OPTIMIZING MILK PRODUCTION, MILK COMPOSITION, AND METHANE EMISSION IN DAIRY COWS : FEEDING OILS AND RUMEN UNDEGRADABLE PROTEIN

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การเพิ่มประสิทธิภาพ ผลผลิตน้ำนม องค์ประกอบของน้ำนม และ การปล่อยแก๊สมีเทนในโคนม : การเสริมน้ำมัน และโปรตีนที่ไม่ย่อยสลายในกระเพาะหมัก

นายลัม เฟือก ทั่น

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรดุษฎีบัณฑิต
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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.

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การทดลองที่ 1 และ 2 ดำเนินการเพื่อประเมินผลของการเสริมน้ำมันลินสีด (LO) หรือน้ำมันทานตะวัน (SO) หรือทั้งสองชนิด ร่วมกับน้ำมันปลา (FO) ที่ระดับ 3% ในอาหารโคนม ต่อผลผลิตน้ำนม องค์ประกอบน้ำนม องค์ประกอบของกรดไขมันในน้ำนม ผลผลิตแก๊สและมีเทน การหมักย่อยในกระเพาะหมัก และการย่อยได้ กลุ่มทดลองประกอบด้วย 1) กลุ่มที่ได้รับอาหารพื้นฐานโดยไม่เสริมน้ำมัน (กลุ่มควบคุม) 2) เสริม LO และ FO (LOFO) 3) เสริม SO และ FO (SOFO) และ 4) เสริม LO SO และ FO (MIXO) ไม่พบการเปลี่ยนแปลงผลผลิตน้ำนม แต่ SOFO ลด ($P < 0.05$) ผลผลิตและความเข้มข้นของไขมันนม LOFO เพิ่ม *cis*-9,*trans*-11 CLA และ n-3 PUFA ในน้ำนม ในขณะที่ MIXO เพิ่ม preformed FA and UFA การเสริมส่วนผสมของน้ำมันลด ($P < 0.01$) ผลผลิตแก๊สที่ระยะเวลาบ่ม 48 ชั่วโมง และผลผลิตมีเทนที่ระยะเวลาบ่ม 24 ชั่วโมง กลุ่ม LOFO และ SOFO ลด ($P < 0.05$) MCP IVTD IVOMD และ IVNDFD หากต้องการเพิ่ม UFA ในน้ำนม และลดมีเทน โดยไม่มีผลกระทบต่อกระบวนการหมักย่อยในกระเพาะหมัก ควรทำการเสริม MIXO

การทดลองที่ 3 และ 4 ดำเนินการเพื่อทดสอบผลของการทดแทนอาหารข้นในอาหารโคนมด้วย SBM หรือ CDDGS หรือทั้งสองอย่าง ต่อผลผลิตน้ำนม องค์ประกอบของน้ำนม รายได้สุทธิ ผลผลิตแก๊สและมีเทน กระบวนการหมักย่อยในกระเพาะหมัก และการย่อยได้ กลุ่มทดลองประกอบด้วย 1) ได้รับอาหารพื้นฐานโดยไม่มีการทดแทน (กลุ่มควบคุม) 2) ใช้ roasted SBM ทดแทนอาหารข้น (R-SBM) 3) ใช้ CDDGS ทดแทนอาหารข้น (DDGS) และ 4) ใช้ roasted SBM และ CDDGS ทดแทนอาหารข้น (SB-DG) กลุ่มทดลองไม่มีผลกระทบต่อองค์ประกอบของน้ำนม ในขณะที่ผลผลิตน้ำนมเพิ่มขึ้น ($P < 0.01$) ในกลุ่ม SB-DG รายได้สุทธิเพิ่มขึ้น ($P < 0.05$) จากสัปดาห์ที่ 5 หลังการเสริม ซึ่งให้มูลค่าสูงสุดในกลุ่ม SB-DG ทุกกลุ่มการทดลองให้ผลผลิตมีเทนต่ำกว่าเมื่อเปรียบเทียบกับกลุ่มควบคุม กลุ่ม DDGS ลดความเข้มข้นของ VFA MCP และ IVOMD ($P < 0.05$) เพื่อปรับปรุงผลผลิตน้ำนม และลดการปล่อยมีเทน โดยไม่มีผลกระทบต่อกระบวนการหมักย่อยในกระเพาะหมัก การทดแทนอาหารข้นด้วยส่วนผสมของทั้ง roasted SBM และ CDDGS บางส่วนเป็นมาตรการที่ดี

การทดลองที่ 5 และ 6 เพื่อหาผลของการเสริมส่วนผสมของน้ำมัน และโปรตีนที่ไม่ย่อยสลายในกระเพาะหมัก ในโคนม ต่อผลตอบสนองของผลผลิตน้ำนม ประสิทธิภาพการใช้อาหาร องค์ประกอบของกรดไขมัน ผลผลิตแก๊สและมีเทน กระบวนการหมักย่อยในกระเพาะหมัก และการย่อยได้ กลุ่มทดลองประกอบด้วย 1) ได้รับอาหารพื้นฐานโดยไม่มีน้ำมันและโปรตีนที่ไม่ย่อยสลายในกระเพาะหมักการทดแทน (กลุ่มควบคุม) และ 2) ใช้ roasted SBM และ CDDGS ทดแทนอาหารข้นเสริม LO SO และ FO ที่ระดับ 3% ในอาหารโคนม ทุกกลุ่มทดลองไม่มีผลกระทบต่อผลผลิตน้ำนม แต่ลดผลผลิตไขมันในน้ำนม ($P < 0.001$) ประสิทธิภาพการใช้อาหาร (Milk/DMI) ดีขึ้น ($P = 0.06$) ในทุกกลุ่มทดลอง และกลุ่มทดลองมีสัดส่วนและผลผลิตของ *cis*-9,*trans*-11 CLA และ n-3 PUFA ทุกชนิดสูงกว่า ($P < 0.001$) แต่มีสัดส่วนของ n-6/n-3 ต่ำกว่า ($P < 0.001$) กลุ่มทดลองลดผลผลิตแก๊ส ($P < 0.001$) ในทุกระยะเวลาบ่ม และลดผลผลิตมีเทน ($P < 0.001$) ที่ระยะเวลาบ่ม 24 ชั่วโมง กลุ่มทดลองลดความเข้มข้นของ total VFA ($P < 0.001$) IVTD และ IVNDFD ในขณะที่ไม่พบว่ากลุ่มทดลองมีผลกระทบต่อสัดส่วนโมลาร์ของ VFA แต่ละชนิด สรุปรวมทั้งหมดได้ว่า การเพิ่มกรดไขมันที่เป็นประโยชน์ต่อสุขภาพและลดผลผลิตมีเทนในโคนมสามารถทำได้โดยการเสริม LO SO และ FO ที่ระดับ 3% ร่วมกับ roasted SBM และ CDDGS ทดแทนอาหารข้นบางส่วน อย่างไรก็ตาม การเสริมส่วนผสมของน้ำมันที่ไม่ได้ป้องกันการย่อยสลายในกระเพาะหมักอาจส่งผลเสียต่อการสังเคราะห์ไขมันในน้ำนม

สาขาวิชาเทคโนโลยีการผลิตสัตว์ ลงชื่อนักศึกษา_____

ปีการศึกษา 2557 อาจารย์ที่ปรึกษา _____

LAM PHUOC THANH : OPTIMIZING MILK PRODUCTION, MILK COMPOSITION, AND METHANE EMISSION IN DAIRY COWS : FEEDING OILS AND RUMEN UNDEGRADABLE PROTEIN. THESIS ADVISOR : ASSOC. PROF. WISITIPORN SUKSOMBAT, Ph.D., 217 PP.

DAIRY COW/MILK PRODUCTION/MILK COMPOSITION/MILK FATTY ACID/METHANE PRODUCTION/OIL/RUMEN UNDEGRADABLE PROTEIN

Experiments 1 and 2 were conducted to evaluate the effects of supplementing either linseed oil (LO) or sunflower oil (SO) or both together with fish oil (FO) at 3% DM in dairy cattle diet on milk yield, milk composition, milk fatty acid (FA) profiles, gas production, methane (CH_4) production, ruminal fermentation, and digestibility. The treatments included : 1) basal diet without oil addition (Control), 2) LO and FO (LOFO), 3) SO and FO (SOFO), and 4) LO, SO and FO (MIXO). No change was detected for milk yield, but the SOFO depressed ($P < 0.05$) milk fat yield and concentration. The LOFO increased milk *cis*-9,*trans*-11 conjugated linoleic acid (CLA) and n-3 polyunsaturated fatty acids (PUFA) while the MIXO improved preformed FA and unsaturated fatty acids (UFA). Inclusion of oil mixtures reduced ($P < 0.01$) gas production at 48 h and CH_4 production at 24 h incubation. The LOFO and SOFO reduced ($P < 0.05$) microbial crude protein (MCP), *in vitro* true digestibility (IVTD), *in vitro* organic matter digestibility (IVOMD), and *in vitro* neutral detergent fiber digestibility (IVNDFD). To improve milk UFA and to reduce CH_4 production without affecting digestibility, an ideal oil inclusion would be MIXO.

Experiments 3 and 4 were conducted to test the effects of replacing concentrate in dairy cattle diet with either roasted soybean meal (SBM) or corn distiller dried grains with solubles (CDDGS) or both on milk yield, milk composition, net income, gas production, CH_4 production, ruminal fermentation, and digestibility. The treatments

included : 1) basal diet without feed substitution (Control), 2) roasted SBM replaced for concentrate (R-SBM), 3) CDDGS replaced for concentrate (DDGS), and 4) roasted SBM plus CDDGS replaced for concentrate (SB-DG). The treatments had no effect on milk composition while milk yield increased ($P < 0.01$) in the SB-DG. Net income was increased ($P < 0.05$) after 5th week feeding with the greatest value in the SB-DG. All treatments had lower ($P < 0.001$) CH_4 production compared with the control. The DDGS reduced total volatile fatty acid (VFA) concentration, MCP, and IVOMD ($P < 0.05$). To improve milk yield and net income and to mitigate CH_4 emission without affecting rumen fermentation, the SB-DG would be an ideal approach.

Experiments 5 and 6 were designed to determine the effects of feeding oil mixture and rumen undegradable protein (RUP) in dairy cattle on milk yield responses, feed efficiency, milk FA profiles, gas production, CH_4 production, ruminal fermentation, and digestibility. The diets included : 1) basal diet without oil and RUP addition (Control) and 2) roasted SBM plus CDDGS replaced for concentrate then supplemented with a mixture of LO, SO and FO at 3% DM (Treatment). The treatment had no effect on milk yield, but reduced milk fat yield ($P < 0.001$). Feed efficiency (Milk/DMI) was improved ($P = 0.06$) by the treatment. The treatment had higher ($P < 0.001$) proportions and yields of milk *cis*-9,*trans*-11 CLA and n-3 PUFA, but lower ($P < 0.001$) n-6/n-3 ratio. The treatment reduced ($P < 0.05$) gas and CH_4 production. The treatment also decreased ($P < 0.001$) total VFA concentration, IVTD, and IVNDFD. Overall, enrichment of healthy FA in milk and mitigation of CH_4 production in dairy cattle was achieved by feeding LO, SO, and FO at 3% DM along with roasted SBM and CDDGS partially replaced for concentration. However, feeding unprotected oil mixture at 3% DM and RUP could cause detrimental effects on milk fat synthesis.

School of Animal Production Technology Student's Signature _____

Academic Year 2014 Advisor's Signature _____

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LIST OF ABBREVIATIONS

AI	=	Atherogenicity index
c	=	*cis*
CDDGS	=	Corn distiller dried grains with solubles
cfu	=	Colony-forming unit
CHO	=	Carbohydrate
DDGS	=	Treatment containing only corn distiller dried grains with solubles
ECM	=	Energy corrected milk
FA	=	Fatty acid or fatty acids
FAME	=	Fatty acid methyl ester
FCM	=	Fat corrected milk
FO	=	Fish oil
GHG	=	Green house gases
HR-SBM	=	High roasted soybean meal
IVDOM	=	*In vitro* digestible organic matter
IVNDFD	=	*In vitro* neutral detergent fiber digestibility
IVTD	=	*In vitro* true digestibility
LO	=	Linseed oil
LOFO	=	Linseed oil and fish oil
LS	=	Linseed
MCP	=	Microbial crude protein
MFD	=	Milk fat depression

LIST OF ABBREVIATIONS (Continued)

MIXO	=	Linseed oil, sunflower oil, and fish oil
MR-SBM	=	Medium roasted soybean meal
NEB	=	Negative energy balance
NE_L	=	Net energy for lactation
NFC	=	Non-fiber carbohydrates
NI	=	Nitrogen intake
RDP	=	Rumen degradable protein
RDPI	=	Rumen degradable protein intake
Ref	=	References
R-SBM	=	Roasted soybean meal
RUP	=	Rumen undegradable protein
RUPI	=	Rumen undegradable protein intake
SB-DG	=	Roasted soybean meal and corn distiller dried grains with solubles
SBM	=	Soybean meal
SF	=	Sunflower
SO	=	Sunflower oil
SOFO	=	Sunflower oil and fish oil
t	=	*trans*
TI	=	Thrombogenicity index
TNF-α	=	Tumor necrosis factor alpha
ω-3	=	Omega-3 or n-3
ω-6	=	Omega-6 or n-6

CHAPTER I

INTRODUCTION

1.1 Rationale of the study

Public health guidelines in most developed countries have recommended population-wide decreases in saturated and *trans* fatty acids (FA) and an increase in alpha-linolenic acid (C18:3n-3, ALA), eicosapentaenoic acid (C20:5n-3, EPA), and docosahexaenoic acid (C22:6n-3, DHA) in the human food chain to reduce the incidence of chronic diseases (WHO, 2003). Dietary consumption of omega-3 fatty acids (n-3 FA) is beneficial for human health (Gebauer et al., 2006), and conjugated linoleic acids (CLA) from ruminant fat has been shown to exert anti-carcinogenic benefits in experimental various animal models (Gebauer et al., 2011; Huth et al., 2006). There is growing interest in elevating n-3 FA and CLA contents in ruminant products, and supplementation of ruminant diets with oilseeds rich in ALA has been shown to increase n-3 FA and CLA contents in milk (Caroprese et al., 2010; Chilliard et al., 2009). In cattle, linseed oil (LO) inclusion in the diet increases *trans*-11 C18:1, *cis*-9,*trans*-11 CLA, and C18:3n-3 at the duodenum (Doreau et al., 2009; Loor et al., 2002), whereas fish oil (FO) supplement results in greater flows of *trans*-11 C18:1, EPA, and DHA (Kim et al., 2008; Lee et al., 2008; Shingfield et al., 2011).

However, the transformation of unsaturated fatty acids (UFA) to saturated fatty acids (SFA), or biohydrogenation (BH), in ruminants represents a major human health issue. The BH process has long been known to occur in the rumen as the result

of microbial metabolic activity (Lee and Jenkins, 2011). Thus, if ruminal BH of UFA can be minimized, it may be possible to improve post-ruminal supply of polyunsaturated fatty acids (PUFA) and therefore the healthiness of ruminant meats and milk. Inclusion of FO containing EPA and DHA to *in vitro* incubations resulted in incomplete BH of linolenic acid (C18:2n-6, LA) and ALA with accumulation of *cis*-9,*trans*-11 CLA and *trans* C18:1 isomers (Boeckaert et al., 2007). Moreover, supplementing the diet of dairy cows with incremental amounts of FO increased the flow of not only EPA and DHA but also *trans* C18:1 and *trans* C18:2 at the omasum (Shingfield et al., 2012). Results suggest that one or more FA in FO inhibit the reduction of *trans* C18:1 and *trans* C18:2 intermediates by ruminal microorganisms (Shingfield et al., 2012). Consequently, FO supplement along with sources of LA and ALA may be a potential strategy to improve healthy FA including EPA, DHA, CLA isomers, and ALA in dairy cows' milk.

In another thing, production of greenhouse gases (GHG) from livestock and their impact on climate change is a currently major concern (O'Mara, 2011). Livestock associated emissions will increase as world population and food demand increases. It has been reported that enteric methane (CH_4) is one of the most important anthropogenic GHG emitted at the farm level in ruminant production systems. It is the main contributor to livestock GHG ranging from 48 to 65% in bovine milk production systems and from 56 to 65% in New Zealand dairy farms (Basset-Mens et al., 2009). The enteric CH_4 emission was projected to increase by over 30% from 2000 to 2020 (O'Mara, 2011). Moreover, energy loss from animals due to CH_4 production ranges from 2 to 12% of gross energy intake in mature cattle (Johnson and Johnson, 1995). Thus, the development of feeding strategies to mitigate

these CH_4 emissions may bring not only environmental friendly for the planet but also nutritional benefits for the animal. Up to now, although there are many GHG mitigation strategies with available technologies, current approaches are cost implications which lead to limit implementation. Therefore, suitable technologies to cost-effective implement should be developed. Webb et al. (2014) suggested that livestock production should be configured to maintain production for human food demand while meeting points to abate GHG emissions. Among the technologies to mitigate GHG from livestock systems, incorporating oil sources to cattle diets has been shown as a proper method to reduce enteric CH_4 emissions (Knapp et al., 2014; Martin et al., 2010).

However, feeding high amounts of oil sources in the ruminant diets could cause adverse effect on feed intake (Boerman and Lock, 2014; Lima et al., 2014), and therefore animal performance (Chilliard et al., 2009). In most situations, total dietary lipid in ruminant diets should not exceed 6-7% of dietary dry matter (DM) (NRC, 2001). Meanwhile, milk yield and reproductive performance are the most important factors in determining profitability of dairy cows, and high milk production is more important to get high profitability than low feeding cost. In early lactating cows moreover, disparities between energy consumption and that required for production can cause a state of negative energy balance (NEB) and disturb metabolic status that predisposes the animals to reduce reproductive and productive performance cumulating in decreased profitability at the farm level (McArt et al., 2013). Therefore, minimizing the extent and duration of NEB might be beneficial for both productive and reproductive performance from dairy cows. Improvements in NEB and adverse effects of oil supplementation on productive performance of

lactating animals are possible through feeding diet high in rumen undegradable protein (RUP). The RUP feeding to lactating animals results in a proportionate increase of amino acid (AA) supply to the host animals for reproduction and production. Some previous studies on dairy cattle and buffaloes reported that feeding RUP increased growth rate and milk yield by 15-25% and 10-15%, respectively (Shelke et al., 2012).

Therefore, feeding of FO in combination with LO or/and SO along with RUP sources to dairy cattle may increase milk healthy FA composition, milk yield and composition as well as mitigate CH_4 production. However, there are still few studies to test the effects of feeding oil mixtures rich in PUFA along with RUP in lactating cows on milk FA composition, milk yield, milk composition, and CH_4 production.

1.2 Research objectives

1) To study the effects of supplementing FO in combination with either LO or SO or both on milk yield, milk composition, milk FA profiles, nutrient digestibility, ruminal fermentation, and CH_4 production of dairy cows.
2) To study the effects of partially replacing concentrate with either roasted soybean meal (SBM) or corn distiller dried grains with solubles (CDDGS) or both on milk yield, milk composition, feed efficiency, income over feed costs, nutrient digestibility, ruminal fermentation, CH_4 production of dairy.
3) To study the effects of supplementing oil mixture rich in PUFA along with RUP on milk yield, milk composition, milk FA composition, nutrient digestibility, ruminal fermentation, and CH_4 production of dairy cows.

1.3 Research hypotheses

1) Dairy cows fed the diet supplemented with oil mixtures high in PUFA mitigated CH_4 production and increased the escape of UFA from rumen BH resulting in enhanced milk UFA composition.
2) Dairy cows fed the diet high in RUP content improved feed efficiency use for milk production resulting in increased milk yield and net income over feed costs without affecting milk composition.
3) Dairy cows fed the diet supplemented with oil mixtures high in PUFA along with RUP abated CH_4 production, improved milk UFA composition, and enhanced feed efficiency use for milk production.

