Hour 2	6.74	6.75	6.76	0.006	0.97
Hour 4	6.31	6.30	6.30	0.006	0.98
Hour 6	6.51	6.53	6.57	0.003	0.57
NH_3-N	..................mg/l....................				
Hour 0	26.51	26.84	26.72	0.023	0.15
Hour 2	41.60	42.22	42.70	0.059	0.63
Hour 4	35.44	34.72	35.56	0.069	0.62
Hour 6	23.74	23.52	24.42	0.059	0.48

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Table 6.6 Effect of rumen-protected rice bran oil supplementation on volatile fatty acids (mol/100 mol) in fistulated cattle.

Item	Control	100 RP-RO	200 RP-RO	SEM	P-Value
Acetate (mol/100mol)					
Hour 0	73.40	73.61	73.57	0.033	0.220
Hour 2	72.51	72.52	72.37	0.058	0.729
Hour 4	72.56	72.88	72.90	0.042	0.893
Hour 6	73.70	73.56	73.44	0.039	0.391
Propionate (mol/100mol)					
Hour 0	18.52	18.16	18.28	0.043	0.334
Hour 2	19.26	19.20	19.50	0.077	0.754
Hour 4	19.01	18.49	18.90	0.028	0.605
Hour 6	18.05	18.18	18.37	0.033	0.488
Butyrate (mol/100mol)					
Hour 0	8.06	8.22	8.14	0.010	0.363
Hour 2	8.22	8.26	8.12	0.026	0.828
Hour 4	8.43	8.62	8.19	0.014	0.289
Hour 6	8.24	8.37	8.06	0.006	0.080
Acetate : Propionate				.	
Hour 0	3.96	4.06	4.03	0.011	0.301
Hour 2	3.77	3.78	3.72	0.018	0.744
Hour 4	3.81	3.94	3.85	0.008	0.733
Hour 6	4.08	4.04	4.00	0.010	0.496

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6.5.4 Degradability of DM, and CP

Rumen-protected rice bran oil supplements had no effect on dry matter degradability (DMD)of concentrate and rice straw (Table 6.7 and Table 6.8).For crude protein degradability (CPD) of concentrate, rumen-protected plant oil supplements had no effects on crude protein degradability (CPD) (Table 6.9).

In this study, rumen-protected rice bran oil supplementation with very low solubility in the rumen resulted in higher pH than 6.0,therefore, the lipid supplement did not affect DM and CP degradation of concentrate and rice straw at 0, 3, 6, 12, 24, 48 and 72 h after feeding.

Table 6.7 Effect of rumen-protected rice bran oil supplementation on dry matter degradability (DMD) of concentrate in fistulated cattle.

Item	Control	100 RP-RO	200 RP-RO	SEM	P-Value
0	20.05	20.05	20.05	-	-
2	33.95	32.82	32.53	0.018	0.065
4	37.30	37.28	37.24	0.068	0.841
6	40.82	39.61	39.59	0.065	0.477
12	44.42	43.66	43.48	0.033	0.414
24	52.50	52.52	53.39	0.126	0.586
48	68.60	69.96	70.02	0.037	0.214

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Table 6.8 Effect of rumen-protected rice bran oil supplementation on dry matter degradability (DMD) of rice straw infistulated cattle.

Item	Control	100 RP-RO	200 RP-RO	SEM	P-Value
0	9.59	9.59	9.59	-	-
2	19.50	19.76	19.64	0.041	0.871
4	20.97	20.51	20.80	0.048	0.741
6	24.52	24.57	24.51	0.019	0.670
12	30.17	29.95	29.98	0.011	0.303
24	42.65	42.31	42.43	0.051	0.360
48	51.87	51.91	51.96	0.075	0.645
72	55.96	56.19	56.15	0.030	0.362

Table 6.9 Effect of rumen-protected rice bran oil supplementation on supplementation on crude protein degradability (CPD) of concentrate infistulated cattle.