1.4 Scope and limitation of the thesis

1) Crossbred Holstein Friesian cows (1-3 lactation, early- and mid-lactation) from Dairy farm and incubation systems from The Center of Scientific and Technological Equipment of Suranaree University of Technology were used in the studies of optimizing milk production, milk composition, and CH_4 emission in dairy cows : feeding oils and RUP from May 2013 to May 2014.
2) Animal used and feed processing prepared were taken much time, intensive labors, and budgets.

1.5 Expected results

1) To know the effects of oil mixtures rich in PUFA on milk yield, milk composition, milk FA profiles, nutrient digestibility, ruminal fermentation, and CH_4 production of dairy cows.

2) To know the effects of feeding different sources of RUP on milk yield, milk composition, feed efficiency, net income over feed costs, nutrient digestibility, ruminal fermentation, CH_4 production of dairy cows.

3) To increase milk healthy FA prolife, milk yield and feed efficiency, and to mitigate CH_4 production by supplementing oil mixture along with RUP in dairy cows.

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CHAPTER II

LITERATURE REVIEW

2.1 Fatty acids

Fatty acids are aliphatic compounds comprising a carboxyl group and a hydrocarbon chain of varying length and degree of saturation. Natural FA commonly have straight chains of an even number of 4 to 28 carbon atoms. Saturated fatty acids have no double bonds in the acyl chain, whereas UFA contain at least one double bond. Fatty acids containing 2 or more double bonds are referred to as PUFA. The PUFA are classified in 2 principal families, the n-6 (or ω-6) and the n-3 (or ω-3) families, according to the position of the terminal double bond. The parent FA of these families, LA and ALA which cannot be synthesized in mammals; they must be provided by the diet and are therefore defined as essential FA (Glaser et al., 2010). Short chain (saturated) fatty acids (SCFA) include acetic (C2:0), propionic (C3:0), and butyric (C4:0) acids, which are formed during fiber fermentation in the rumen and colon. Medium chain (saturated) fatty acids (MCFA) include caproic (C6:0), caprylic (C8:0), and capric (C10:0) acids. Long chain (saturated) fatty acids (LCFA) include lauric (C12:0), myristic (C14:0), palmitic (C16:0), and stearic (C18:0) acids have significant atherogenic and thrombogenic potential. Very long chain (saturated) fatty acids (VLCFA) include arachidic (C20:0), behenic (C22:0), lignoceric (C24:0), cerotic (C26:0), montanic (C28:0), and melissic (C30:0) acids, which appear in significant concentrations in inherited

metabolic diseases, e.g. Zellweger syndrome, X-linked adrenoleucodystrophy, Refsum's disease, and Menkes' disease (Tvrzicka et al., 2011).

2.1.1 Sources of edible fatty acids

The main sources of the SCFA are palm oil and cottonseed oil. Almost the fat sources contain adequate amount of LCFA. Omega-7 in form of palmitoleic acid (C16:1) often occurs in avocado, butter, cashew, macadamia nut, olive, peanut, and pecan. The common sources of LA or n-6 FA are flaxseed, hemp, nuts, pumpkin seeds, safflower, sesame seeds, soybean, and sunflower seeds. The main sources of ALA or n-3 FA are in vegetable oils such as canola, corn, hemp, linseeds, nuts, olive, safflower, and soybean. Omega-3 FA including EPA and DHA are often found in fish sources such as anchovies, herring, mackerel, salmon, sardines, trout, and tuna, whereas plant sources of n-3 PUFA contain neither EPA nor DHA. Fatty acid composition of some edible sources is presented in the Tables 2.1 and 2.2.

Table 2.1 Major fatty acid composition of some plant oils (g/100 g of total FA)

Oil source	Stearic acid (C18:0)	Oleic acid (C18:1n-9)	Linoleic acid (C18:2n-6)	Linolenic acid (C18:3n-3)
Grass[1]	3.29	5.74	14.0	49.2
Grass silage[1]	2.90	6.32	14.5	46.2
Corn silage[2]	3.07	17.7	45.8	6.54
Rapeseed oil[3]	2.10	60.5	20.8	9.20
Soya oil[3]	4.10	22.3	53.5	7.00
Canola oil[4]	1.93	63.4	20.5	5.89

Table 2.1 Major fatty acid composition of some plant oils (g/100 g of total FA) (conc.)

Oil source	Stearic acid (C18:0)	Oleic acid (C18:1n-9)	Linoleic acid (C18:2n-6)	Linolenic acid (C18:3n-3)
Corn oil[4]	2.17	28.9	55.5	1.06
Cottonseed oil[4]	2.45	19.5	51.8	0.18
Olive oil[4]	2.76	70.0	10.2	0.14
Soybean oil[4]	4.06	22.4	51.2	6.58
Sunflower oil[4]	3.60	29.4	58.0	0.40
Linseed oil[5]	3.08	15.8	13.7	62.0
Safflower oil[5]	2.22	11.7	76.2	3.47

Sources : [1]French et al. (2000), [2]Cabrita et al. (2007), [3]Glasser et al. (2008), [4]Yalcin et al. (2012), and [5]Li et al. (2012).

Table 2.2 EPA and DHA contents of some fish oils (g/100 g of total FA)

Oil source	EPA (C20:5n-3)	DHA (C22:6n-3)
Marine fish oil[1]	35.8	28.4
Menhaden fish oil[2]	19.9	16.3
Crude fish oil[3]	13.5	14.3
Farmed bluefin tuna fish oil (*Thunnus thynnus*)[4]	8.66	10.4
Wild bluefin tuna fish oil (*Thunnus thynnus*)[4]	6.51	16.2
Yellowfin tuna fish oil (*Thunnus Albacares*)[5]	2.39	16.9
Bigeye tuna fish oil (*Thunnus Obesus*)[5]	3.27	20.2

Sources : [1]Mattos et al. (2004), [2]Bharathan et al. (2008), [3]Duckett and Gillis (2010), [4]Topic Popovic et al. (2012), and [5]Peng et al. (2013).

2.1.2 Metabolism of n-3 fatty acids in the rumen

Long-chain PUFA (LC-PUFA) are provided by the diet, but can also be synthesized in human and animal metabolism from the precursor essential FA, such as LA and ALA, by the action of desaturases and elongases (Figure 2.1). Linoleic acid and ALA serve as substrates for other important FA. By insertion of additional double bonds into the acyl chain and by elongation of the acyl chain, LC-PUFA are synthesized endogenously from LA and ALA. Both these FA have analogous reaction pathways catalyzed by the same enzymes. Therefore, a competition exists between both FA families for metabolism. However, these enzymes appear to give preference to the n-3 over the n-6 pathway (Guil-Guerrero, 2002). Linoleic acid and ALA can be converted by Δ-6 desaturation to GLA and stearidonic acid (SDA), respectively. This step is rate limiting and is followed by elongation of GLA to DGLA and of SDA to eicosatetraenoic acid (C20:4n-3, ETA). In addition to these common pathways, Park et al. (2009) reported an alternative pathway via elongation of LA and ALA to n-6 eicosadienoic acid (C20:2n-6) and n-3 eicosatrienoic acid (C20:3n-3), followed by a Δ-8 desaturation of these PUFA to DGLA and ETA, respectively. Both PUFA can be further elongated, leading to the production of arachidonic acid and EPA. A further important LC-PUFA is DHA, the end-product of the n-3 family (Figure 2.1). The conversion of ALA to DHA requires several elongation and desaturation steps, all taking place in the endoplasmatic reticulum. However, the last step requires a compartmental translocation to peroxisomes, the unique place for β-oxidation of LC-PUFA (Sprecher, 2000). This restriction may explain why the conversion rate of docosapentaenoic acid (C22:5n-3), the elongation product of EPA, to DHA is low (Burdge, 2004).

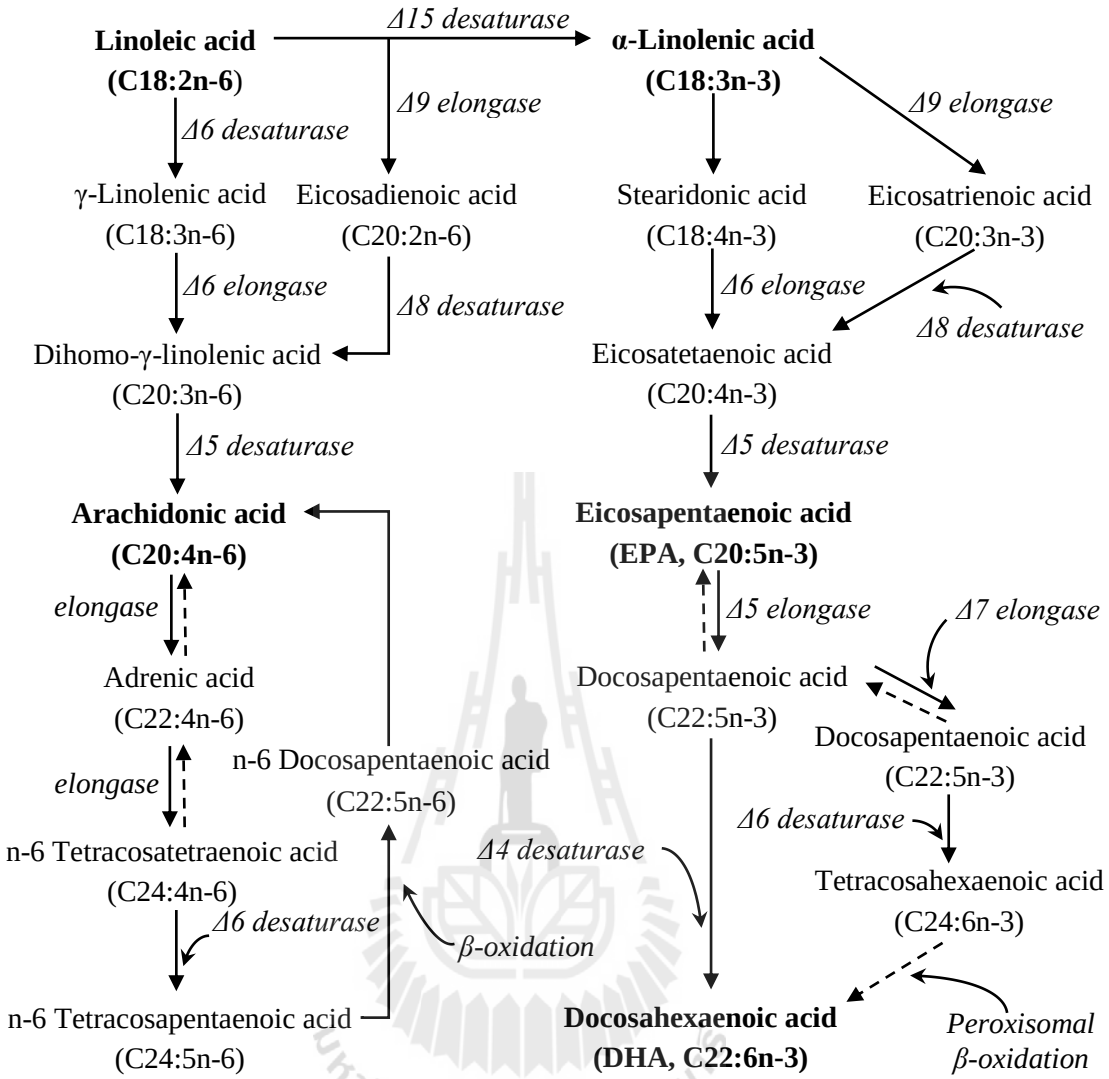

Figure 2.1 The common biosynthesis pathways of n-6 and n-3 PUFA. Adapted from Guil-Guerrero (2007), Park et al. (2009), and Conklin et al. (2010).

2.1.3 Synthesis of CLA isomers in the ruminants

The presence of CLA in milk fat from ruminants relates to the isomerization and BH of dietary UFA by rumen bacteria as well as the Δ9–desaturase activity in the mammary gland (Figure 2.2). The *cis*-9,*trans*-11 CLA contributes to 75-90% of total CLA and is derived from LA and ALA (Bauman et al., 2003). Linoleic acid (*cis*-9,*cis*-12 C18:2) is first isomerized to the *cis*-9,*trans*-11 CLA by *cis*-12,*trans*-11

isomerase and then hydrogenated by *Butyrivibrio fibrisolvens* to vaccenic acid (VA, *trans*-11 C18:1) in the rumen (Kepler and Tove, 1967). These initial steps occur rapidly. A strong positive correlation between the *trans* isomers of C18:1 (VA, *trans*-13–14, *trans*-15, and *trans*-16) in milk fat and the level of LA in the diet was first found by Loor et al. (2002). The hydrogenation of VA to stearic acid appears to involve a different group of organisms and occurs at a slow rate (Harfoot and Hazelwood, 1997). For this reason, VA typically accumulates in the rumen. This main *trans* FA is responsible for the formation of the *cis*-9,*trans*-11 CLA, which occurs by desaturation (Δ9-desaturase) of the ruminally derived VA in the mammary gland (Griinari et al., 2000; Piperova et al., 2000). The pathway for the formation of the *cis*-9,*trans*-11 CLA from ALA (*cis*-9,*cis*-12,*cis*-15 C18:3) in the rumen involves an initial isomerization to a conjugated triene (*cis*-9,*trans*-11,*cis*-15 C18:3), followed by reduction of double bonds at carbons 9, 15, and 11 to yield the *trans*-11,*cis*-15 C18:2, *trans*-11 C18:1, and C18:0 FA, respectively, but not *cis*-9,*trans*-11 CLA, as intermediates (Wilde and Dawson, 1966). Kraft et al. (2003) hypothesized that ALA is the indirect precursor of another CLA (*trans*-11,*cis*-13). The pathway from *trans*-11,*cis*-15 FA to the *trans*-11,*cis*-13 CLA isomer is as yet unclear. Bauman and Griinari (2003) described that under certain dietary situations, the rumen environment is altered and a portion of BH occurs via a pathway that produces *trans*-10,*cis*-12 CLA and *trans*-10 C18:1 (dotted arrows, left side; Figure 2.2). *Bifidobacterium, Propionibacterium, Lactococcus, Streptococcus,* and *Lactobacillus* isolates from other habitats have been reported to produce *trans*-10,*cis*-12 CLA. As these genera occur in the rumen, although generally at rather low numbers, they may contribute to BH and specifically to *trans*-10,*cis*-12 CLA

formation in the rumen. *Propionibacterium, Streptococcus*, and *Lactobacillus* are also more numerous in the rumen with concentrate diets (Jenkins et al., 2008), which would again be consistent with greater *trans*-10,*cis*-12 CLA production with concentrate diets. Therefore, dietary situations causing MFD alter the pathways of rumen BH resulting in changes in the specific *trans* C18:1 and CLA isomers available for uptake by the mammary gland and incorporation into milk fat.

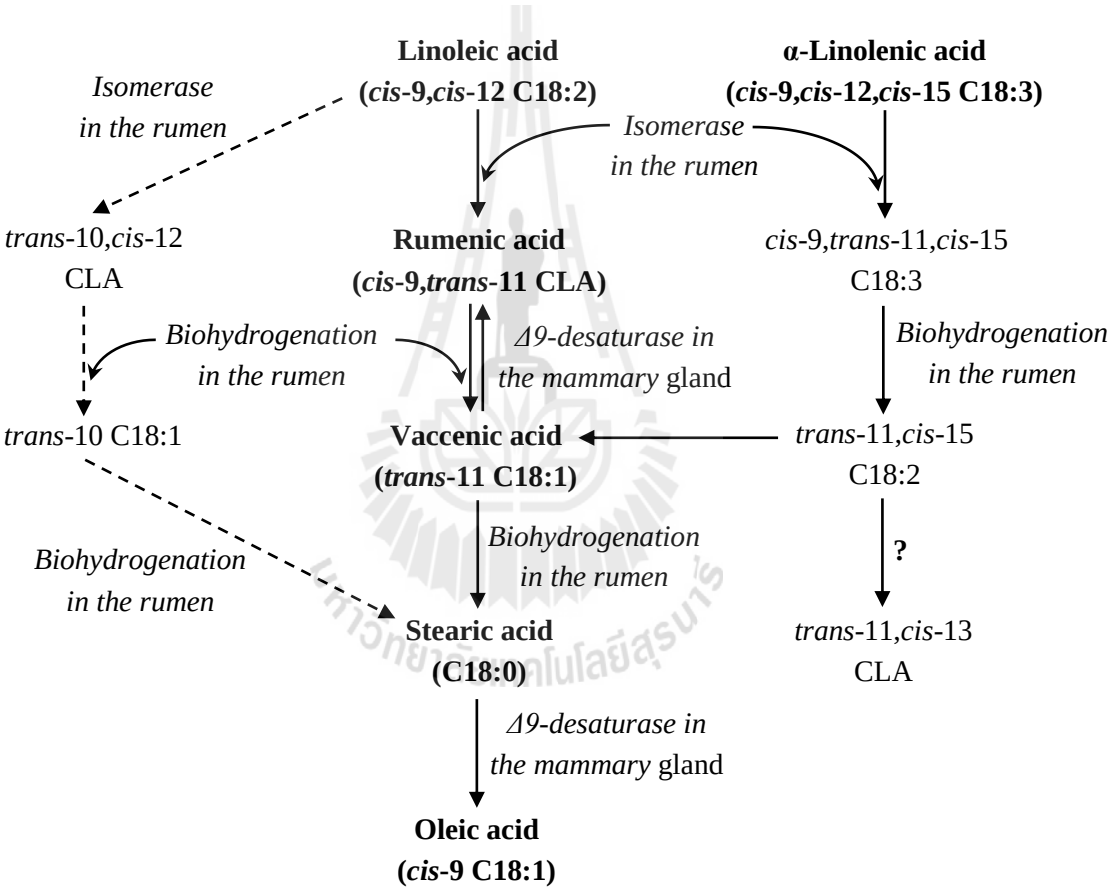

Figure 2.2 Metabolic pathways for formation of CLA isomers. Adapted from Bauman and Griinari (2003) and Collomb et al. (2006).

As shown in Figure 2.2, this '*trans*-10 shift' in BH pathways, and the associated increase in the *trans*-10 C18:1 content of milk fat, is indicative of the

complex changes in ruminal BH pathways characteristic of MFD. Hinrichsen et al. (2006) reported that there was the negative correlation between the content of *trans*-10 C18:1 in milk fat and milk fat percent. Although *trans*-10 C18:1 does not directly inhibit mammary synthesis of milk fat (Lock and Bauman, 2007), it is relatively easy to analyze compared to *trans*-10,*cis*-12 CLA and other CLA isomers. Therefore, in general, this FA can serve as a surrogate marker for the type of alterations in rumen BH that characterize diet-induced MFD.

2.1.4 Roles of n-3 PUFA on human health

Omega-3 PUFA are naturally occurring LCFA. The two main n-3 PUFA such as EPA and DHA are found predominantly in fish such as salmon, mackerel, sardines, and tuna. The parent C18:3n-3 PUFA, ALA, is commonly found in vegetable oils. The DHA and EPA are thought to play an important function in the prevention and treatment of inflammatory, cardiovascular, autoimmune diseases, mental disorders, and cancer because of their immune-suppressive action, whereas DHA is crucial for normal functional development of the brain and retina (Simopoulos, 2002; Uauy and Dangour, 2006). The EPA and DHA are not well converted in humans and other mammals from their precursor as ALA due to the moderate activity of some enzymes involved in these conversions (Horrocks and Yeo, 1999; Uauy and Dangour, 2006; Zamaria, 2004), and the amount of these FA required appears to be unattainable by this route. Therefore, in consequence of their importance, they are recommended to be included in the diet as conditionally essential nutrients (Hull, 2011).

There is evidence that n-3 PUFA improve cardiovascular outcomes (De Caterina, 2011) and that they have efficacy in rheumatological conditions (Bhangle

and Kolasinski, 2011). There is now emerging evidence that n-3 PUFA may also have anti-cancer activity, particularly against colorectal cancer (Cockbain et al., 2011). Several different molecular and cellular activities have been proposed, based mainly on *in vitro* observations, in order to explain the anti-neoplastic activity of n-3 PUFA. Hull (2011) reported that these mechanisms include : 1) inhibition of cyclooxygenase activity; 2) production of novel anti-inflammatory lipid mediators, including resolvins, protectins, and maresins; 3) direct FA signaling via G protein-coupled receptors; 4) alteration of membrane dynamics and cell surface receptor function; and 5) increase of cellular oxidative stress. Omega-3 PUFA have recognized immunomodulatory activity including alteration of T-cell activation and cytokine production (Calder, 2007).

2.1.5 Roles of CLA on human health

Positive health effects attributed to CLA are mainly based on cell culture models and animal studies with comparatively less scientific evidences from direct studies on humans (Rainer and Heiss, 2004). Being the predominant isomers, *cis*-9,*trans*-11 CLA and *trans*-10,*cis*-12 CLA are the primary focus of most of the studies evaluating the biological activities of CLA, which are primarily derived from LA (Figure 2.2). The fundamental basis for major health benefits attributed to CLA including anti-carcinogenesis, anti-atherosclerosis, anti-obesity, born formation, and immunomodulation (Park, 2009).

Anti-carcinogenesis : Isomers of CLA have been shown to reduce cancer in animal models, such as skin, fore-stomach, colon, mammary, and liver. The inclusion of CLA at 1% of the diet, for 30 weeks, reduced 1,2-dimethylhydrazine-induced tumor incidence in the colon of rats, possibly through induction of apoptosis (Park et

al., 2001). It has been suggested that CLA not only reduce initiation, promotion, and progression steps of cancer development, but also reduce metastasis of cancer. It is suggested that CLA may be involved in reducing eicosanoids production, interfering with cell signaling pathways, inhibiting DNA synthesis, enhancing apoptosis, as well as inhibiting angiogenesis as shown in reduced matrix metallo-proteinases and vascular endothelial growth factors (Bhattacharya et al., 2006; Kelley et al., 2007; Lee et al., 2005b).

Anti-atherosclerosis : CLA has been reported to reduce atherosclerotic lesions in hamsters and rabbits (Kritchevsky et al., 2004; Nicolosi et al., 1996). CLA reduced total cholesterol, triacylglycerides, low-density lipoprotein cholesterol and increased high-density lipoprotein cholesterol in a number of animal models (Lee et al., 2005a; Stangl, 2000). CLA affects these parameters through reduced blood pressure, or involvement of peroxisome proliferator-activated receptor, sterol regulatory element-binding proteins, and/or steroyl-CoA desaturase (Bhattacharya et al., 2006). In human, intake of 3 g/day of CLA (50 : 50 *cis*-9,*trans*-11 and *trans*-10,*cis*-12) for 8 weeks increased high-density lipoprotein concentration and reduced ratio of low-density lipoprotein to high-density lipoprotein in type 2 diabetic patients (Moloney et al., 2004).

Anti-obesity : Considering anti-obesitic and hypolipidemic effects, it is possible to modify body composition by supplementing CLA to the diet (Benjamin and Spener, 2009). Since then it was confirmed that the *trans*-10,*cis*-12 CLA is the isomer responsible for this activity (Park et al., 2009). CLA's effect on body fat reduction is suggested to be the result of multiple mechanisms : increasing energy expenditure, reducing lipid accumulation in adipose tissues and/or adipocytes

differentiation, increasing adipocyte apoptosis, modulating adipokines and cytokines, such as leptin, TNF-α, adiponectin, or interleukins, and increasing FA β-oxidation in skeletal muscle (Park and Pariza, 2007).

Bone formation : Although CLA has been reported to improved bone mass as reported by ash weights, bone density, bone mineral contents, bone dry weights, bone length, or calcium, magnesium or phosphate contents, effects of CLA on body ash or bone mass have not been consistent (Park, 2009). This inconsistency of CLA on bone mass may be due in part to interaction between CLA and calcium in the diet (Park and Pariza, 2008). It has been reported that CLA decreased activities of osteoclasts thus reducing bone resorption (Rahman et al., 2007) while Rahman et al. (2006) reported no effects of CLA on markers of bone resorption.

Immunomodulations : Anti-inflammatory properties of CLA have been reviewed by reducing colonic inflammation, decreasing antigen-induced cytokine production in immune-competent cells, and modulating the production of cytokines, prostaglandins, and leukotrien B4 (Park, 2009). However, Poirier et al. (2006) reported that the *trans*-10,*cis*-12 isomers induced inflammatory responses in white adipose tissue. Park (2009) concluded that CLA improves immune related responses by modulating tumor necrosis factor-α (TNF-α), cytokines (i.e. interleukin-1, 4, 6, or 8), prostaglandins, or nitric oxides while reducing allergic type immune responses.

2.1.6 Oil supplement in ruminants

Effect of oil supplement on methane production : Dietary FA, particularly PUFA, are among the most promising dietary alternatives able to depress ruminal methanogenesis (Martin et al., 2008). The PUFA decrease CH_4 production through different working mechanisms, e.g., stimulation of propionate, direct inhibition of

methanogens, and a toxic effect on microorganisms involved in fibre digestion and hydrogen production such as protozoa and cellulolytic bacteria (Castro-Montoya et al., 2012; Martin et al., 2010). This effect, observed with all long-chain PUFA, is probably through an action on the cell membrane particularly of gram-positive bacteria. It has been shown *in vitro* that ALA (predominant FA in LO) is particularly toxic for the 3 cellulolytic bacterial species (*Fibrobacter succinogenes*, *Ruminococcus albus*, and *Ruminococcus flavefaciens*), because it disrupts cell integrity (Maia et al., 2007). In addition, a direct toxic effect of PUFA on methanogens that use hydrogen for CH_4 production may have occurred, as shown *in vitro* with linseed oil hydrolysate (Prins et al., 1972). In this case, free hydrogen may accumulate in the gas mixture, resulting in growth inhibition of cellulolytic bacteria (Wolin et al., 1997). The remarkable lower daily CH_4 output (g/d) was found in the animals supplemented with linseed oil, offered FA level at a dose of 5.7 g/kg DM, as compared to the control diet and other linseed forms (Martin et al., 2008). Chung et al. (2011) reported that the lower enteric CH_4 production in the non-lactating cows supplemented with ground linseed (150 g/kg DM) compared to the animals fed basal diet based on barley silage. The PUFA in free oil probably interact more rapidly with microorganisms in the rumen than FA in seeds due to evidence by a more pronounced shift of the VFA pattern toward propionate for oils than for seeds (Jouany et al., 2000).

An *in vitro* study showed that fish oil decreased CH_4 production about 30-50% (Castro-Montoya et al., 2012). Fish oil FA responsible for CH_4 production are EPA and DHA, where the high EPA (181 g/kg FA) and DHA (119 g/kg FA) source of fish oil caused a stronger reduction of CH_4 than the low EPA–DHA (EPA, 54 g/kg FA; DHA, 75 g/kg FA) source (Fievez et al., 2003). Besides effects of the concentration of

EPA and DHA in fish oil on CH_4 inhibition, their lipolysis is also of importance because free fatty acids are more toxic to bacteria and more potent enteric CH_4 inhibitors than esterified FA and triacylglycerols (Hristov et al., 2009).

In term of LA sources, McGinn et al. (2004) reported that adding sunflower oil at 50 g/kg DM diet for fattening steers substantially decreased CH_4 emissions. Similarly, Pilajun et al. (2010), conducting with different coconut and sunflower oil ratios offered at a dose of 50 g/kg DM to fattening steers, observed that CH_4 concentration linearly decreased as sunflower oil proportion increased. The potential of soybean oil to decrease CH_4 production from the rumen has been also demonstrated when it was added to diets for dairy goats (Li et al., 2009) and fattening lambs (Mao et al., 2010). Blanco et al. (2012) reported *in vitro* that soapstocks of soybean and sunflower oils caused a higher decrease in gas production (7 and 6% lower than control, respectively) and in CH_4 production (22 and 25% lower than the control, respectively). The soapstocks seemed to decrease ruminal fermentability, as evidenced by the lower gas production, although VFA production was not affected by any soapstock. Methane production per mole of total VFA was linearly decreased with higher doses of soapstocks from palm, soybean, and sunflower oils, indicating that the reduction in gas and CH_4 production cannot be explained solely by a reduction in fermentation of OM, and suggesting that a specific inhibitory effect on methanogenesis may have occurred (Blanco et al., 2012). *In vitro* studies have found FA used in combination have the greatest suppression of methanogenesis due to a synergistic effect (Dohme et al., 2001; Soliva et al., 2004). Therefore, it is likely that oil supplementation would provide a more dramatic depression of CH_4 production than individual FA (Soliva et al., 2004).

Effects of oil supplement on milk production and components : The responses of milk production and components on supplementing oils are dependents on many factors, such as type of added oils, percentage of lipid in the diet as well as duration of feeding oils (Rabiee et al., 2012). The effects of oil supplementation mainly focusing on linseed oil, sunflower oil, and fish oil on milk yield, milk composition, and milk fatty acid profiles from some previous studies are presented in Tables 2.3 and 2.4.

Table 2.3 Milk yield and components in response to oil addition in dairy cows

Ref	Treatment (% EE in the diet)	Start of study (Duration)	Milk yield (kg/d)	Milk Fat (%)	Milk protein (%)
Linseed oil addition					
[1]	Calcium salts of palm oil (3.6)	38 DIM	24.8^{b}	3.49	3.92^{a}
	whole unprocessed LS (6.6)	(35 d)	32.1^{a}	3.63	3.87^{ab}
	whole unprocessed SF seed (6.7)		25.9^{b}	3.30	3.74^{bc}
	Megalac (6.2)		31.5^{a}	3.35	3.68^{c}
[2]	Control (1.84)	150 DIM	18.9	3.23^{q}	3.03^{q}
	LO 170 g/d (3.47)	(21 d)	18.5	3.44	3.19
	LO 340 g/d (5.32)		19.6	3.35	3.12
	LO 510 g/d (7.21)		19.1	3.27	3.08
[3]	Extruded SBM (5.8)	28 DIM	45.6	2.86^{a}	3.00^{b}
	Extruded LS (6.3)	(112 d)	44.5	2.65^{b}	3.04^{a}
[4]	Control (2.6)	213 DIM	23.0^{a}	4.11^{a}	3.40
	Crude LS (5.2)	(28 d)	21.5^{a}	4.54^{a}	3.46
	Extruded LS (5.7)		20.8^{ab}	3.53^{b}	3.33
	LO (8.0)		18.9^{b}	3.23^{b}	3.47

Table 2.3 Milk yield and components in response to oil addition in dairy cows (conc.)

Ref	Treatment (% EE in the diet)	Start of study (Duration)	Milk yield (kg/d)	Milk Fat (%)	Milk protein (%)
[5]	Calcium salts of FA (5.8)	24 d pre-calving	49.5[b]	3.63[a]	2.94
	Extruded LS (5.8)	(124 d)	52.9[a]	3.23[b]	2.97
[6]	Control (2.9)	65 DIM	26.1[l]	3.90	3.40[l]
	LO 2% (4.9)	(28 d)	27.3	3.70	3.30
	LO 3% (5.7)		27.4	3.90	3.30
	LO 4% (6.6)		28.4	3.70	3.20
[7]	Crushed LS (6.2)	52 DIM	33.1	4.30[a]	3.18
	Extruded whole LS (6.5)	(21 d)	31.4	4.75[a]	3.27
	Formaldehyde-treated LO (5.5)		33.7	4.67[a]	3.26
	DHA addition to LO (5.6)		29.7	3.27[b]	3.09
[8]	Control, corn silage (2.62)	117 DIM	25.3	3.38[l,q]	3.04
	Extruded LS 5% DMI (4.45)	(28 d)	25.2	3.07	2.89
	Extruded LS 10% DMI (6.16)		23.6	2.71	2.99
	Extruded LS 15% DMI (7.84)		25.1	3.12	3.06
[9]	Protected palm oil (5.0)	Calving	39.3	4.17[a]	3.22
	Extruded LS (5.1)	(42 d)	40.1	3.55[b]	3.28
Sunflower oil addition					
[10]	30 g/kg SO	mid-lactation	22.1	2.06[l]	2.98
	20 g/kg SO + 10 g/kg FO	(21 d)	21.6	2.28	2.92
	10 g/kg SO + 20 g/kg FO		20.3	2.62	2.88
	30 g/kg FO		19.8	2.64	2.96
[11]	Control (3.35)	169 DIM	27.1	4.60[a]	3.61[a]
	3.0% SO + 1.5% FO (6.30)	(28 d)	26.4	2.90[b]	3.33[b]

Table 2.3 Milk yield and components in response to oil addition in dairy cows (conc.)

Ref	Treatment (% EE in the diet)	Start of study (Duration)	Milk yield (kg/d)	Milk Fat (%)	Milk protein (%)
[12]	Confinement, corn silage-alfalfa hay	195 DIM	23.1[a]	2.51	3.34
	Grazing, alfalfa-grass pasture	(21 d)	19.6[b]	2.95	3.35
	(both groups received 640 g/d FO and SO (1 : 3 w/w)				
[13]	Control	297 DIM	27.3	3.81	3.16
	1.5% SO + 0.5% FO	(38 d)	31.3	3.50	3.08
	3.0% SO + 0.5% FO		26.6	3.63	3.17
	4.5% SO + 0.5% FO		29.5	3.39	3.06
[14]	Control, 400 g/d animal fat (8.83)	105 DIM	32.7	3.70	3.07
	300 g/d SO + 100 g/d FO (8.42)	(21 d)	33.1	3.74	2.99
[15]	Control	202 DIM	22.0	3.77	3.44
	255 g/d SO	(54 d)	23.9	3.53	3.40
	255 g/d SO + 52.5 g/d FO		22.9	3.56	3.40
	105 g/d FO		24.5	3.42	3.33
[16]	Control	145 DIM	22.2	3.75[a]	3.51
	0.5 kg/d rapeseed oil	(28 d)	21.9	3.33[b]	3.45
	0.5 kg/d SO		22.0	3.27[b]	3.45
	0.5 kg/d LO		22.2	3.59[a]	3.43
Fish oil addition					
[17]	Control (3.2)	48 DIM	31.7[l,q]	2.97[l]	3.17
	1% FO (4.5)	(35 d)	34.2	2.79	3.19
	2% FO (5.4)		32.3	2.37	3.21
	3% FO (5.8)		27.4	2.30	3.17

Table 2.3 Milk yield and components in response to oil addition in dairy cows (conc.)

Ref	Treatment (% EE in the diet)	Start of study (Duration)	Milk yield (kg/d)	Milk Fat (%)	Milk protein (%)
[18]	1% FO + 2% saturated fat (5.75)	73 DIM	35.8	3.14[a]	3.04
	1% FO + 2% OA-high SF seed (5.42)	(28 d)	36.3	2.81[b]	3.03
	1% FO + 2% SF seed (5.70)		34.9	2.66[b]	3.10
	1% FO + 2% LS (5.48)		35.0	3.08[a]	3.08
[19]	Control	90 DIM	26.4[a]	2.48[a]	3.13[a]
	160 g/d FO	(28 d)	25.9[ab]	2.99[b]	3.08[a]
	320 g/d FO		24.6[b]	2.34[c]	2.89[b]
[20]	Control	30 d pre-calving	36.5	3.56[a]	2.96
	200 g/d protected microalgae	(125 d)	35.6	3.40[a]	2.97
	200 g/d protected FO		39.1	3.00[b]	2.86

[a-c] Means within a column with different superscripts are significantly different at $P < 0.05$.

[l] Linear contrast statement was significant difference at $P < 0.05$.

[q] Quadratic contrast statement was significant difference at $P < 0.05$.

[l,q] Both linear and quadratic contrast statements were significant difference at $P < 0.05$.

Sources : [1](Petit et al., 2004), [2](Flowers et al., 2008), [3](Fuentes et al., 2008), [4](Chilliard et al., 2009), [5](Zachut et al., 2010), [6](Benchaar et al., 2012), [7](Sterk et al., 2012), [8](Ferlay et al., 2013), [9](Jahani-Moghadam et al., 2015), [10](Palmquist and Griinari, 2006), [11](Shingfield et al., 2006), [12](AbuGhazaleh et al., 2007), [13](Cruz-Hernandez et al., 2007), [14](AbuGhazaleh, 2008), [15](Murphy et al., 2008), [16](Rego et al., 2009), [17](Donovan et al., 2000), [18](AbuGhazaleh et al., 2003), [19](Rego et al., 2005), and [20](Vahmani et al., 2013).

Table 2.4 Milk fatty acid profiles in response to oil addition in dairy cows

Ref	Treatment (% EE in the diet)	Start of study (Duration)	CLA (g/100 g FA)			n-3 PUFA (g/100 g FA)		
			Total	*c*9,*t*11	*t*10,*c*12	ALA	EPA	DHA
Linseed oil addition								
[1]	Control (1.84)	150 DIM	-	1.12[l,q]	-	0.59[l,q]	0.05	0.14
	LO 170 g/d (3.47)	(21 d)	-	1.18	-	0.78	0.04	0.19
	LO 340 g/d (5.32)		-	1.39	-	1.01	0.04	0.12
	LO 510 g/d (7.21)		-	1.65	-	1.03	0.04	0.11
[2]	Extruded SBM (5.8)	28 DIM	0.70[b]	-	-	0.47[b]	-	-
	Extruded LS (6.3)	(62 d)	1.02[a]	-	-	1.11[a]	-	-
[3]	Control (2.6)	213 DIM	0.84[b]	0.77[b]	-	0.67[b]	0.05[a]	-
	Crude LS (5.2)	(28 d)	0.48[c]	0.44[b]	-	0.65[b]	0.05[a]	-
	Extruded LS (5.7)		1.33[a]	1.27[a]	-	1.20[a]	0.04[a]	-
	LO (8.0)		0.66[bc]	0.65[b]	-	0.54[b]	0.02[b]	-
[4]	Calcium salts of FA (5.8)	24 d pre-	-	-	-	0.29[b]	0.06	-
	Extruded LS (5.8)	calving	-	-	-	1.47[a]	0.10	-
		(124 d)						
[5]	Control (5.56)	153 DIM	-	0.63	0.01	0.02	0.05	0.01
	Rapeseed oil (9.80)	(23 d)	-	0.91	0.02	0.01	0.03	0.01
	Soybean oil (8.96)		-	1.07	0.02	0.02	0.04	0.01
	LO (9.38)		-	1.05	0.02	0.02	0.04	0.02
	Three oils (1 : 1 : 1) (9.39)		-	1.15	0.03	0.02	0.04	0.01
[6]	Control (2.9)	65 DIM	-	0.36[l]	0.02	0.33[l,q]	0.04[l,q]	0.02
	LO 2% (4.9)	(28 d)	-	0.67	0.02	0.79	0.06	0.01
	LO 3% (5.7)		-	0.87	0.02	0.86	0.06	0.01
	LO 4% (6.6)		-	1.22	0.02	0.86	0.05	0.01

Table 2.4 Milk fatty acid profiles in response to oil addition in dairy cows (conc.)