Item	Control	100 RP-RO	200 RP-RO	SEM	P-Value
0	12.97	12.97	12.97	-	-
2	58.11	58.03	58.16	0.071	0.878
4	63.43	63.23	63.31	0.063	0.743
6	68.95	69.14	69.22	0.023	0.451
12	73.13	73.07	73.24	0.021	0.202
24	79.20	79.04	79.17	0.123	0.949
48	87.53	87.74	87.26	0.062	0.751

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6.6 Conclusion

Supplementation of rumen-protected rice bran oil had no effects on pH, Ammonia nitrogen, VFA, DMD, and CPD when compare to non-supplemented control. However, The proportion of ruminal C18:1n-9c, C18:2n-6, and *c9,t11*-C18:2 was increased. while The proportion of ruminal C18:0 decreased when supplemented 200 g/d of rumen-protected rice bran oil compare to other treatments.

6.7 Reference

AbuGhazaleh, A.A., Schingoethe, D.J., Hippen, A.R., Kalscheur, K.F., and Whitlock, L.A. (2002). Fatty acid profiles of milk and rumen digesta from cows fed fish oil, extruded soybeans or their blend. **Journal of Dairy Science**. 85(9): 2266-2276.

Andrae, J.G., Duckett, S.K., Hunt, C.W., Pritchard, G.T., and Owens, F.N. (2001). Effects of feeding high-oil corn to beef steers on carcass characteristics and meat quality. **Journal of Animal Science.** 79(3): 582-588.

Bergman, E.N. (1990). Energy contribution of volatile fatty acids from the gastrointestine tract in various species. **Physiological reviews.** 70(2): 567-590

Chalupa, W. (1986). Ruminal fermentation in vivo as influenced by long-chain fatty acids. **Journal of Dairy Science**. 69(5): 1293-1301.

Chow, T.T., Fievez, V., Moloney, A.P., Raes K., Demeyer D., and Smet, S. De. (2004). Effect of fish oil on *in vitro* rumen lipolysis, apparent bio-hydrogenation of linoleic and linolenic acid and accumulation of bio-hydrogenation intermediates. **Animal Feed Science and Technology**. 117(1): 1-12.

Cortes, C., da Silva-Kazama, D. C., Kazama,R., Gagnon,R., Benchear,C., Santos,G.T.D., Zeoula,L.M., and Petit,H.V. (2010). Milk composition, milk fatty acid profile, digestion, and ruminal fermentation in dairy cows fed whole flaxseed and calcium salts of flaxseed oil. **Journal of Dairy Science**. 93 (7-8): 3146-3157.

Dairy Records Management Systems. (2014). DHI Glossary, Accessed April 2014. http://www.drms.org/PDF/materials/glossary.pdf.

Elmeddah, Y., Doreau, M., and Michalet-Doreau, B. (1991). Interaction of lipid supply and carbohydratein the diet of sheep with digestibility and ruminal digestion. **Journal of Agricultural Science**. 116(3): 437-445.

Griinari, J.M., Corl, B.A., Lacy, S.H., Chouinard, P.Y., Nurmela, K.V.V., and Bauman, D.E. (2000). Conjugated linolenic acid is synthesized endogenously in lactatind dairy cows by Δ9-desaturase. **Journal of Nutrition**. 130(9): 2285-2291.

Hazlewood, G.P ., Kemp, P ., Lander, D .,and Dawson, R.M.(1976).C18 unsaturated fatty acid hydrogenation patterns of some rumen bacteria and their ability to hydrolyse exogenous phospholipid.**The British Journal of Nutrition** .35(2): 293-297

Harfoot, C.G., and Hazlewood G.P. (1997). Lipid metabolism in the rumen. In: P.N. Hobson, C.S. Stewart (Editors). **The Rumen Microbial Ecosystem**. Stewart Published by Blackie Academic and Professional.London, pp. 382-419.