Ref	Treatment (% EE in the diet)	Start of study (Duration)	CLA (g/100 g FA)			n-3 PUFA (g/100 g FA)		
			Total	*c*9,*t*11	*t*10,*c*12	ALA	EPA	DHA
[7]	Crushed LS (6.2)	52 DIM	0.57[b]	0.56[b]	0.01	0.87[b]	-	-
	Extruded whole LS (6.5)	(21 d)	0.35[b]	0.35[b]	0.00	0.83[b]	-	-
	DHA added to LO (5.6)		1.45[a]	1.45[a]	0.00	0.46[b]	-	-
	Formaldehyde-treated LO (5.5)		0.45[b]	0.43[b]	0.01	3.19[a]	-	-
[8]	Control, corn silage (2.62)	117 DIM	0.67[l]	0.61[l]	0.00[l,q]	0.51[l]	0.04	-
	Extruded LS 5% DMI (4.45)	(28 d)	0.81	0.71	0.02	1.00	0.04	-
	Extruded LS 10% DMI (6.16)		0.96	0.77	0.02	1.65	0.02	-
	Extruded LS 15% DMI (7.84)		1.05	0.92	0.01	1.58	0.03	-
Sunflower oil addition								
[9]	30 g/kg SO	mid-	-	4.02[q]	0.11	0.84	0.12[l]	0.00[l]
	20 g/kg SO + 10 g/kg FO	lactation	-	6.09	0.10	0.93	0.14	0.00
	10 g/kg SO + 20 g/kg FO	(21 d)	-	5.84	0.10	0.83	0.58	0.06
	30 g/kg FO		-	3.43	0.12	0.88	0.68	0.09
[10]	Control (3.35)	169 DIM	0.50[b]	0.44[b]	0.00[b]	0.21[a]	0.03[b]	0.00[b]
	3.0% SO + 1.5% FO (6.30)	(28 d)	3.47[a]	3.04[a]	0.08[a]	0.23[b]	0.11[a]	0.07[a]
[11]	Corn silage-alfalfa hay	195 DIM	0.87[b]	0.84[b]	0.00	0.16	0.04	0.05
	Alfalfa-grass pasture (both groups received 640 g/d FO and SO (1 : 3 w/w)	(21 d)	1.61[a]	1.53[a]	0.01	0.16	0.03	0.04
[12]	Control	297 DIM	0.66[c]	-	-	0.44[a]	0.05[b]	0.03[b]
	1.5% SO + 0.5% FO	(38 d)	1.90[b]	-	-	0.41[a]	0.06[a]	0.04[a]
	3.0% SO + 0.5% FO		2.36[b]	-	-	0.36[b]	0.04[b]	0.03[b]
	4.5% SO + 0.5% FO		3.87[a]	-	-	0.31[c]	0.04[b]	0.03[b]

Table 2.4 Milk fatty acid profiles in response to oil addition in dairy cows (conc.)

Ref	Treatment (% EE in the diet)	Start of study (Duration)	CLA (g/100 g FA)			n-3 PUFA (g/100 g FA)		
			Total	*c*9,*t*11	*t*10,*c*12	ALA	EPA	DHA
[13]	400 g/d animal fat (8.83)	105 DIM	0.97[b]	0.83[b]	-	0.68	0.10	0.05[b]
	300 g/d SO + 100 g/d FO (8.42)	(21 d)	1.75[a]	1.55[a]	-	0.60	0.10	0.07[a]
[14]	Control	202 DIM	-	1.76	-	0.71	-	-
	255 g/d SO	(54 d)	-	1.87	-	0.74	-	-
	255 g/d SO + 52.5 g/d FO		-	2.36	-	0.65	-	-
	105 g/d FO		-	2.16	-	0.64	-	-
[15]	Control	145 DIM	1.35[b]	1.19[b]	0.00[a]	0.60[a]	0.07[a]	-
	0.5 kg/d rapeseed oil	(28 d)	1.36[b]	1.14[b]	0.01[a]	0.38[c]	0.05[bc]	-
	0.5 kg/d SO		1.82[a]	1.61[a]	0.00[b]	0.42[c]	0.04[b]	-
	0.5 kg/d LO		1.92[a]	1.54[a]	0.01[a]	0.53[b]	0.05[b]	-
Fish oil addition								
[16]	Control (3.2)	48 DIM	0.71[l,q]	0.60[l,q]	-	0.18	0.05[l]	0.02
	1% FO (4.5)	(35 d)	1.71	1.58	-	0.36	0.22	0.06
	2% FO (5.4)		2.53	2.23	-	0.24	0.32	0.26
	3% FO (5.8)		2.12	1.90	-	0.22	0.40	0.20
[17]	2% saturated fat (5.75)	73 DIM	-	-	-	0.75[b]	0.14[a]	0.16[a]
	2% OA-high SF seed (5.42)	(28 d)	-	-	-	0.71[b]	0.10[c]	0.13[b]
	2% SF seed (5.70)		-	-	-	0.65[c]	0.10[c]	0.13[b]
	2% LS (5.48)		-	-	-	1.19[a]	0.12[b]	0.15[a]
	(all groups received 1% FO)							
[18]	Control	90 DIM	2.25[a]	-	-	0.99	0.07[b]	0.06[b]
	160 g/d FO	(28 d)	3.23[ab]	-	-	1.06	0.18[ab]	0.17[b]
	320 g/d FO		3.64[a]	-	-	1.03	0.33[a]	0.43[a]

Table 2.4 Milk fatty acid profiles in response to oil addition in dairy cows (conc.)

Ref	Treatment (% EE in the diet)	Start of study (Duration)	CLA (g/100 g FA)			n-3 PUFA (g/100 g FA)		
			Total	*c*9,*t*11	*t*10,*c*12	ALA	EPA	DHA
[19]	Control	30 d pre-	0.92[b]	0.62	0.01	0.49	0.06[b]	0.02[c]
	200 g/d protected microalgae	calving	1.12[a]	0.74	0.01	0.51	0.14[a]	0.14[b]
	200 g/d protected FO	(125 d)	1.24[a]	0.87	0.01	0.51	0.07[b]	0.20[a]

[a-c] Means within a column with different superscripts are significantly different at $P < 0.05$.

[l] Linear contrast statement was significant difference at $P < 0.05$.

[l,q] Both linear and quadratic contrast statements were significant difference at $P < 0.05$.

Sources : [1](Flowers et al., 2008), [2](Fuentes et al., 2008), [3](Chilliard et al., 2009), [4](Zachut et al., 2010), [5](Jacobs et al., 2011), [6](Benchaar et al., 2012), [7](Sterk et al., 2012), [8](Ferlay et al., 2013), [9](Palmquist and Griinari, 2006), [10](Shingfield et al., 2006), [11](AbuGhazaleh et al., 2007), [12](Cruz-Hernandez et al., 2007), [13](AbuGhazaleh, 2008), [14](Murphy et al., 2008), [15](Rego et al., 2009), [16](Donovan et al., 2000), [17](AbuGhazaleh et al., 2003), [18](Rego et al., 2005), and [19](Vahmani et al., 2013).

2.2 Rumen undegradable protein

2.2.1 Metabolism of protein in the rumen

Dietary protein is divided into rumen degradable protein (RDP) and rumen RUP with RDP comprises of true protein and non-protein N (Bach et al., 2005). True protein is degraded to peptides and AA and eventually deaminated into ammonia N or incorporated into microbial protein. Non-protein N is composed of N present in RNA, DNA, AA, ammonia, and small peptides. The N sources from AA, peptides, and ammonia are used for microbial growth. Rumen output consists of ammonia N, undegraded protein, and microbial protein (Bach et al., 2005). Protein degradation in

the rumen includes attachment of bacteria to feed particles, followed by activity of cell-bound microbial proteases (Brock et al., 1982) (Figure 2.3).

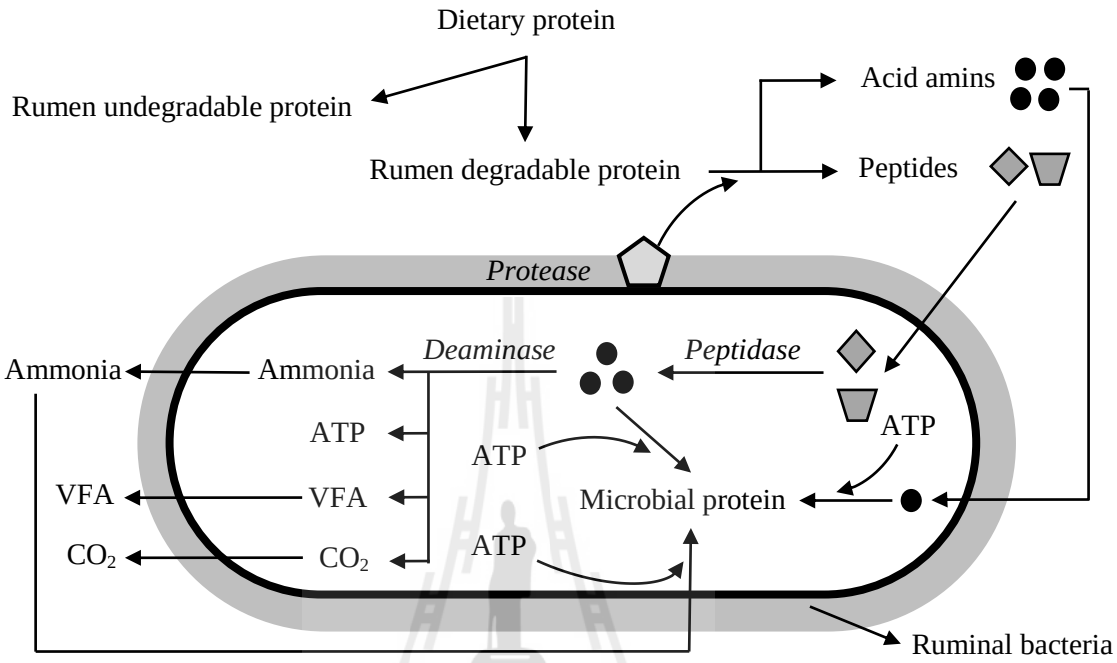

Figure 2.3 Protein degradation and fate of end products in the rumen. Adapted from Bach et al. (2005).

Approximately 70-80% of ruminal microorganisms attach to undigested feed particles in the rumen (Craig et al., 1987), and 30 to 50% of those have proteolytic activity (Prins et al., 1983). Protein is converted to peptides and AA by cell-bound microbial proteases (Brock et al., 1982). The rate and extent at which protein degradation occurs will depend on proteolytic activity of the ruminal microflora and the type of protein (Bach et al., 2005). Peptides and AA resulting from the extracellular rumen proteolytic activity are transported inside microbial cells. Peptides can be further degraded by peptidases into AA, and the latter can be incorporated into microbial protein or further deaminated to VFA, CO_2, and ammonia (Tamminga,

1979). The fate of absorbed peptides and free AA inside of microbial cells will depend on the availability of energy. If energy is available, AA will be transaminated or used directly for microbial protein synthesis. However, if energy is limiting, AA will be deaminated, and their carbon skeleton will be fermented into VFA (Figure 2.3).

Broderick et al. (1991) demonstrated that rapidly degraded proteins may result in the accumulation of peptides and AA within the first 2 h after feeding, suggesting that rates of peptidolysis and deamination play an important role in the control of protein degradation. Recently, Cardozo et al. (2004) found that the concentration of peptides, AA, and ammonia were within the same range for up to 8 h after feeding. *In vivo*, protozoa keep a major role in protein degradation. The most important aspect of protozoa is their ability to engulf large molecules, protein, carbohydrate, or even ruminal bacteria (Van Soest, 1994). In addition, protozoa play a role in regulating bacterial N turnover in the rumen, and they supply soluble protein to sustain microbial growth. Because protozoa are not able to use ammonia N (Onodera et al., 1977), a fraction of previously engulfed insoluble protein is later returned to the rumen fluid in the form of soluble protein (Dijkstra, 1994). This is one of the main reasons why defaunation decreases ammonia N concentration in the rumen (Eugène et al., 2004).

The most important factors affecting microbial protein degradation include the type of protein, interactions with other nutrients (mainly CHO within the same feedstuff and within the rumen contents), and the predominant microbial population (dependent on the type of ration, ruminal passage rate, and ruminal pH) (Bach et al., 2005). Solubility of proteins is a key factor determining their susceptibility to microbial proteases and, thus, their degradability. The structure of the protein is also important. In high growing and high yielding animals microbial supply is limited,

then the demand of AA at the tissue level, so to support the demand, it is necessary to provide proteins in the form of RUP or rumen protected proteins by modification of protein structure.

2.2.2 Sources of rumen undegradable protein

High RDP sources are ingested by ruminants, resulting in large amount of ammonia production. This ammonia is wasted by urea through urine excretion even the ruminant can particularly convert the ammonia into liver urea. For this reason, the RUP technique can be used to enhance the efficiency of protein utilization from the high RDP sources, so that AA from these protein feed sources are directly digested and absorbed in the small intestine for protein synthesis in the tissue as well as gluconeogenesis pathway in the liver (Walli, 2005).

The RUP sources are found in a few naturally feeds such as coconut meal, cotton seed meal, fish meal, maize gain, and maize gluten meal. Medium RDP is often detected in deoiled rice bran, linseed meal, soybean meal, and *Leucocaenea* leaf meal, high RDP is occurred in the mustard meal and groundnut meal (Shelke et al., 2011; Walli, 2005). Negi et al. (1989) reported that 50-70% of total nitrogen (N) in tree forages may be presented as protected protein. However, these feed sources contain high condensed tannins that bind the protein compound, leading to reduce efficiency of protein utilization. Therefore, tree forages can be used as RUP sources when only detoxification of tannins by some biological, biotechnological, or chemical methods. Meanwhile, the high or medium degradable proteins as above concerning need protection against attack of proteolytic enzymes in the rumen to improve efficiency of their protein, and RUP sources do not need any protection (Shelke et al., 2012b).

2.2.3 Methods of rumen undegradable protein

There are several methods which help to increase the RUP in the animal feeds and by-products. Most of researches were carried out by heat treatment of high rumen degradable meals. The limitation with heat treatment is that it may increase the feed cost, and protein can become over-protected (Sengar and Mudgal, 1982). Walli (2005) found that heat treatment at 150°C for 2 h was the optimal temperature time combination for SBM. The heat treatment (roasting) at 130°C for 20-25 min could help to protect soybean protein from ruminal degradation (Sirohi et al., 2011). Another method was also used to improve RUP of feedstuffs was formaldehyde treatment. This method has proved to be an efficient and cheaper approach for protecting the high RDP sources (Chaturvedi and Walli, 2001). The effects of feedstuffs treated with formaldehyde on the productive performance of dairy animals were also confirmed in the some previous studies (Sahoo and Walli, 2007; Shelke et al., 2011; Shelke et al., 2012a). However, the use of formaldehyde as chemical treatment to protect feed protein can cause a hazard to animal health.

2.2.4 Effect of RUP on animal performance

The feeding of RUP in low milk yielders is essential due to the more energy supply to these animals, because AA supplied from RUP are synthesized to glucose in the liver. Thus, feeding the RUP will increase not only the efficient of protein but also energy utilization in the ruminants. The previous studies showed that feeding of cotton seed meal and maize gluten-meal in the diets of lactating ruminants increased milk yield (Chaturvedi and Walli, 2001; Walli, 2005). Sahoo and Walli (2007) found that feeding mustard cake treated by formaldehyde to lactating goats increased the milk production to 1.44 kg/day as compared with 1.31 kg/day in the control group.

Garg et al. (2003) reported that milk yield and milk fat were enhanced when lactating buffaloes fed diet supplemented with sunflower seed meal treated formaldehyde (optimal bypass with 75% RUP) as compared to formaldehyde-untreated sunflower seed meal (30% RUP). Feeding the low-degradable protein diet including only corn gluten meal in the diet of early-lactation goats for a 135-day period significantly increased milk fat, protein, and casein (3.65, 3.87, and 2.82%, respectively) compared to the animals fed the high-degradable protein diet containing SBM, sunflower meal, and urea 3.28, 3.36, and 2.55%, respectively) (Laudadio and Tufarelli, 2010). Shelke et al. (2011) concluded that lactating buffaloes supplemented with a combination of formaldehyde treated cakes and protected fat at 2.5% DMI from 60 days pre-partum to 90 days postpartum significantly increased milk yield and FCM yield by 2.01 and 3.50 kg/day, respectively, as compared to the animals fed untreated cakes. Sirohi et al. (2011) reported that crossbred dairy cows fed diet supplemented with 1 kg/day roasted soybean for a 90-day period significantly increased milk and FCM yields (10.4 and 11.1 kg/day, respectively) as compared to the control group fed raw soybean (9.60 and 10.2 kg/day, respectively).

2.3 References

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CHAPTER III

MILK RESPONSES AND FATTY ACID PROFILES IN DAIRY COWS FED OIL MIXTURES RICH IN POLYUNSATURATED FATTY ACIDS

3.1 Abstract

To evaluate the effects of supplementing linseed oil (LO) or/and sunflower oil (SO) mixed with fish oil (FO) on milk yield, milk composition, and FA profiles of dairy cows fed a high-concentrate diet, 24 crossbred primiparous Holstein Friesian lactating dairy cows in early lactation averaging 26.67 ± 9.20 days in milk, 12.25 ± 0.57 kg of milk, and 347.21 ± 30.80 kg body weight, were assigned to a completely randomized design experiment. All cows were fed a high-concentrate diet and 0.38 kg DM molasses per day. The dietary treatments composed : 1) basal diet without oil supplement (Control), 2) 3% linseed and fish oils (1 : 1, w/w, LOFO), 3) 3% sunflower and fish oils (1 : 1, w/w, SOFO), and 4) 3% mixture (1 : 1 : 1, w/w) of linseed, sunflower, and fish oils (MIXO). The animals fed SOFO had a 13.12% decrease ($P < 0.05$) in total DMI compared with the control diet. No significant change was detected for milk yield; however, the animals fed diet supplemented with SOFO depressed ($P < 0.05$) milk fat yield (35.42%) and milk fat concentration (27.20%) compared to those fed the control diet. Milk *cis*-9,*trans*-11 CLA proportion increased ($P < 0.01$) by 198.11% in the LOFO group, whereas these values were 39.62 and 77.36% in the

SOFO and MIXO, respectively, relative to the control group. Milk C18:3n-3 proportion was enhanced ($P < 0.001$) by 227.27% by supplementing LOFO relative to the control group. The proportion of milk DHA was increased ($P < 0.001$) in the cows fed LOFO (0.38%) and MIXO (0.23%) compared to the control group (0.01%). Dietary inclusion of LOFO mainly increased milk *cis*-9,*trans*-11 CLA and *n*-3 PUFA, whereas feeding MIXO improved preformed FA and UFA. The lowest n-6/n-3 ratio was found in the LOFO while the decreased AI and TI seemed to be more extent in the MIXO. Therefore, to maximize milk *cis*-9,*trans*-11 CLA and n-3 PUFA and to minimize milk n-6/n-3 ratio, AI, and TI, an ideal diet would look to be either LOFO or MIXO. Nevertheless, feeding MIXO to dairy cows to enhance milk beneficial UFA seems to be greater economic rather than feed them with LOFO.

3.2 Introduction

The inclusion of saturated fats in human diets may bring the risk of cardiovascular diseases (Joyce et al., 2009). However, for milk processors, milk with high SFA may improve the keeping quality but may be detrimental to human health. The negative effects of SFA seem to outweigh their positive functions in milk, so more research is needed to reduce the amount of SFA in milk (Nantapo et al., 2014). It has been showed that consumption of dietary n-3 FA, mainly ALA, EPA, and DHA is useful for human health, especially cardio- and cerebro-vascular diseases (Molendi-Coste et al., 2011; Siegel and Ermilov, 2012; Weintraub, 2013), and CLA from ruminant fat has been reported to exert anti-carcinogenic benefits in various experimental animal models and human cancer cells (Gebauer et al., 2011; Grądzka et al., 2013). Dairy cows' diets supplemented with LO rich in ALA has been shown to

increase milk PUFA, especially ALA and *cis*-9,*trans*-11 CLA proportions (Hurtaud et al., 2013; Mach et al., 2013). AbuGhazaleh (2008) reported that SO and FO inclusion in dairy cattle diet led to increase milk *cis*-9,*trans*-11 CLA and VA proportions.

Nevertheless, ruminal BH, which transforms UFA into SFA, is a major concern due to SFA is detrimental for human health. The BH process has long been known to occur in the rumen as the result of microbial metabolic activity (Lee and Jenkins, 2011; Lock and Bauman, 2007). Thus, if ruminal BH of UFA to further form stearic acid is controlled to become an incomplete process, it may be possible to improve the healthiness of ruminant meats and milk by increasing their UFA composition in general (Shelke and Thakur, 2011) and CLA (Sultana et al., 2008) and the n-3 PUFA in particular (Côrtes et al., 2010). The ALA inclusion decreased the BH of both EPA and DHA (Shingfield et al., 2011) and increased *trans*-11 C18:1, precursor of *cis*-9,*trans*-11 CLA in tissues, accumulation *in vitro* study (Boeckaert et al., 2007; Chow et al., 2004; Wasowska et al., 2006) while inclusion of FO containing EPA and DHA has been shown to inhibit the complete BH of C18 UFA, resulting in an increase of *trans* C18:1 available for incorporation in tissue lipids and milk fat triacylglycerides (Lee et al., 2008; Shingfield et al., 2010; Shingfield et al., 2003). Consequently, the EPA and DHA supplement mixed with LA (precursor of CLA isomers) and ALA sources may improve EPA, DHA, CLA isomers, and ALA in animals' products.