Hawke, J.C., and Silcock, W.R. (1969). Lipolysis and hydrogenation in the rumen. **Biochemical Journal.** 112(1): 131.

Huerta-Leidenz, N. O., Cross, H.R., Lunt, D.K., Pelton, L.S., Savell, J.W., and Smith,S.B. (1991). Growth, carcass traits and fatty acid profiles of adipose tissues from steers fed whole cottonseed. **J. Anim. Sci.**, 69: 3665-3672.

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. **Journal of Animal Science**. 86: 397-412.

Kemp,P., White,R.W., and Lander,D.J.,(1975). The Hydrogenation of Unsaturated Fatty Acids by Five Bacterial Isolates from the Sheep Rumen, Including a New Species. **Microbiology.** 90: 100-114

Kemp, P., and Lander, D.J. (1984). Hydrogenation in vitro of a-linolenic acid to stearic acid by mixed cultures of pure strains of rumen bacteria. **Journal of general microbiology**. 130(3): 527-533.

Kepler, C.R., Hirons, K.P., McNeil,l J.J., and Tove, S.B. (1966). Intermediates and products of the biohydrogenation of linoleic acid by *Butyrinvibrio fibrisolvens*. **Journal of Biological Chemistry**. 241(6): 1350-1354.

Kepler,C.R. and Tove,S.B. (1967). Purification and properties of a linoleate Δ^{12}-*cis*,Δ^{11}-*trans*-isomerase from *butyrivibrio fibrisolvens*. **Journal of Biological Chemistry.**242: 5686-5692.

Maia,M.R.G., Chaudhary,L.C., Figueres,L., and Wallace,R.J.(2007).Metabolism of polyunsaturated fatty acids and their toxicity to the microflora of the rumen. **Springer Science**. 91(4): 303-314

McDonald, P., Edwards, R.A., Greenhalgh, J. F. D., and Morgan, C. A. (1995). **Animal Nutrition**. 5th Ed. Longman Scientific & Technical. New York. 607 pp.

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. **Journal of Dairy Science**. 97(3): 1543-1557.

Peters, J.P., Shen, R.Y., Robinson, J.A., and Chester, S.T. (1990). Disappearance and passage of propionic acid from the rumen of the beef steer. **Journal of animal science.** 68(10): 3337-3349.

Piperova,L.S., Teter,B.B., Bruckental, I., Joseph Sampugna, J., Mills, S.E.,Yurawecz,M.P., Fritsche,J., Ku,K., and Erdman,R.A.(2000). Mammary Lipogenic Enzyme Activity, *trans* Fatty Acids and Conjugated Linoleic Acids Are Altered in Lactating Dairy Cows Fed a Milk Fat–Depressing Diet. **The Journal of Nutrition.** 130(10): 2568-2574.

Pond, W.G., Church, D.C., Pond, K.R.,and Schokecht, P.A.(2005). **Basic Animal Nutrition and Feeding**. Inc. 580 pp.

SAS. (2002). **SAS software User's Guide, release 9.0**. SAS Institute. Inc., Cary, NC

Satter, L. D., and Slyter, L. L. (1974). Effect of ammonia concentration on rumen microbial production in vitro. **British Journal of Nutrition**. 32: 199.

Schauff, D.J., and Clark, J.H. (1992). Effects of Feeding Diets Containing Calcium Salts of Long-Chain Fatty Acids to Lactating Dairy Cows1. **Journal of Dairy Science**. 75(11): 2990-3002.

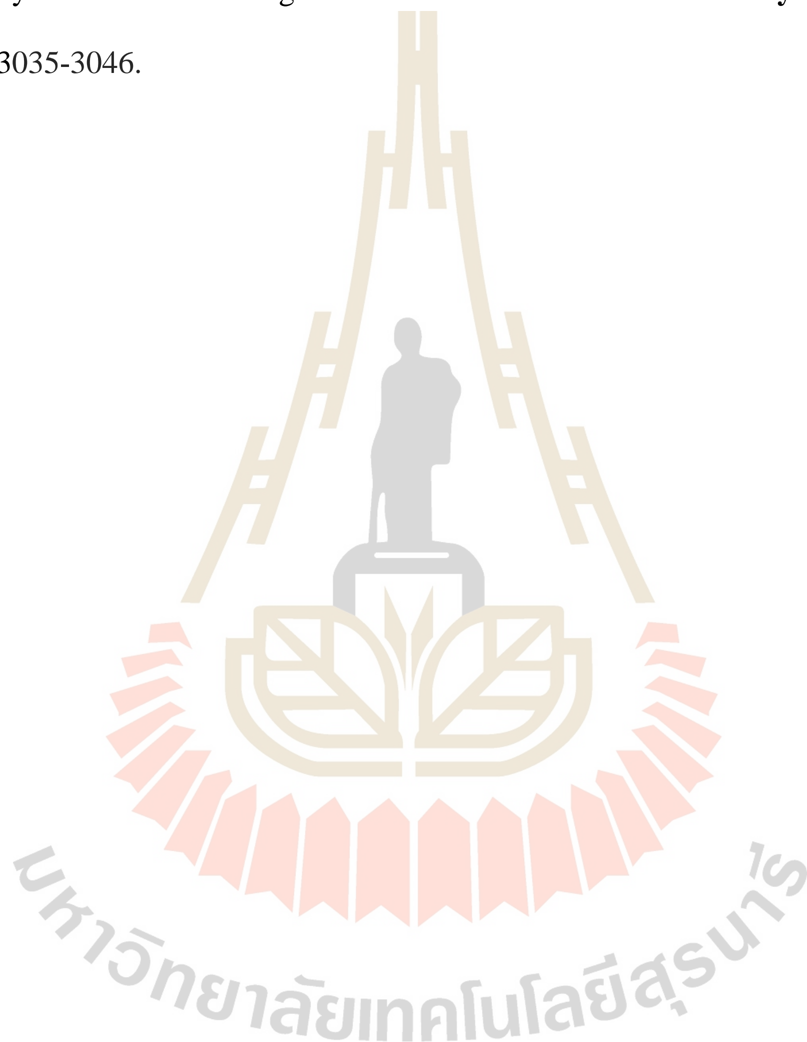
Shelke, S. K., Thakur, S. S. and Shete, S. M. (2012). Productive and reproductive performance of Murrah buffaloes (Bubalusbubalis) supplemented with rumen protected fat and protein**. Indian Journal of Animal Nutrition**, 29: 317-323.

Steel, R. G. D., and Torrie, J. H. (1980). **Principles and Procedures of Statistics: a biometric approach (2nd Ed)**, New York :McGrowhill.

Smith, S.B., Lunt, D.K., Chung, K.Y., Choi, C.B., Tume, R.K., and Zembayashi, M. (2006). Adiposity, fatty acid composition, and delta- 9 desaturase activity during growth in beef cattle. **Animal Science Journal.** 77(5): 478-486.

Ward,P.F.V., Scott,T.W., and Dawson,R.M.C.(1964). The hydrogenation of unsaturated fatty acids in the ovine digestive tract. **Biochemical Journal.** 92(1): 60-68.

Wu, Z., and Palmquist, D.L. (1991). Synthesis and Biohydrogenation of Fatty Acids by Ruminal Microorganisms In Vitro1. **Journal of Dairy Science.** 74(9): 3035-3046.



CHAPTER VII

OVERALL CONCLUSIONS AND RECOMMENDATION

7.1 Conclusions

The purposes of the present study were to investigate the effect of rumen protected fat containing high oleic acid supplementation on carcass quality, sensory evaluation and fatty acid profile of beef from crossbred Wagyu beef steers and effects of rumen protected fat containing oleic acid supplementation on ruminal bio-hydrogenation and fermentation in fistulated cattle. The present studies were successful.