Hence, feeding FO in combination with LO or/and SO to dairy cattle diet may increase milk beneficial FA as concerned above. However, inclusion of oil high in PUFA in the ruminant diets could induce adverse effects on feed intake and nutrient digestibility (Bernard et al., 2009; Bhatt et al., 2011), resulting in decrease milk yield and milk fat yield (Hutchinson et al., 2012; Lunsin et al., 2012). For this reason,

feeding proper oil mixture rich in PUFA in dairy cows should be studied so that it enhances milk beneficial PUFA for human health without affecting animal performance. The objectives of this study were to measure the effects of feeding different oil mixtures rich in PUFA on milk yield, milk composition, and FA profiles of dairy cows fed a high-concentrate diet. The hypothesis of this experiment was that dairy cows' diet supplemented with oil compounds containing high n-3 PUFA and CLA precursors could improve n-3 PUFA and CLA isomers in cows' milk.

3.3 Materials and methods

3.3.1 Animals, experimental design, and diets

All experimental procedures were conducted following the Ethical Principles and Guidelines for the Use of Animals issued by National Research Council of Thailand. Twenty four crossbred primiparous Holstein Friesian lactating dairy cows in early lactation averaging 26.67 ± 9.20 days in milk, 12.25 ± 0.57 kg of milk, and 347.21 ± 30.80 kg body weight, housed in individual tie stalls and offered daily rations as equal meals at 06:00 and 17:00 h. The animals had free access to water and mineral block, and they had enough space to walk. The animals were assigned to a completely randomized design with six replicates per each treatment. The experiment lasted for 6 weeks consisting a former 2-week for adjustment, followed by a latter 4-week for sample collection. The cows were fed a high-concentrate basal diet (R : C 40 : 60) and 0.38 kg DM molasses per day. The dietary treatments composed : 1) basal diet without oil supplement (Control), 2) 3% linseed and fish oils (1 : 1, w/w, LOFO), 3) 3% sunflower and fish oils (1 : 1, w/w, SOFO), and 4) 3% mixture (1 : 1 : 1, w/w) of linseed, sunflower, and fish oils (MIXO). Three oil compounds were daily blended

as above ratios and then mixed with a 21% CP concentrate before feeding. The concentrate was formulated to meet the nutrient requirements of dairy cows (NRC, 2001). Corn silage was offered *ad libitum* as a main roughage source. Molasses was added as a top-dressing on corn silage.

3.3.2 Sampling, measurements, and chemical analysis

Feeds offered and the residuals were recorded daily during the collection period, and feed samples were collected for two consecutive days weekly to calculate daily feed intake. Feed samples were taken and dried at 60°C for 48 h. At the end of the experimental period, feed and oil samples were pooled and representative samples were taken for further chemical analysis. Samples were ground through a 1-mm screen and subjected to proximate analysis. Crude protein (CP) was determined by Kjeldahl method, procedure 928.08 of AOAC (1998). Ether extract was determined using petroleum ether in a Soxtec System, procedure 948.15 of AOAC (1998). 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. The NDF analysis used sodium sulfite in the neutral detergent solution. Both NDF and ADF are expressed inclusive of residual ash. The net energy for lactation (NE_L; Mcal/kg DM) of feeds and oils was calculated according to the equations of NRC (2001). All chemical components were expressed on dry matter (DM) basis. The animals were weighed at the start and end of the experiment.

The dairy cows were milked daily at 5:00 and 16:00 h, and milk yields were recorded at each milking. Milk from both morning and afternoon milking were sampled weekly in 2 consecutive milking days. The morning milk samples were pooled to one composite sample; afternoon samples were also pooled. The composite

milk samples of both milking times were analyzed for milk composition mainly including fat, protein, casein, lactose, solid not fat, total solid, urea, free fatty acid, citric acid, and acidity using MilkoScan™ FT2 infrared automatic analyser (Foss, Hillerød, Denmark).

To measure milk FA composition, milk samples were taken from individual cow on two consecutive days (d 41 and 42) of the experiment and stored at – 20°C until further analysis. Lipid in milk samples was extracted in a mixture of dichloromethane and methanol (2 : 1, v/v) following a method of Romeu-Nadal et al. (2004) with small adjustments, whereas lipid in feed samples was extracted in a mixture of chloroform and methanol (2 : 1, v/v) according to a modified method described by Folch et al. (1957). After lipid extraction, 10 ml of the extracts were transferred to new culture tubes fitted with a teflon-lined screw cap, evaporated to exact dryness under a N_2 stream, and then methylated. Approximately 30 mg of the extracted lipid were added with 1 ml of internal standard (2 mg C17:0/ml hexane). The samples were mixed for 30 sec by a vortexer. Then, 1.5 ml of 0.5 M NaOH in methanol was added, and the tubes were heated in a water bath at 90°C for 30 min. Two ml of 14% BF_3 in methanol were added, and the mixture was left at room temperature (25°C) for 30 min to prevent intra-isomerization of CLA isomers (Werner et al., 1992). Ten ml of deionized water were added. The top-layer solution was transferred to a 40 ml centrifuged tube, and 5 ml of hexane were added for extraction of FA methyl esters. The mixture was centrifuged at 2,000 g, at 10°C, for 20 min, and the hexane layer was then dried over Na_2SO_4. Finally, 1 ml of sample at top-layer was transferred into vial for analyzing FA by gas chromatography (Hewlett-Packard 7890A series, Agilent Technology, Palo Alto, CA, USA) equipped with a

100 m × 0.25 mm × 0.2 μm film fused silica capillary column (SP1233, Supelco Inc, Bellefonte, PA, USA) and a flame ionization detector. 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 for 27 min, then increased at 4°C/min to 215°C and held for 17 min, then increased at 4°C/min to 240°C and held for 10 min. Fatty acids were identified by comparison of retention times with external FAME standards (Food Industry 37 FAME mix, 35077 Restek Co., Bellefonte, PA, USA). The CLA mixture (Sigma–Aldrich, Louis, MO, USA) contained *cis*-9,*trans*-11 CLA, *trans*-10,*cis*-12 CLA, *cis*-9,*cis*-11 CLA, and *trans*-9,*trans*-11 CLA.

3.3.3 Calculations

Dietary FA, FA intake and milk FA yield from different experimental diets were calculated following formula described by Stergiadis et al. (2014) :

Dietary FA = % DM of feed × [feed lipid content (g/100 g DM)/100] × [% of FA (g/100 g total FA in feed)/100] / dietary lipid content.

FA intake = feed intake (g DM) × [feed lipid content (g/100 g DM)/100] × [% of FA (g/100 g total FA in feed)/100].

Milk FA yield (g) = milk yield (g) × [milk fat content (g/100 g milk)/100)] × [FA (g/100 g total FA) × 0.933)/100)], where 0.933 was correction factor representing % of FA in total milk fat (Glasser et al., 2007).

Atherogenecity index (AI) and thrombogenicity index (TI) were calculated using equations proposed by Ulbricht and Southgate (1991) :

AI = [C12:0 + 4 (C14:0) + C16:0] / [MUFA + PUFA]

TI = [C14:0 + C16:0 + C18:0] / [(MUFA + n-6 PUFA)/2 + 3 (n-3 PUFA) + (n-3 PUFA/n-6 PUFA)]

The apparent transfer of FA = 100 × (FA yield in milk, g) / (FA intake, g).

3.3.4 Statistical analysis

Data on feed intake, milk yield, and milk composition were analyzed according to a completely randomized design with the repeated measures (weeks) using PROC MIXED procedure of SAS (2002) with the statistical model $Y_{ijk} = \mu + T_i + W_j + (T \times W)_{ij} + \varepsilon_{ijk}$, where Y_{ijk} = the dependent variable, μ = the overall mean, T_i = the effect of treatment, W_j = the effect of week, $(T \times W)_{ij}$ = the effect of interaction between treatment and week, and ε_{ijk} = the random residual error. The treatment, week, and interaction between treatment and week were considered as fixed effects, whereas cow within treatment was included as a random effect.

Data on live weight and milk FA were analyzed by ANOVA procedure of SAS (2002) for a completely randomized design with the following statistical model $Y_{ij} = \mu + T_i + \varepsilon_{ij}$, where Y_{ij} = the dependent variable, μ = the overall mean, T_i = the treatment effect, and ε_{ij} = the random residual error. Overall differences between treatment means were considered to be significant as $P < 0.05$. Significant differences among treatment means were assessed by Tukey's multiple comparison tests after a significant F-test. Data are expressed as mean ± SEM, which represents the pooled SEM for the model.

3.3.5 Site and period of the study

The experiment was conducted at Dairy cattle farm and The Center of Scientific and Technological Equipment of Suranaree University of Technology from 22 August 2013 to 06 October 2013. The daily temperature and relative humidity at the farm during the study were 27.85 ± 1.72 and 82.69 ± 5.81, respectively (n = 42).

3.4 Results

3.4.1 Chemical compositions of experimental feeds and diets

The dietary ingredients and chemical compositions of the individual feeds and experimental diets used in the current study are presented in Tables 3.1 and 3.2. Concentrate was used as the main source of protein (21.13%), whereas corn silage was fed as the major source of fiber due to its high NDF content (65.47%). Linseed, sunflower, and fish oils were selected as sources of supplemented oil in the diets. Table 3.1 shows that LO was particularly great in ALA (55.82%) while SO was rich in LA (59.10%), and only FO was greater source of EPA and DHA (8.12 and 36.30%, respectively). Therefore, LOFO mixture led to increase three main n-3 FA, such as ALA, EPA, and DHA contents in the diet; SOFO induced to improve LA, EPA, and DHA; and MIXO resulted in a perfectly potential compound rich in n-3 PUFA (ALA, EPA, and DHA) and LA, which is precursor of *cis*-9,*trans*-11 CLA (Table 3.2).

Table 3.1 Chemical and major fatty acid compositions of individual feeds

Item	Experimental feed					
	Concentrate[1]	**Corn silage**	**LO**	**SO**	**FO**	**Molasses**
Chemical composition (% of DM unless otherwise noted)						
DM (%)	91.18	23.45	-	-	-	75.63
OM	90.84	90.97	-	-	-	88.44
CP	21.13	9.75	-	-	-	1.23
EE	3.79	1.84	100	100	100	-
Ash	9.16	9.03	-	-	-	11.56
NFC[2]	24.54	13.91	-	-	-	-
NDF	41.38	65.47	-	-	-	-
ADF	28.43	41.69	-	-	-	-

Table 3.1 Chemical and major fatty acid compositions of individual feeds (conc.)

Item	Experimental feed					
	Concentrate[1]	**Corn silage**	**LO**	**SO**	**FO**	**Molasses**
Lignin (sa)	3.60	4.40	-	-	-	-
NE_L (Mcal/kg)[3]	1.79	1.35	4.39	4.39	4.39	1.88
FA composition (g/100 g FA)[4]						
C12:0	20.16	2.98	0.01	0.03	0.07	-
C14:0	6.62	4.78	0.07	0.11	3.80	-
C16:0	14.38	33.04	5.56	6.52	22.84	-
C18:0	2.92	17.38	3.20	3.38	6.21	-
c-9 C18:1	26.56	12.46	17.92	27.19	12.51	-
c-9,*c*-12 C18:2	23.66	20.15	16.40	59.10	1.67	-
C18:3n-3	2.03	0.00	55.82	1.60	0.10	-
C20:5n-3	0.00	0.00	0.00	0.00	8.12	-
C22:6n-3	0.00	0.00	0.00	0.00	36.30	-
SFA	47.51	67.38	9.06	10.28	37.52	-
UFA	52.49	32.62	90.94	89.72	62.48	-
MUFA	26.80	12.47	18.05	27.30	13.66	-
PUFA	25.69	20.15	72.89	62.42	48.82	-
n-3	2.03	0.00	56.09	1.80	44.69	-
n-6	23.66	20.15	16.80	60.62	4.13	-

[1] Contained (as DM basis) : 32% cassava distillers dried meal, 20% soybean meal, 17.5% corn distillers dried grains with solubles, 10% rice bran, 10% wheat bran, 8% molasses, and 2.5% mineral and vitamin mix. Mineral and vitamin mix : 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] Calculated as 100 − (CP + NDF + EE + ash).

[3] Calculated using published formulas of NRC (2001).

[4] Zero values indicate proportions of FA in feed ingredients were <0.01% total FA or undetectable.

Table 3.2 Chemical and major fatty acid composition of dietary treatments

Item	Experimental treatment[1]			
	Control	**LOFO**	**SOFO**	**MIXO**
Ingredient composition (% DM)				
Concentrate	55.07	54.00	53.92	54.25
Corn silage	41.51	39.30	39.17	39.08
Linseed oil	-	1.47	-	0.98
Sunflower oil	-	-	1.48	0.98
Fish oil	-	1.47	1.48	0.98
Molasses	3.42	3.76	3.94	3.72
Chemical composition (% of DM unless otherwise noted)				
DM (%)	62.53	61.30	61.33	61.44
OM	90.81	88.13	88.10	88.12
CP	15.73	15.29	15.26	15.32
EE	2.85	5.71	5.73	5.72
Ash	9.19	8.93	8.93	8.93
NFC	19.29	18.72	18.68	18.75
NDF	49.96	48.08	47.96	48.03
ADF	32.96	31.74	31.66	31.72
Lignin (sa)	3.81	3.67	3.66	3.67
NE_L (Mcal/kg)	1.61	1.70	1.70	1.70
Fatty acid composition (g/100 g FA)[2]				
C12:0	15.56	7.62	7.59	7.63
C14:0	6.13	3.97	3.97	3.66

Table 3.2 Chemical and major fatty acid composition of dietary treatments (conc.)

Item	Experimental treatment[1]			
	Control	**LOFO**	**SOFO**	**MIXO**
C16:0	19.38	16.65	16.88	15.31
C18:0	6.79	5.67	5.71	5.43
c-9 C18:1	22.78	18.93	21.31	21.00
c-9,*c*-12 C18:2	22.72	15.69	26.70	24.28
C18:3n-3	1.49	15.12	1.16	10.61
C20:5n-3	0.00	2.09	2.10	1.49
C22:6n-3	0.00	9.35	9.40	6.23
SFA	52.83	37.56	37.78	35.29
UFA	47.17	62.44	62.22	64.71
MUFA	22.96	19.35	21.73	21.33
PUFA	24.21	43.09	40.49	43.38
n-3	1.49	26.67	12.76	18.35
n-6	22.72	16.42	27.73	25.03

[1] Control : basal diet without oil supplement; LOFO : 3% linseed and fish oils at 1 : 1; SOFO : 3% sunflower and fish oils at 1 : 1; MIXO : 3% mixture of linseed, sunflower, and fish oils at 1 : 1 : 1.

[2] Zero values indicate proportions of FA in feed ingredients were <0.01% total FA or undetectable.

3.4.2 Intakes of main components and major fatty acids

Supplementation of SOFO did result in a 13.12% decrease ($P < 0.05$) of total DMI compared with the control diet (Table 3.3). As the result of DMI reduction, crude protein intake (CPI) was decreased ($P < 0.001$) by supplementing oil mixtures,

the lowest value was in the animals fed the SOFO diet (1.48 kg/d) versus the greatest one in those fed the control diet (1.76 kg/d). The SOFO-supplemented group led to increase ($P < 0.001$) intake of *cis*-9,*cis*-12 C18:2 while intake of C18:3n-3 was remarkably improved ($P < 0.001$) in the animals fed LOFO compared to the control group. Oil inclusion in the basal diet led to increase ($P < 0.001$) intake of total FA as compared with the control diet.

Table 3.3 Intakes of main components and major fatty acids

Item	**Treatment**[1]				**SEM**	***P*-value**
	Control	**LOFO**	**SOFO**	**MIXO**		
Live weight (LW, kg)	350.67	337.17	338.25	341.25	34.64	0.903
Intake of main components						
DM (kg/d)						
Total	11.05[a]	10.06[ab]	9.60[b]	10.15[ab]	1.10	0.019
Concentrate	6.09[a]	5.43[b]	5.18[b]	5.51[b]	0.38	0.001
Corn silage	4.59	3.96	3.76	3.97	1.00	0.106
Molasses	0.38	0.38	0.38	0.38	-	-
Added oil	0.00[b]	0.30[a]	0.28[a]	0.30[a]	0.02	<0.001
Added oil/DMI (%)	0.00[b]	2.95[a]	3.00[a]	2.97[a]	0.27	<0.001
C^2 (%)	57.74	58.12	58.59	58.63	6.20	0.969
R^2 (%)	42.26	41.88	41.41	41.37	6.20	0.969
DMI/LW (g/kg)	31.84	30.09	28.79	29.87	4.50	0.581
CP (kg/d)	1.76[a]	1.56[b]	1.48[b]	1.57[b]	0.13	<0.001
NE_L (Mcal/d)	17.80	17.08	16.30	17.24	1.84	0.234

Table 3.3 Intakes of main components and major fatty acids (conc.)

Item	Treatment[1]				SEM	*P*-value
	Control	LOFO	SOFO	MIXO		
Intake of FA (g/d)						
C12:0	49.03^{a}	43.81^{b}	41.75^{b}	44.38^{b}	3.00	<0.001
C14:0	19.31^{b}	22.84^{a}	21.86^{a}	21.28^{a}	1.52	<0.001
C16:0	61.07^{b}	95.66^{a}	92.89^{a}	89.00^{a}	7.78	<0.001
C18:0	21.41^{b}	32.58^{a}	31.41^{a}	31.55^{a}	3.56	<0.001
c-9 C18:1	71.79^{c}	108.77^{b}	117.27^{ab}	122.05^{a}	7.44	<0.001
c-9,*c*-12 C18:2	71.59^{c}	90.12^{b}	146.92^{a}	141.13^{a}	8.69	<0.001
C18:3n-3	4.68^{c}	86.87^{a}	6.40^{c}	61.64^{b}	4.50	<0.001
C20:5n-3	0.00^{c}	12.01^{a}	11.57^{a}	8.10^{b}	0.68	<0.001
C22:6n-3	0.00^{c}	53.68^{a}	51.71^{a}	36.28^{b}	3.05	<0.001
SFA	166.49^{b}	215.77^{a}	207.90^{a}	205.14^{a}	16.70	<0.001
UFA	148.63^{c}	358.72^{ab}	342.34^{b}	376.06^{a}	23.39	<0.001
MUFA	72.36^{c}	111.17^{b}	119.54^{ab}	123.95^{a}	7.56	<0.001
PUFA	76.28^{c}	247.55^{a}	222.79^{b}	252.11^{a}	16.03	<0.001
n-3	4.68^{d}	153.21^{a}	70.21^{c}	106.61^{b}	8.10	<0.001
n-6	71.59^{c}	94.35^{b}	152.59^{a}	145.50^{a}	8.95	<0.001
Total FA	315.12^{b}	574.49^{a}	550.23^{a}	581.20^{a}	38.33	<0.001

[1] Control : basal diet without oil supplement; LOFO : 3% linseed and fish oils at 1 : 1; SOFO : 3% sunflower and fish oils at 1 : 1; MIXO : 3% mixture of linseed, sunflower, and fish oils at 1 : 1 : 1.

[2] C, concentrate and R, roughage were calculated as percent to total concentrate and corn silage intakes.

[a-d] Means within a row with different superscripts are significantly different at $P < 0.05$ ($n = 6$).

3.4.3 Milk yield and composition

In contrast to DM and CP intakes, no significant change ($P > 0.05$) was detected for milk yield (Table 3.4). However, the animals fed diet supplemented with SOFO induced to depress ($P < 0.05$) milk fat yield (35.42%) and milk fat concentration (27.20%) compared to those fed the control diet. Further analysis across week of experiment showed that added oils had effect on milk fat percentage after 2-week feeding. The lowest milk fat concentration was observed in the SOFO group at 4-week supplementation ($P < 0.05$, Figure 3.1). While milk contents of protein, casein, lactose, and solid not fat remained unchanged ($P > 0.05$) among the treatments reduced milk fat content reflected to decrease ($P < 0.05$) milk total solid content in the animals supplemented with SOFO, compared to the control group. Similar trend to MFD, milk total FA was decreased ($P < 0.05$) in the SOFO group (2.62%) compared to 3.60% in the control group. The supplementing of oils in the cattle diets had no effect on milk urea concentration; however, added oils increased ($P < 0.05$) milk citric acid concentration, accompanied by decreasing ($P < 0.05$) milk acidity in the SOFO group related to the control group. Feed efficiency was not affected ($P > 0.05$) by feeding oil mixtures in the current study.

Table 3.4 Milk yield and composition

Item	Treatment[1]				SEM	*P*-value
	Control	LOFO	SOFO	MIXO		
Yield						
Milk (kg/d)	12.41	11.29	11.02	12.02	1.21	0.128
3.5% FCM[2] (kg/d)	13.14[a]	11.26[ab]	9.77[b]	11.30[ab]	2.27	0.015
ECM[3] (Mcal/d)	12.89[a]	11.11[ab]	9.92[b]	11.29[ab]	1.90	0.014

Table 3.4 Milk yield and composition (conc.)

Item	Treatment[1]				SEM	*P*-value
	Control	LOFO	SOFO	MIXO		
Fat (kg/d)	0.48[a]	0.39[ab]	0.31[b]	0.38[ab]	0.13	0.014
Protein (kg/d)	0.35[a]	0.31[ab]	0.31[b]	0.33[ab]	0.03	0.034
Casein (kg/d)	0.26[a]	0.22[b]	0.23[b]	0.24[ab]	0.03	0.015
Lactose (kg/d)	0.53	0.49	0.48	0.51	0.06	0.339
Solid-not-fat (kg/d)	1.00	0.88	0.90	0.99	0.11	0.017
Total solid (kg/d)	1.48[a]	1.27[b]	1.21[b]	1.36[ab]	0.16	0.006
Composition						
Fat (%)	3.86[a]	3.52[ab]	2.81[b]	3.12[ab]	1.03	0.039
Protein (%)	2.82	2.73	2.79	2.75	0.14	0.447
Casein (%)	2.12	1.96	2.07	1.99	0.26	0.390
Lactose (%)	4.29	4.31	4.33	4.24	0.21	0.718
Solid-not-fat (%)	8.09	7.82	8.17	8.21	0.60	0.178
Total solid (%)	11.95[a]	11.34[ab]	10.98[b]	11.33[ab]	0.99	0.035
Urea N (mg/dl)	18.56	16.41	15.98	16.29	3.39	0.337
FFA (mekv/l)	0.71	0.78	0.75	0.86	0.39	0.781
Citric acid (%)	0.17[b]	0.19[ab]	0.21[ab]	0.22[a]	0.04	0.037
Acidity (^{o}TH)	17.79[a]	15.67[ab]	14.91[b]	14.97[ab]	2.46	0.035
Fatty acid (%)	3.60[a]	3.28[ab]	2.62[b]	2.91[ab]	0.57	0.039
Feed efficiency						
Milk/DMI	1.14	1.13	1.16	1.20	0.17	0.814
3.5% FCM/DMI	1.21	1.23	1.03	1.12	0.24	0.368
ECM/DMI	1.18	1.11	1.04	1.12	0.21	0.478
ECM/NE_Lintake	0.74	0.65	0.61	0.66	0.12	0.175

[1] Control : basal diet without oil supplement; LOFO : 3% linseed and fish oils at 1 : 1; SOFO : 3% sunflower and fish oils at 1 : 1; MIXO : 3% mixture of linseed, sunflower, and fish oils at 1 : 1 : 1.

[2] 3.5% FCM = [0.432 × milk (kg)] + [16.216 × fat (kg)] (Dairy Records Management Systems, 2014).

[3] ECM = [0.327 × milk (kg/d)] + [12.86 × fat (kg/d)] + [7.65 × protein (kg/d)] (Peterson et al., 2012).

[a-b] Means within a row with different superscripts are significantly different at $P < 0.05$ ($n = 6$).

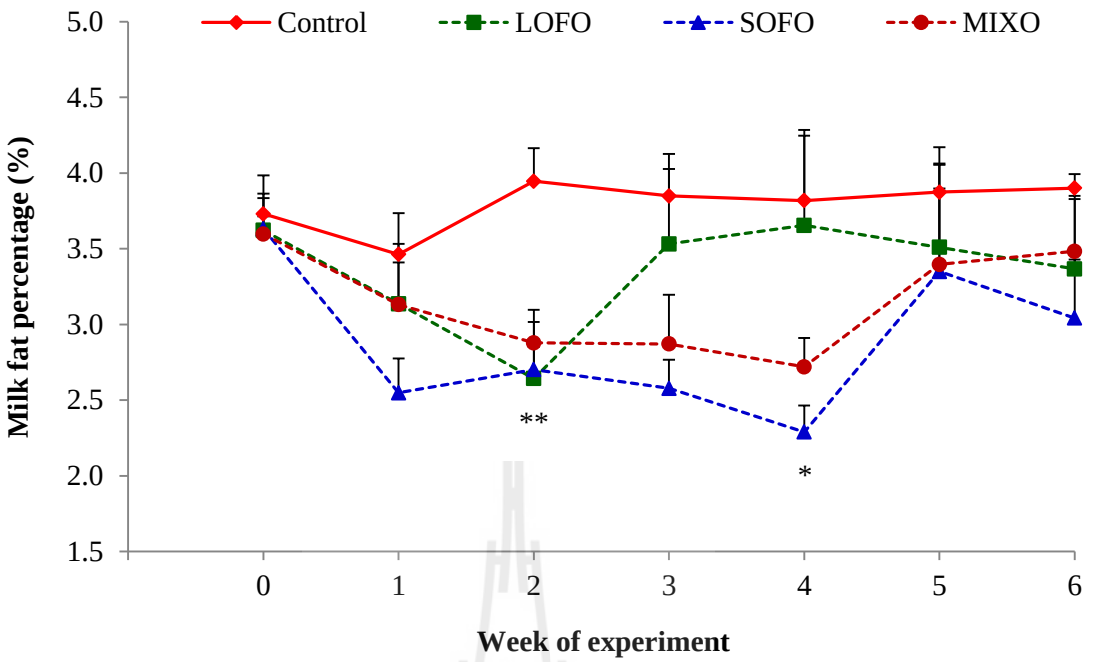

Figure 3.1 Milk fat percentage changes during the experiment. Diets were statistical different (P = 0.03), but no difference was detected for week and treatment × week interaction (P > 0.05). The standard error of the mean is indicated by the error bars over each point. * : P < 0.05 and ** : P < 0.01 (n = 6).

3.4.4 Milk FA composition

Milk FA profiles were strongly modified by oil supplementation (Table 3.5). The proportion of total C18:1n-9 in the milk fat was remarkably enhanced (P < 0.001) to 29.85, 31.00, and 33.67% corresponding to the LOFO, SOFO, and MIXO while in the control group it was only 21.60%. Milk *cis*-9,*trans*-11 CLA increased (P < 0.01) by 198.11% in the LOFO group relative to the control group. In the current study, milk proportion of *trans*-10,*cis*-12 CLA was increased (P < 0.001) because of oil supplementation rich in PUFA, regardless of different oil mixtures. Milk C18:3n-3 proportion was enhanced (P < 0.001) by 227.27% by supplementing LOFO relative to the control group. It was found in the current study that the proportion of DHA was

increased (P < 0.001) in the cows fed the LOFO (0.38%) and MIXO (0.23%) compared with the control group (0.01%), but the SOFO supplementation didn't have any significant additive effect.

Table 3.5 Milk composition of individual fatty acids

FA[1] (g/100 g FA)	Treatment[2]				SEM	*P*-value
	Control	LOFO	SOFO	MIXO		
C4:0	2.41^{a}	2.10^{ab}	1.69^{b}	1.74^{b}	0.33	0.003
C6:0	1.78^{a}	1.05^{b}	0.93^{bc}	0.79^{c}	0.15	<0.001
C8:0	1.12^{a}	0.55^{b}	0.49^{b}	0.40^{b}	0.12	<0.001
C10:0	2.44^{a}	1.25^{b}	1.11^{b}	0.88^{b}	0.33	<0.001
C11:0	0.32^{a}	0.13^{b}	0.10^{bc}	0.07^{c}	0.02	<0.001
C12:0	7.06^{a}	5.48^{b}	5.13^{b}	4.47^{b}	0.64	<0.001
C13:0	0.25^{a}	0.17^{b}	0.15^{b}	0.12^{c}	0.02	<0.001
C14:0	12.51^{a}	11.00^{b}	10.11^{bc}	9.23^{c}	0.75	<0.001
c-9 C14:1	1.08	0.96	0.87	0.69	0.27	0.133
C15:0	1.02	1.02	1.05	0.97	0.07	0.270
C16:0	35.83	34.01	34.18	32.55	3.03	0.343
c-9 C16:1	2.24	2.29	2.37	2.00	0.48	0.590
c-10 C17:1	0.19	0.23	0.22	0.23	0.04	0.342
C18:0	7.73^{a}	5.06^{b}	7.07^{a}	8.02^{a}	1.10	<0.001
C18:1n-9	21.60^{b}	29.85^{a}	31.00^{a}	33.67^{a}	2.54	<0.001
c-9 C18:1	17.91^{ab}	15.06^{b}	15.31^{b}	19.18^{a}	2.19	0.009
t-9,*t*-12 C18:2	0.19^{ab}	0.27^{a}	0.15^{b}	0.22^{ab}	0.07	0.040

Table 3.5 Milk composition of individual fatty acids (conc.)

FA[1] (g/100 g FA)	Treatment[2]				SEM	*P*-value
	Control	LOFO	SOFO	MIXO		
c-9,*c*-12 C18:2	0.99[b]	0.18[ab]	1.06[b]	1.38[a]	0.17	0.004
c-9,*t*-11 CLA	0.53[b]	1.58[a]	0.74[b]	0.94[ab]	0.44	0.003
t-10,*c*-12 CLA	0.02[b]	0.16[a]	0.21[a]	0.18[a]	0.04	<0.001
c-9,*c*-11 CLA	0.01[b]	0.02[b]	0.07[a]	0.05[a]	0.01	<0.001
t-9,*t*-11 CLA	0.08[b]	0.21[a]	0.16[a]	0.16[a]	0.05	<0.001
C18:3n-3	0.11[c]	0.36[a]	0.20[b]	0.25[b]	0.06	<0.001
C20:0	0.15	0.14	0.18	0.16	0.03	0.091
c-11 C20:1	0.08[b]	0.22[a]	0.19[a]	0.18[a]	0.03	<0.001
c-11,*c*-14 C20:2	0.03	0.07	0.04	0.06	0.04	0.212
C20:3n-6	0.07	0.05	0.06	0.05	0.03	0.649
C20:4n-6	0.09	0.13	0.12	0.13	0.04	0.331
C24:0	0.03	0.06	0.14	0.07	0.11	0.339
C20:5n-3	0.00	0.00	0.08	0.08	0.08	0.131
C22:6n-3	0.01[c]	0.38[a]	0.19[bc]	0.23[ab]	0.11	<0.001

[1] Zero values indicate proportions of FA in feed ingredients were <0.01% total FA or undetectable.

[2] Control : basal diet without oil supplement; LOFO : 3% linseed and fish oils at 1 : 1; SOFO : 3% sunflower and fish oils at 1 : 1; MIXO : 3% mixture of linseed, sunflower, and fish oils at 1 : 1 : 1.

[a-c] Means within a row with different superscripts are significantly different at $P < 0.05$ ($n = 6$).

Table 3.6 shows that oil supplementation led to reduce ($P < 0.001$) proportions of short- and medium chain FA (<16 carbons) in the milk fat compared to the control diet, the extent of the decrease was greatest for the animals fed the

MIXO diet. In contrast, preformed FA (>16 carbons) proportion in the milk fat was increased ($P < 0.001$) in the animals fed MIXO compared with the control and LOFO animals. Feeding oil mixtures improved ($P < 0.001$) C18 UFA proportion in the milk fat by 43.84-57.00% relative to the control, the greatest increase was observed for MIXO group. The oil inclusion in the diet led to reduce ($P < 0.001$) SFA, AI, and TI but increased UFA including MUFA and PUFA compared to the control diet. The greater increase ($P < 0.001$) of the total n-3 FA led to decrease ($P < 0.001$) the ratio of n-6 to n-3 FA in the milk fat of the animals fed diets added oils (2.28-3.46%) compared to the control animals (11.63%). Further calculation of yield of some selected FA in milk fat showed that milk yields of *cis*-9,*trans*-11 CLA, C18:3n-3, EPA+DHA, n-3 PUFA were greater ($P < 0.05$) in the LOFO group than those in the control group. In contrast, the transfer of C18:2n-6, C18:3n-3, and PUFA from feed to milk fat was higher ($P < 0.001$) in the control group compared to the other groups (Table 3.7).

Table 3.6 Milk composition of fatty acid groups and indices

Item	Treatment[1]				SEM	*P*-value
	Control	LOFO	SOFO	MIXO		
FA groups (g/100 g FA)						
De novo[2]	30.00[a]	23.72[b]	21.51[bc]	19.38[c]	1.86	<0.001
Mixed[3]	38.08	36.30	36.55	34.56	3.30	0.356
Preformed[4]	31.93[c]	39.98[b]	41.95[ab]	46.07[a]	3.21	<0.001
C18 UFA	23.63[b]	33.99[a]	33.79[a]	37.10[a]	2.70	<0.001

Table 3.6 Milk composition of fatty acid groups and indices (conc.)

Item	Treatment[1]				SEM	*P*-value
	Control	LOFO	SOFO	MIXO		
SFA (S)	72.68^{a}	62.04^{b}	62.26^{b}	59.49^{b}	2.50	<0.001
UFA (U)	27.32^{b}	37.96^{a}	37.74^{a}	40.51^{a}	2.50	<0.001
MUFA (M)	25.19^{b}	33.54^{a}	34.65^{a}	36.78^{a}	2.33	<0.001
PUFA (P)	2.13^{c}	4.42^{a}	3.09^{b}	3.72^{ab}	0.58	<0.001
n-6	1.34^{b}	1.63^{ab}	1.38^{b}	1.78^{a}	0.19	0.002
n-3	0.12^{b}	0.74^{a}	0.48^{a}	0.57^{a}	0.17	<0.001
EPA+DHA	0.01^{b}	0.38^{a}	0.27^{a}	0.31^{a}	0.14	<0.001
Indices						
U/S	0.38^{b}	0.62^{a}	0.61^{a}	0.68^{a}	0.06	<0.001
M/S	0.35^{b}	0.55^{a}	0.56^{a}	0.62^{a}	0.05	<0.001
P/S	0.03^{c}	0.07^{a}	0.05^{b}	0.06^{ab}	0.01	<0.001
n-6/n-3	11.63^{a}	2.28^{b}	3.21^{b}	3.46^{b}	1.12	<0.001
AI	3.42^{a}	2.24^{b}	2.10^{b}	1.83^{b}	0.31	<0.001
TI	4.11^{a}	2.54^{b}	2.59^{b}	2.35^{b}	0.39	<0.001

[1] Control : basal diet without oil supplement; LOFO : 3% linseed and fish oils at 1 : 1; SOFO : 3% sunflower and fish oils at 1 : 1; MIXO : 3% mixture of linseed, sunflower, and fish oils at 1 : 1 : 1.

[2] *De novo* FA originate from mammary *de novo* synthesis (<16 carbons).

[3] Mixed FA originate from both sources (C16:0 and C16:1).

[4] Preformed FA originate from extraction from plasma (>16 carbons).

[a-c] Means within a row with different superscripts are significantly different at $P < 0.05$ ($n = 6$).

Table 3.7 Milk fatty acids secreted relative to corresponding dietary fatty acids

Item	Treatment[1]				SEM	*P*-value
	Control	LOFO	SOFO	MIXO		
Yield of milk fatty acids (g/d)						
c-9,*c*-12 C18:2	5.11	4.84	3.56	5.48	1.28	0.085
c-9,*t*-11 CLA	2.39^{b}	5.95^{a}	2.41^{b}	3.38^{ab}	1.97	0.013
C18:3n-3	0.48^{b}	1.30^{a}	0.59^{b}	0.91^{ab}	0.32	0.001
C20:5n-3 + C22:6n-3	0.05^{b}	1.37^{a}	0.79^{ab}	1.09^{a}	0.48	<0.001
PUFA	9.61^{b}	16.29^{a}	8.96^{b}	13.30^{ab}	3.79	0.011
n-3 PUFA	0.53^{c}	2.67^{a}	1.40^{bc}	2.00^{ab}	0.69	<0.001
n-6 PUFA	6.06	5.96	4.02	6.33	1.53	0.061
Transfer into milk (g/100 g intake)						
C18:2n-6[2]	7.20^{a}	5.40^{ab}	2.43^{c}	3.88^{bc}	1.33	<0.001
c-9,*t*-11 CLA[3]	3.16^{ab}	3.37^{a}	1.40^{b}	1.68^{ab}	1.16	0.014
C18:3n-3	10.27^{a}	1.52^{b}	9.15^{a}	1.47^{b}	2.05	<0.001
C20:5n-3 + C22:6n-3	-	2.11	1.24	2.46	-	-
PUFA	12.72^{a}	6.61^{b}	4.02^{b}	5.27^{b}	2.09	<0.001
n-3 PUFA	11.41^{a}	1.77^{b}	1.98^{b}	1.87^{b}	1.64	<0.001
n-6 PUFA	8.54^{a}	6.34^{ab}	2.63^{c}	4.34^{bc}	1.51	<0.001

[1] Control : basal diet without oil supplement; LOFO : 3% linseed and fish oils at 1 : 1; SOFO : 3% sunflower and fish oils at 1 : 1; MIXO : 3% mixture of linseed, sunflower, and fish oils at 1 : 1 : 1.

[2] Calculated as 100 × (Sum of milk *cis*-9,*cis*-12 C18:2 and *trans*-9,*trans*-12 C18:2) / *cis*-9,*cis*-12 C18:2 intake.

[3] Calculated as 100 × (milk *cis*-9,*trans*-11 CLA (g) / (sum of *cis*-9,*cis*-12 C18:2 and C18:3n-3 intakes).

[a-c] Means within a row with different superscripts are significantly different at $P < 0.05$ ($n = 6$).

3.5 Discussion

3.5.1 Intakes

The adverse effects of FA from high oil supplementation (total dietary fat more than 5%) on feed intake and animal performance are foremost consideration (Patra, 2013). The decreased total DMI in the current study was in agreement with some previous studies (Chilliard et al., 2009; Martin et al., 2008). However, the lesser effect as total lipid concentration in the diet higher than 6% DM was also documented (Angulo et al., 2012; Benchaar et al., 2012; Huang et al., 2008). Diets supplemented with oil sources rich in unprotected PUFA often causes a decrease in DMI, and the mechanisms of this effect are attributed to the effects on ruminal fermentation, gut motility, palatability of added fat diets, release of gut hormones, and oxidation of fat in the liver (Allen, 2000). In the current study, the lower DMI in the SOFO diet might highly relate to some causes concerning above.