The first experiment was to investigate the effects of rumen protected fat containing oleic acid including palm oil, rice bran oil, and corn oil supplementation on carcass quality, sensory evaluation and fatty acid profile of beef from crossbred Wagyu beef steers. The results revealed that feeding dietary treatment that inclusion of 200 g/d of rumen-protected plant oils (palm oil, rice bran oil and corn oil) did not negatively affect any of performance and carcass quality of steers. The overall feed consumption of the steers was unaffected when dietary oil was provided. RP-plant oils supplement did also not influence muscle sensory and physical characteristics. RP-RO increased the percentage of C18:1n-9) in the LD fat and lowered the C18:2n-6t in beef. RP-RO and RP-CO increased beef marbling score. Thus, it can be concluded that 200 g/d RP-RO can be safety supplemented to diets of steers to enrich beef with potential health beneficial FA.

The second experiment was carried out to investigate the effect of rumen-protected rice bran oil supplementation on carcass quality, sensory evaluation and fatty acid profile of beef from crossbred Wagyu beef steers. The results revealed that Feeding dietary treatment that inclusion of 100 or 200 g/d of rumen-protected rice bran oils did not negatively affect any of performance and carcass quality of steers. The overall feed consumption of the steers was unaffected when dietary rumen-protected rice bran oil was provided. RP-RO supplement did also not influence muscle sensory and physical characteristics with the exception of an increase in beef tenderness score of both LD and SM fat. RP-RO increased the percentage of C18:1n-9 and beef marbling score in the LD and SM fat. Thus, it can be concluded that RP-RO addition can be safety supplemented to diets of steers to enrich beef with potential health beneficial FA.

The third experiment was to determine the effects of rumen protected fat containing oleic acid including palm oil, rice bran oil, and corn oil supplementation on ruminal fermentation in fistulated cattle. The results demonstrated that supplementation of rumen-protected plant oils had no effects on pH, ammonia nitrogen, VFA, DMD, and CPD when compare to non-supplemented control. However, at 4 and 6 hours after feeding, there were a change in the proportion of fatty acids in the rumen. It was found that the proportion of ruminal C16: 0 in cattle fed RP-PO diet was significantly higher than those cattle fed RP-RO and RP-CO diets ($p<0.05$).The proportion of ruminal C18: 1n-9c was significantly higher in RP-RO cattle than in RP-PO and RP-CO cattle ($p<0.001$). While the ruminal proportion of C18: 2n-6c was significantly higher in RP-CO cattle than RP-PO and RP-RO cattle.

The fourth experiment was to determine effect of rumen-protected rice bran oil supplementation on ruminal bio-hydrogenation and fermentation in fistulated cattle. The results demonstrated that Supplementation of rumen-protected rice bran oil had no effects on pH, ammonia nitrogen, VFA, DMD, and CPD when compare to non-supplemented control. However, The proportion of ruminalC18:1n-9c, C18:2n-6,and *c9,t11*-C18:2was increased. while The proportion of ruminal C18:0 decreased when supplemented 200 g/d of rumen-protected rice bran oil compare to other treatments.

7.2 Recommendation

The present study suggests that the fattening crossbred Wagyu beef steers should be supplemented with high oleic acid source including 200 g/d RP-RO. The supplementing period being beneficial to RP-RO supplemented steers is at least 70 days before slaughtering since at this stage and at this supplementing period RP-RO can increased the percentage of C18:1n-9 and beef marbling score in the LD fat but cause no effect on growth performance in steer.