3.5.2 Milk yield and composition

Oil supplementation at 3% DMI in the current study had no effect on milk yield. This result was supported by Angulo et al. (2012) and Vafa et al. (2012). A similar result was also recently published by Neveu et al. (2014). The decrease in milk fat percentage and yield in the animals fed SOFO was consistent with some previous studies in bovines (Angulo et al., 2012; Murphy et al., 2008). This effect is known as MFD and often occurs in dairy cows fed oil/fats rich in PUFA (Huang et al., 2008; Shingfield et al., 2006). The MFD is related to an alteration of ruminal BH resulting from the production of different ruminal intermediates that has a negative effect on the gene expression of lipogenic enzymes (Bauman and Griinari, 2001). In general, oils with a high degree of unsaturation are able to disturb ruminal fermentation and fiber

digestibility, leading to lower acetate production and therefore milk fat synthesis (Coppock and Wilks, 1991). Moreover, some studies where cows were fed milk fat-depressing diets including low forage and high-oil diets show down regulation of genes involved in mammary lipid synthesis, which was associated with increased milk *trans*-10 C18:1 and *trans*-10,*cis*-12 CLA (Ahnadi et al., 2002; Angulo et al., 2012; Harvatine and Bauman, 2006), but *cis*-9,*trans*-11 CLA did not (Baumgard et al., 2000). In the current study, therefore increase of *trans*-10,*cis*-12 CLA proportion in milk fat was found in the SOFO group which resulted in the strong depression of milk fat percentage and yield. The molecular mechanism mediating the inhibitory effect of CLA isomers, particularly *trans*-10,*cis*-12 CLA, on MFD is not well understood up to now. Nevertheless, Harvatine and Bauman (2006) showed that the sterol response element binding protein transcription factor system, by binding to response elements located in lipogenic enzyme genes, may be a central signaling pathway by which CLA regulates FA synthesis in the mammary gland. Thyroid hormone responsive spot 14, which is down regulated during diet-induced MFD, may also be involved in the molecular mechanism of MFD, possibly as a secondary cellular signal for sterol response element binding protein 1. In this study, unchanged milk fat content in the control group contrary to the lower values in the other oil-supplemented groups over weeks of feeding suggested that added oils were a main factor to affect milk fat synthesis. The greater milk fat content in the LOFO than other oil-supplemented treatments after 3rd week feeding revealed that the animals and rumen microbes in the LOFO could be greater and faster capacity to recovery themselves with negative effect of oil inclusion. Milk urea N in this study (15.98-18.56 mg/dl) was in normal range for lactating dairy cows (16.0 ± 4.99 mg/dl) as reported by Fatehi et al. (2012). It has often

been suggested that milk urea N could be used as a diagnostic of on-farm efficiency of N utilization. No significant difference of milk urea N among the treatments revealed that the oil supplementation did't affect efficiency of N utilization in the animals.

3.5.3 Milk FA composition

In the current study, increases in milk fat C18:1n-9, isomers of C18:1, when dairy cows were fed oil mixtures rich in C18 UFA was in agreement with Bu et al. (2007) and Huang et al. (2008). The lower proportion of C18:0 in milk fat from cows fed LOFO could be ascribed to an incomplete BH process in the rumen of either C18:3n-3 or C18:1 to C18:0, resulting in increased milk C18:3n-3, C18:1 isomers, and CLA isomers. The LOFO had additive effects on *cis*-9,*trans*-11 CLA and C22:6n-3 proportions in milk fat, but the SOFO did not have clear additive effect, indicating that a quantity of *trans*-11 C18:1, *cis*-9,*trans*-11 CLA, and C22:6n-3 was able to bypass ruminal BH in the LOFO group while this was not in the SOFO group. Milk C18:3n-3 proportion was increased to the greatest extent by feeding LO in combination with FO, indicates that ruminal BH of C18:3n-3 was diminished.

Short- and medium chain FA proportions (<16 carbons) were decreased while the long-chain FA (particularly C18 UFA) were increased in diets blended with LO, SO, and FO as compared to the control diet (Table 3.6). These suggested that the supplementation of oil mixtures rich in PUFA had strongly inhibited the *de novo* synthesis of FA in mammary fat tissues, because almost milk C4:0–C14:0 and about half of C16:0 are synthesized *de novo* by the mammary epithelial cells. Dietary supply of long-chain UFA has been shown to improve their secretion in milk fat and decrease the *de novo* synthesis of short- and medium-chain FA in the mammary gland (Grummer, 1991), and it is probable that the decreased part in endogenous mammary

FA synthesis when UFA are fed is related to increase formation of specific BH intermediates in the rumen. The improved proportion of milk C18 UFA in the current study was in agreement with some previous studies (Caroprese et al., 2010; Lerch et al., 2012). This was also supported by Chow et al. (2004) that FO inclusion in the *in vitro* study containing SO and LO led to increase the accumulation of C18:1 UFA. Milk from cows receiving oil inclusion rich in PUFA showed an improvement in UFA proportion and a decrease in SFA proportion. These were in agreement with some previous studies (Caroprese et al., 2010; Lerch et al., 2012; Neveu et al., 2014).

Some FA can help to prevent or promote coronary thrombosis and atherosclerosis based upon their effects on low-density lipoprotein-cholesterol concentrations and serum cholesterol (Ulbricht and Southgate, 1991). The equations proposed by Ulbricht and Southgate (1991) for the atherogenic and thrombogenic indices showed that C12:0, C14:0, and C16:0 FA are atherogenic while C14:0, C16:0, and C18:0 are thrombogenic. The n-3 PUFA, n-6 PUFA, and MUFA are anti-atherogenic and anti-thrombogenic. The ratio between the saturated and unsaturated FA was used to calculate the atherogenic and thrombogenic (Table 3.6). The supplementation of oil mixtures rich in PUFA in the current study resulted in reduced AI, TI, and n-6 to n-3 ratio that could counteract the detrimental effect of high SFA and n-6 FA in the milk. Decreased milk atherogenic and thrombogenic indices in the present study were supported by Huang et al. (2008). That the transfer efficiency of selected FA including C18:2n-6, C18:3n-3, PUFA, n-3 PUFA, and n-6 PUFA in milk was higher for cows fed the control diet is in agreement with Dewhurst et al. (2003) and Côrtes et al. (2011) who reported that the lower apparent transfer was observed in the diets containing higher UFA concentration.

3.6 Conclusions

A supplementation of dairy cow diet with LOFO or MIXO had no effects on total DMI, milk yield, and milk composition. In contrast, the diet supplemented with SOFO caused negative effects on total DMI, milk fat percentage, and yields of milk fat, protein, and total solid. Dietary supplementation with LOFO mainly increased milk *cis*-9,*trans*-11 CLA and n-3 PUFA whereas feeding MIXO improved preformed FA and UFA. The lowest n-6/n-3 ratio was found in the LOFO, but the decreased AI and TI seemed to be remarkable in the MIX-O. To maximize milk *cis*-9,*trans*-11 CLA and n-3 PUFA and to minimize milk n-6/n-3 ratio, AI, and TI, an ideal supplement would look to be either LOFO or MIXO. Nevertheless, feeding MIXO to dairy cows to enhance milk beneficial UFA seems to be greater economic rather than feed them with LOFO due to the price of sunflower oil (75 Thai Baht/kg) was cheaper than those in the linseed oil and fish oil (160 and 95 Thai Baht/kg, respectively).

3.7 References

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CHAPTER IV

EFFECTS OF LINSEED OIL AND SUNFLOWER OIL ALONE OR BOTH WITH FISH OIL ON *IN VITRO* RUMEN FERMENTATION AND GAS PRODUCTION

4.1 Abstract

This study was conducted to test the effects of supplementing different oils on *in vitro* gas and CH_4 production, ruminal fermentation, and digestibility. The study was carried out as a completely randomized design using rumen fluid obtained from three non-lactating Holstein Friesian dairy cows. The dietary treatments included : 1) high-concentrate diet without oil addition (Control), 2) linseed oil (LO), 3) sunflower oil (SO), 4) linseed oil and fish oil (LOFO), 5) sunflower oil and fish oil (SOFO), and 6) mixture of linseed oil with sunflower oil as well as fish oil (MIXO). The amount of added oils were at 3% DM. Cumulative gas production was recorded at 2, 4, 6, 8, 10, 12, 18, 24, 36, and 48 h incubation. *In vitro* digestibilities were determined after 48 h incubation. Ruminal pH, NH_3-N, VFA, and CH_4 values were measured at 0, 2, 4, 6, and 24 h post incubation. Cumulative gas production at 48 h incubation and protozoa population were lower ($P < 0.01$) in the oils added in combination, such as LOFO, SOFO, and MIXO. Methane production was remarkably reduced ($P < 0.001$) after 24 h incubation by oil inclusion (except SO). Inclusion of SOFO had lower total VFA concentration, lower acetate proportion, and higher propionate proportion than the

control. Supplementation of LOFO and SOFO declined ($P < 0.05$) microbial protein (MCP) synthesis and *in vitro* digestibilities of true DM, OM, and NDF. Based on this study, it suggests that inclusion of MIXO could maintain ruminal fermentation and digestibility, but could decrease gas and CH_4 production.

4.2 Introduction

Conjugated linoleic acid, a group of geometric and positional isomers of LA with conjugated double bonds, is a FA which can detect easily in ruminants-derived foods. Among CLA isomers in dairy products, *cis*-9,*trans*-11 has been known to exert various potent physiological functions such as anti-carcinogenic, anti-diabetic, anti-hypertensive, and anti-obese properties (Koba and Yanagita, 2014). Omega-3 PUFA including ALA, EPA, and DHA have important roles in anti-atherogenic, anti-inflammatory, immunemodulatory, and anti-arrhythmic properties (Sekikawa et al., 2015). It has been growing interest in elevating *cis*-9,*trans*-11 CLA and n-3 PUFA contents in ruminant meats and milk due to the beneficial effects of these FA for human health. Supplementation of vegetable oils such as LO and SO improved the contents of CLA and ALA in milk (Benchaar et al., 2012; Rego et al., 2009), whereas FO addition in the dairy cattle diet increased milk n-3 PUFA (Vahmani et al., 2013). Moreover, lipid supplementation has been researching extensively as an enteric CH_4 mitigation strategy in ruminant feeding system (Hristov et al., 2013; Knapp et al., 2014). Climate Change Central (2010) in Alberta, Canada already recognizes oil addition as a strategy to abate CH_4 emission from dairy cattle under their protocols. However, supplemental oil may cause to reduce digestibilities of DM and NDF (Patra, 2014), reflecting in decreased animal performance, which may limit the use of oils to

mitigate CH_4 emissions in ruminants. The effect of added oils on CH_4 production depends on the source, FA profile, and inclusion level (Knapp et al., 2014). Therefore, an ideal oil addition which doesn't have negative effects on digestibility and ruminal fermentation, but still has greater lowering influence on CH_4 production should be studied. This study aimed to investigate the effects of supplementing different oils on *in vitro* gas and CH_4 production, ruminal fermentation, and digestibility.

4.3 Materials and methods

4.3.1 Experimental design and treatments

This experiment was carried out using a syringe gas production technique. The experiment was conducted as a completely randomized design with the treatments included : 1) high-concentrate diet without oil addition (Control), 2) 15 mg/syringe of linseed oil (LO), 3) 15 mg/syringe of sunflower oil (SO), 4) 15 mg/syringe of oil mixture (1 : 1, w/w) from linseed oil and fish oil (LOFO), 5) 15 mg/syringe of oil mixture (1 : 1, w/w) from sunflower oil and fish oil (SOFO), and 6) 15 mg/syringe of oil mixture (1 : 1 : 1, w/w) from linseed oil, sunflower oil, and fish oil. Addition of oil alone or mixtures in the current experiment was at 3% DM.

4.3.2 Substrates, added oils, and rumen inoculum

Corn silage and concentrate were ground in a Retsch mill (SR200 model, Retsch, Haan, Germany) to pass a 1-mm mesh prior to analyze for chemical compositions and *in vitro* gas production measurements. The incubation substrate consisted of corn silage and concentrate were mixed at a ratio of 40 : 60 (w/w, on DM basis) and stored until incubation. Linseed oil was obtained from T. Charoenchai Hardware, Bangkok, Thailand. Sunflower oil was bought from Makro, Nakhon

Ratchasima, Thailand. Tuna fish oil was obtained from T.C. Union Agrotech Co., Ltd., Bangkok, Thailand. Oils were prepared and added into incubation syringes as an oil-ethanol solution (185 : 15, v/w). Chemical characteristics of feeds and oils used in this study are presented in the Table 4.1.

Table 4.1 Ingredients and chemical composition of feeds used in the experiment

Item	**Concentrate**	**Corn silage**	**LO**	**SO**	**FO**
Ingredient (% DM)					
Cassava distillers dried meal	32.00	-	-	-	-
Soybean meal	20.00	-	-	-	-
CDDGS	17.50	-	-	-	-
Rice bran	10.00	-	-	-	-
Wheat bran	10.00	-	-	-	-
Molasses	8.00	-	-	-	-
Mineral and vitamin mix	2.50	-	-	-	-
Chemical composition (% of DM)					
DM (%)	90.22	23.79	-	-	-
OM	90.86	91.10	-	-	-
CP	21.14	9.61	-	-	-
EE	3.77	1.81	100	100	100
Ash	9.14	8.90	-	-	-
NFC[1]	24.55	14.15	-	-	-
NDF	41.40	65.53	-	-	-
ADF	28.45	42.09	-	-	-
Lignin	3.61	4.44	-	-	-

[1] Calculated as 100 − (CP + NDF + EE + ash).

Rumen contents were obtained before the morning feeding from three fistulated non-lactating Holstein Friesian dairy cows (approximately 500 kg) fed a maintenance diet based on corn silage and 21% CP concentrate (R : C 70 : 30, w/w on DM basis). The animals were fed twice daily at 08:30 and 17:00 for 1-week period before taking the rumen contents. Approximately 1,000 ml of rumen liquor obtaining from donor cows were transported in three thermos flasks to the *in vitro* laboratory within 10 min. The rumen fluid was filtered through 2 layers of cheesecloth into pre-warmed thermos flasks to retain small particles.

4.3.3 Medium preparation

Medium solution was prepared according to Menke and Steingass (1988) with some minor modifications. In detail, 2.5 g tryptone was added into a volumetric flask, followed by respective addition of 0.125 ml micromineral solution (prepared by diluting 13.2 g $CaCl_2.2H_2O$, 10.0 g $MnCl_2.4H_2O$, 1.0 g $CoCl_2.6H_2O$, and 8.0 g $FeCl_2.6H_2O$ in deionized water to a final volume of 100 ml), 250 ml buffer solution (prepared by diluting 4.0 g NH_4HCO_3 and 25.0 g $NaHCO_3$ in deionized water to a final volume of 1 L), 250 ml macromineral solution (prepared by diluting 5.7 g Na_2HPO_4, 6.0 g KH_2PO_4, and 0.6 g $MgSO_4.7H_2O$ in deionized water to a final volume of 1 L), 1.25 ml 0.1% resazurin solution (prepared by dissolving 0.1 g resazurin in deionized water to a final volume of 100 ml), and deionized water to a final volume of 1000 ml. The solution was then placed in water bath (39°C) and gassed CO_2 for 45 min. While still flushing with CO_2, the exact amounts of 0.313 g L-cysteine hydrochloride and 0.313 g sodium sulphide were weighed and added directly to medium. The medium solution was continued to flush with CO_2 for another approximate 30 minutes or until solution turns grey to clear.

4.3.4 *In vitro* fermentation

Substrates were weighed to 500 mg of DM into 100-ml glass syringes then supplemented with 200 µl of oil-ethanol solutions which providing 15 mg of added oil/syringe. Three blank syringes for gas production were added 200 µl of absolute ethanol (99.99%) without oil supplementation and substrate. An overview of substrates and oils added into the incubation syringes is given in Table 4.2. Under continuous CO_2 flushing, the filtrated rumen fluid was mixed (1 : 4, v/v) with pre-warmed (39°C) medium and then introduced (50 ml of rumen fluid and medium mixture) into gastight glass syringes. The lower end of syringes was closed afterward, and the syringes were incubated in a water bath at 39°C for 48 h. Gas volume produced was recorded at 2, 4, 6, 8, 10, 12, 18, 24, 36, and 48 h incubation.

Table 4.2 Amount (mg) of substrate and oil added to each incubation syringe

Item	Control	LO	SO	LOFO	SOFO	MIXO
Substrate	500	500	500	500	500	500
Linseed oil	-	15.0	-	7.5	-	5.0
Sunflower oil	-	-	15.0	-	7.5	5.0
Fish oil	-	-	-	7.5	7.5	5.0
Ethanol (µl)	200	185	185	185	185	185

4.3.5 Sampling, measurements, and chemical analysis

Rumen fluid was collected at 0, 2, 4, 6, and 24 h post incubation. Incubation was stopped by placing the syringes into ice-cold water, and the pH of syringe contents was immediately measured. One milliliter of rumen content was sampled and

mixed with 9 ml of 10% formalin solution. Total protozoa were directly counted using hemocytometer following the methods of Galyen (2010). The syringe contents were filtrated through four layers of cheesecloth. The samples for NH_3–N and VFA analyses were acidified with 1 M sulfuric acid (10/1, v/v), centrifuged at 14,000 × g for 15 min, and the supernatant was then stored at – 20°C.

At 48 h post inoculation, some syringe samples were stopped to determine *in vitro* true digestibility (IVTD) following the method described by Van Soest and Robertson (1985). For this purpose, the incubation content was totally transferred to a 500–ml beaker and repeatedly washed with 100 ml neutral detergent solution. The content was boiled in hot plates for 1 h and filtrated through pre-weighed crucibles. The samples were then dried overnight in hot air oven at 105°C, and the residue was weighed to calculate IVTD and IVNDFD of feed.

Organic matter content was calculated as the difference from ash, determined according to AOAC (1998). Crude protein was determined by Kjeldahl method, procedure 928.08 of AOAC (1998). Ether extract was determined using petroleum ether in a Soxtec System, procedure 948.15 of AOAC (1998). Neutral detergent fiber and ADF were determined using the methods described by Van Soest et al. (1991), adapted for Fiber Analyzer. The NDF analysis used sodium sulfite in the neutral detergent solution. Both NDF and ADF are expressed inclusive of residual ash. All chemical compositions were expressed on DM basis. The rumen NH_3–N was determined by Kjeldahl method (AOAC, 1998). The volatile fatty acids (VFA) were analyzed using gas chromatography (Hewlett Packard GC system HP6890 A; Hewlett Packard, Avondale, PA, USA). Gas chromatography was equipped with a 30 m × 0.32 mm × 0.15 μm film fused silica

capillary column (HP Innowax, AB 002, Agient, USA). Injector and detector temperatures were 250°C. The column temperature was respectively set as follow : kept at 80°C for 5 min, increased at 10°C/min to 170°C, increased at 30°C /min to 250°C and finally held at 250°C for 5 min.

4.3.6 Calculations

Methane concentration was calculated from individual net molar of VFA with the equation proposed by Fievez et al. (2005) as follow :

CH_4 (mmol) = 0.5 × acetate – 0.25 × propionate + 0.5 × butyrate

IVTD (%) = 100 × (DM of feed used for incubation – NDF residue) / DM of feed used for incubation

IVDNFD (%) = 100 × (NDF of feed used for incubation – NDF residue) / NDF of feed used for incubation

The *in vitro* digestible organic matter (IVDOM) was calculated from the gas produced and chemical composition according to equation of Menke et al. (1979) :

IVDOM (g/kg DM) = $(14.88 + 0.889 \times G_p + 0.45 \times CP + 0.0651 \times XA) \times 10$

where CP is the crude protein (% of DM), XA is the ash (% of DM), and G_p is the net gas production (ml) from 200 mg (DM of sample) after 24 h incubation.

Microbial protein production (MP) was calculated as 19.3 g microbial N per kg IVDOM according to Czerkawski (1986).

4.3.7 Statistical analysis

Data on mean values of CH_4 production, protozoa population, pH, NH_3-N, and VFA were analyzed according to a completely randomized design with the repeated measures (hours) using PROC MIXED procedure of SAS (2002) with the statistical model $Y_{ijk} = \mu + T_i + H_j + (T \times H)_{ij} + \varepsilon_{ijk}$, where Y_{ijk} = the dependent variable, μ = the

overall mean, T_i = the fixed effect of treatment, H_j = the fixed effect of incubation time (hour), $(T \times H)_{ij}$ = the fixed effect of interaction between treatment and time, and ε_{ijk} = the random residual error. The replicate within treatment was considered as a random effect.

Data on gas production, MCP, and digestibility were analyzed by ANOVA procedure of SAS (2002) for a completely randomized design with the statistical model $Y_{ij} = \mu + T_i + \varepsilon_{ij}$, where Y_{ij} = the dependent variable, μ = the overall mean, T_i = the treatment effect, and ε_{ij} = the random residual error. Overall differences between treatment means were considered to be significant as $P < 0.05$. Significant differences among treatment means were assessed by Tukey's multiple comparison tests after a significant F-test. Data are expressed as mean ± SEM, which represents the pooled SEM for the model.