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APPENDIX

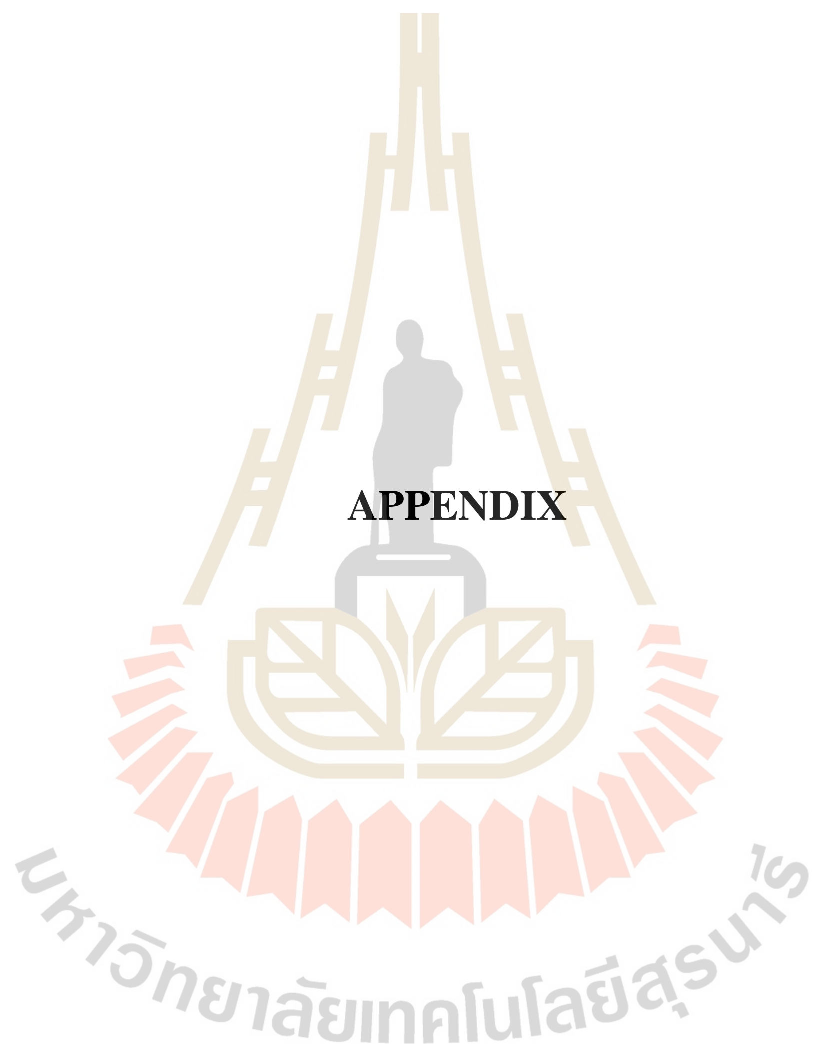

Table 1A Standards and reference compounds of fatty acid methyl esters by gas chromatography (GC) analysis (Supelco 37 Component FAME Mix).

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

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Table 2A degradability of rumen-protected plant oil (% of DM).

Item	RP-PO	RP-RO	RP-CO
Hour 0	4.38	4.69	4.52
Hour 2	11.69	12.03	12.36
Hour4	12.49	13.65	13.72
Hour6	13.22	13.80	14.27
Hour12	13.83	14.30	14.47
Hour24	14.11	14.73	14.93
Hour48	15.09	16.73	16.83

RP-PO = rumen-protected palm oil; RP-RO = rumen-protected rice bran oil; RP-CO = rumen-protected corn oil

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BIOGRAPHY

Rattakorn Mirattanaphrai was born on December 10th, 1987 in Roi-et Province, Thailand. He graduated Bachelor of Science in Animal Production Technology, Institute of Agricultural Technology, Suranaree University of Technology in 2009. He received Master of Science in Animal Production Technology, Institute of Agricultural Technology, Suranaree University of Technology in 20012. He continued to study Doctor of Philosophy in Animal Production Technology, Institute of Agricultural Technology, Suranaree University of Technology.