4.4 Results

4.4.1 Gas production, methane production, and protozoa

The oil supplementation had strongly affected gas production, CH_4 production, and protozoa population (Table 4.3). Particularly, cumulative gas production at 48 h reduced ($P < 0.01$) from 12.61 mmol/g DM in the control to 11.77-11.91 mmol/g DM in the combinative oil groups. The lowering ($P < 0.05$) of gas production was observed from 18 h until the end of the incubation, except at 24 h (Figure 4.1). It was found from this study that oil inclusion as combinative form was more effective to mitigate CH_4 emission rather than single form. Methane production calculated from net molars of VFA showed that SOFO had lower ($P < 0.05$) CH_4 production than the control.

Table 4.3 Gas production, CH_4 production, and protozoa

Item	Treatment						SEM	*P*-value
	Control	LO	SO	LOFO	SOFO	MIXO		
Gas (48 h)								
ml/g DM	282.46^{a}	270.57^{ab}	269.86^{ab}	263.85^{b}	263.68^{b}	266.75^{b}	8.27	0.005
mmol	6.31^{a}	6.04^{ab}	6.03^{ab}	5.89^{b}	5.89^{b}	5.95^{b}	0.19	0.004
mmol/g DM	12.61^{a}	12.08^{ab}	12.05^{ab}	11.78^{b}	11.77^{b}	11.91^{b}	0.37	0.005
Methane								
mmol	0.74^{a}	0.68^{ab}	0.67^{ab}	0.60^{ab}	0.54^{b}	0.63^{ab}	0.10	0.009
mmol/g DM	1.48^{a}	1.35^{ab}	1.33^{ab}	1.21^{ab}	1.09^{b}	1.26^{ab}	0.20	0.009
Protozoa (×10^{5} cfu/ml)	10.80^{a}	8.33^{b}	8.93^{ab}	8.20^{b}	6.93^{b}	7.50^{b}	1.92	0.003

$^{a-b}$ Means within a row with different superscripts are significantly different at $P < 0.05$ ($n = 6$ for gas production and $n = 3$ for methane production and protozoa).

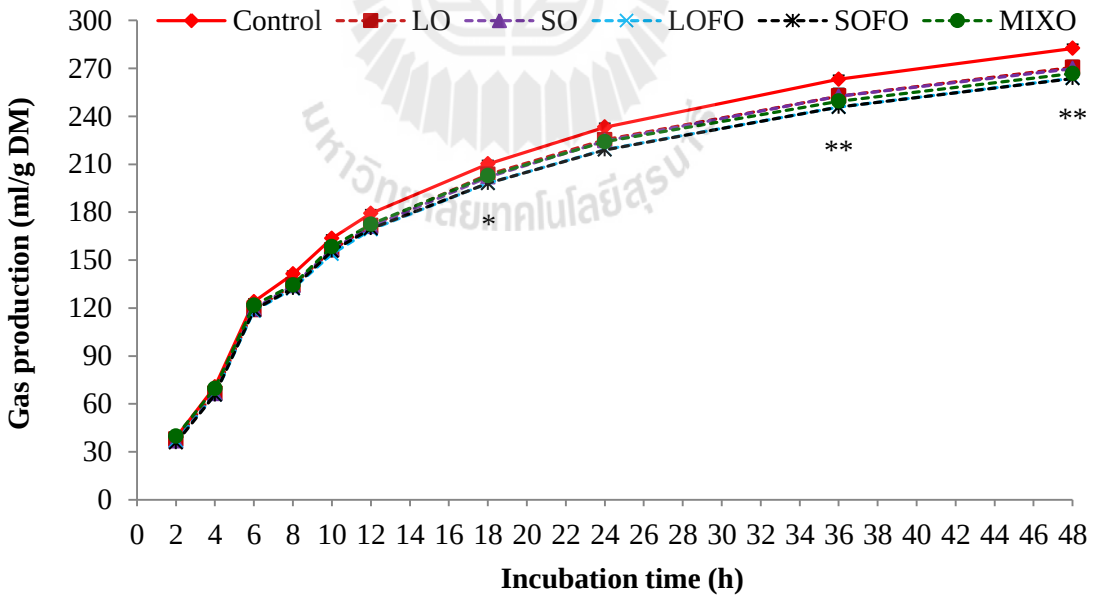

Figure 4.1 Cumulative gas production changes during the incubation. The standard error of the mean is indicated by the error bars over each point. * : $P < 0.05$ and ** : $P < 0.01$ ($n = 6$).

As can we seen from the Figure 4.2 that during the early time points of incubation, the variable of CH_4 production was still small to detect the significant difference, but CH_4 production was remarkably reduced ($P < 0.001$, SE = 0.10) after 24 h incubation by supplementation of oils (except SO). Population of ruminal protozoa were numerously decreased ($P < 0.01$) in the combinative oil groups (6.93-8.20 × 10^5 cfu/ml) compared to 1.08 × 10^6 cfu/ml in the control.

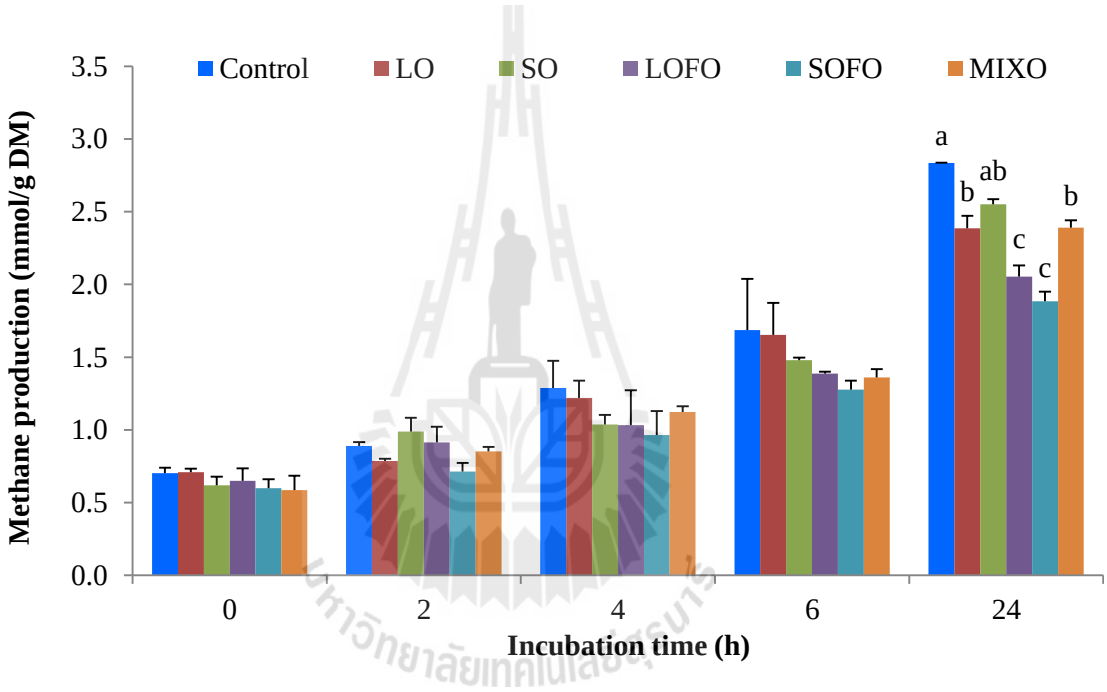

Figure 4.2 Methane production changes during the incubation. The standard error of the mean is indicated by the error bars over each point. The statistical significance is indicated by different letters over the columns ($P < 0.05$, $n = 3$).

4.4.2 Volatile fatty acids, nitrogen metabolism, and digestibility

Oil supplementation influenced on VFA production, microbial protein synthesis as well as nutrient digestibility (Table 4.4). The inclusion of SOFO had lower total VFA concentration (3.02 mmol/g DM) than the control (3.99 mmol/g

DM), the significant change ($P < 0.001$, SE = 0.28) of total VFA concentration was only detected at the last 24 h of incubations (Figure 4.3). The molar proportions of acetate and propionate were strongly modified ($P < 0.01$) by SOFO supplementation, whereas butyrate proportion seemed to be less effect by additional oils ($P = 0.078$). Addition of SOFO resulted in decreased acetate proportion (70.46%) but increased propionate proportion (19.11%) compared to those in the control (71.51 and 18.01%, respectively). These accompanied by decreasing of C2 to C3 ratio in the SOFO (3.74) compared to the control (4.03). In this study, combinative oil inclusion had strongly modified MCP, but individual oil inclusion did not. Supplementation of LOFO and SOFO showed 0.48-0.49 g MCP/kg OM decrease relative to the control ($P < 0.05$). Compared to the control moreover, supplementing LOFO and SOFO declined ($P < 0.05$) IVTD, IVOMD, and IVNDFD. Inclusion of MIXO showed intermediate results of ruminal fermentation, microbial protein synthesis, and digestibility.

Table 4.4 Volatile fatty acid production, nitrogen metabolism, and digestibility

Item	Treatment						SEM	*P*-value
	Control	LO	SO	LOFO	SOFO	MIXO		
pH	6.63^{a}	6.61^{ab}	6.61^{ab}	6.61^{ab}	6.60^{b}	6.60^{ab}	0.02	0.029
Volatile fatty acid								
Total (mmol)	2.00^{a}	1.85^{ab}	1.84^{a}	1.66^{ab}	1.51^{b}	1.74^{ab}	0.27	0.014
Total (mmol/g DM)	3.99^{a}	3.71^{ab}	3.68^{a}	3.33^{ab}	3.02^{b}	3.49^{ab}	0.54	0.014
Acetate, C2 (%)	71.51^{a}	71.21^{a}	70.94^{ab}	70.95^{ab}	70.46^{b}	70.86^{ab}	0.66	0.008
Propionate, C3 (%)	18.01^{c}	18.57^{b}	18.88^{ab}	18.83^{ab}	19.11^{a}	19.08^{a}	0.33	<0.001
Butyrate (%)	10.48	10.22	10.18	10.22	10.43	10.06	0.43	0.078
C2/C3 ratio	4.03^{a}	3.87^{b}	3.82^{bc}	3.83^{bc}	3.74^{c}	3.78^{bc}	0.11	<0.001

Table 4.4 Volatile fatty acid production, nitrogen metabolism, and digestibility (conc.)

Item	Treatment						SEM	*P*-value
	Control	LO	SO	LOFO	SOFO	MIXO		
Nitrogen metabolism								
NH_3-N (mg N/dl)	27.01	26.81	26.68	26.71	26.55	26.68	0.64	0.550
MCP (g/kg OM)	12.51^{a}	12.34^{ab}	12.21^{ab}	12.03^{b}	12.02^{b}	12.20^{ab}	0.26	0.018
Digestibility (%)								
IVTD	63.85^{a}	63.67^{ab}	61.75^{ab}	59.76^{b}	59.74^{b}	62.56^{ab}	1.66	0.032
IVOMD	64.83^{a}	63.93^{ab}	62.28^{ab}	62.31^{b}	62.29^{b}	63.20^{ab}	1.33	0.018
IVNDFD	29.19^{a}	28.83^{ab}	25.07^{ab}	21.17^{b}	21.14^{b}	26.68^{ab}	3.26	0.032

[a-b] Means within a row with different superscripts are significantly different at $P < 0.05$ ($n = 6$ for MCP and IVOMD and $n = 3$ for other parameters).

[a-b] Means within a row with different superscripts are significantly different at $P < 0.05$ ($n = 6$ for MCP and IVOMD and $n = 3$ for other parameters).

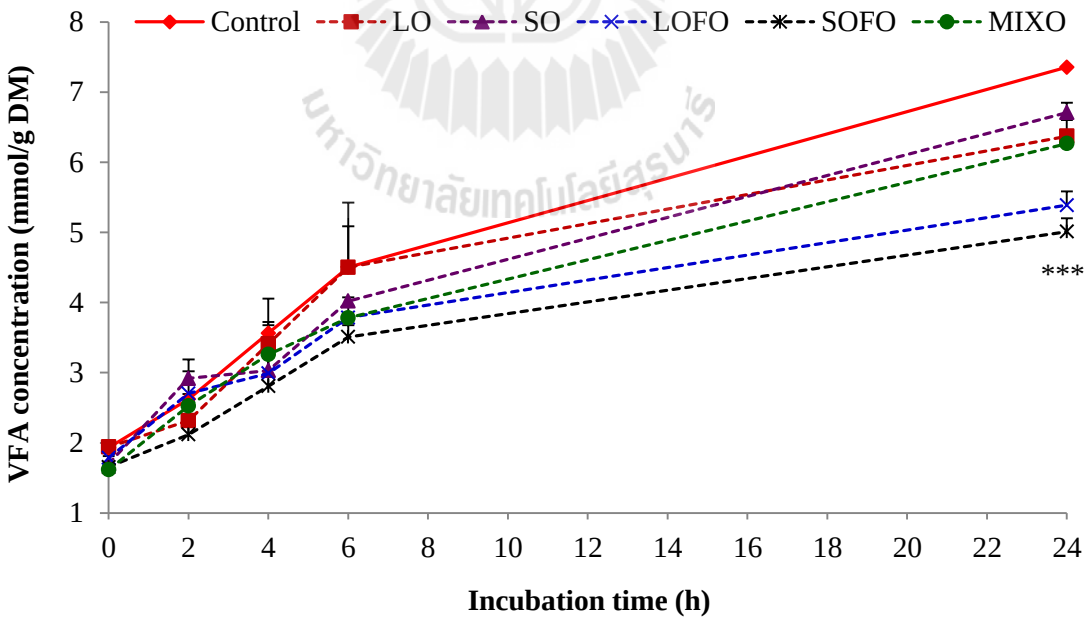

Figure 4.3 Volatile fatty acid concentration changes during the incubation. The standard error of the mean is indicated by the error bars over each point. *** : $P < 0.001$ ($n = 3$).

4.5 Discussion

In this study, we hypothesized that supplementation of oils could enhance the reduction of gas and CH_4 production, without depressing of nutrient digestibility and ruminal fermentation. Some of treatment supported this hypothesis, but others didn't show clear effects.

4.5.1 Gas and methane production

Castagnino et al. (2015) reported that inclusion of soybean oil or linseed oil at 80 g/kg DM strongly decreased CH_4 production, expressed as %/g DM disappeared, through direct effect on rumen methanogens. However, addition of LO or SO alone at 3% DM in the current study didn't show different effect on gas and CH_4 production. This might be due to the lower amounts of linseed and sunflower oils were used in this experiment. As expectation, treatments contained either LO or SO or both combined with FO reduced total gas production after 48 h incubation and protozoa population, whereas only mixture of both SO and FO showed greater reduction of CH_4 production. Similar result was also found in the *in vitro* research of Toral et al. (2009), who reported that supplementation of SO and FO *in vivo* reduced gas production *in vitro*. The greater effects of oil mixtures on gas production and protozoa than those in the control and individual oils might be a result of FO inclusion. Supporting this finding, Fievez et al. (2003) concluded that FO had high potent to mitigate CH_4 production through reduced ruminal methanogenesis. However, Pirondini et al. (2015) didn't see any significant effect on CH_4 production as FO was added at low amount (0.8% DM) into the high or low starch contained-diets. A meta-analysis of Patra (2013) showed that CH_4 depression could be only detected when dietary lipid content more than 5%. In this study

therefore the treatments containing 5.05% EE (2.05% from substrates and 3.00% from added oils) were high enough to observe the depression of gas and CH_4 production. Rumen protozoa are responsible for symbiotic transfers of H_2 with methanogens, which is used to reduce CO_2–CH_4, producing more than 25% of CH_4 production in the rumen (Newbold et al., 1995). The decreased CH_4 production in the SOFO probably happened due to the UFA profile of these oil sources since supplementation of unsaturated oils can increase H_2 consumption by BH, but this process is just 1–2% (Czerkawski, 1972). In addition, UFA may reduce protozoa counts, hence protozoa-associated methanogens, and may be also direct inhibitory effect on the membrane transport of methanogens (Beauchemin et al., 2008). The greater mitigation of ruminal protozoa population, reflecting on reduced methanogens in the treatments of LO or/and SO with FO than those added oils alone seemed the results of synergistic effect of oil combination. This was also supported by Soliva et al. (2004). The observed decrease of ruminal protozoa in this study were a result of oil supplementation rich in UFA. In fact, dietary lipids are almost hydrolyzed in the rumen by microbial lipases, releasing free LCFA that may inhibit activity of ruminal microorganisms. Among of LCFA, UFA are more antimicrobial than SFA (Harfoot and Hazlewood, 1997), BH action therefore can protect microbes from their toxic effect. Microbial toxicity of EPA and DHA has been reported to be greater than those from ALA and LA (Maia et al., 2007). In other words, FO has higher toxicity to rumen microbes than LO and then SO.

4.5.2 Ruminal fermentation and digestibility

Mean pH was significantly lower (6.60) in the SOFO, and reduction was probably large enough to cause a disturbance in bacterial populations due to numerous

decrease occurred on VFA production. The effect of oil supplementation on ruminal VFA content is inconsistent in previous studies. That reduced total VFA concentration in the SOFO was in line with evidence showing low VFA content with the inclusion of SO and FO mixture into rumen incubations (Toral et al., 2009), whereas VFA content showed a higher result with SO supplementation (Razzaghi et al., 2015). AbuGhazaleh and Ishlak (2014) and Pirondini et al. (2015) didn't find any change of total VFA concentration by FO addition. Concerning particular VFA proportions, supplementation of SOFO caused shift of rumen fermentation towards increase of propionate at the expense of acetate with no change in butyrate. The increase in molar proportion of ruminal propionate in the SOFO treatment is a consequence of a decrease in acetate molar proportion rather than an increase in propionate concentration. The unsaturated oil addition has been reported increase in propionate proportion and decrease in acetate proportion (Razzaghi et al., 2015; Shingfield et al., 2011). The reduced acetate proportion by SOFO in this study suggests that the growth of predominant cellulolytic bacteria, such as *Fibrobacter succinogenes*, *Ruminococcus albus*, and *Ruminococcus flavefaciens*, which are considered as acetate-producing bacteria, have been more inhibited by PUFA from these oils (Huws et al., 2010; Maia et al., 2007). The lower production of microbial protein in the LOFO and SOFO suggested that these oil compounds not only affected protozoa counts but also ruminal bacteria which involve microbial protein synthesis. The lower ruminal VFA production could be accompanied by lower digestibility. It was found in this study that the LOFO and SOFO decreased *in vitro* digestibility, especially NDFD, reflecting on the negative influence of double bonds in the UFA present in these treatments. Supplementation of oils rich in UFA such as EPA, DHA, ALA, and LA

can be harmful to microbial membrane in the rumen and cause metabolic disorders, mainly in fibrolytic bacterial populations (Huws et al., 2010; Patra and Yu, 2013; Yang et al., 2009).

4.6 Conclusions

The supplementation of a high-concentrate diet with LOFO, SOFO, and MIXO at 3% DM showed a good strategy to reduce gas and CH_4 production *in vitro*. Both LOFO and SOFO showed disturbances in microbial protein synthesis and nutrient digestibility in the rumen, but only SOFO impaired total VFA concentration. However, rumen function was maintained with MIXO inclusion, revealing that ruminal microorganisms in this treatment were not much disturbed, and they were able to degrade the diet normally. Therefore, to reduce gas and CH_4 production without affecting ruminal fermentation and digestibility, an ideal oil addition would be MIXO at 3% DM. Moreover, to understand deeply the effects of this oil compound on shift of ruminal FA and microbial diversity, further aspects involving ruminal BH and molecular-based studies would be advisable.

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CHAPTER V

MILK RESPONSES AND NET INCOME IN DAIRY COWS FED RUMEN UNDEGRADABLE PROTEIN SOURCES

5.1 Abstract

The aims of this study were to determine the effects of feeding medium-roasted soybean meal (SBM) and corn dried distillers grains with solubles (CDDGS) in dairy cows on milk yield, milk composition, and net income over feed costs. A randomized complete block design experiment was conducted with 24 crossbred multiparous Holstein Friesian dairy cows in early- and mid-lactation. The dietary treatments included : 1) basal diet without feed substitute (Control), 2) 7.17% DM roasted SBM replaced for concentrate (R-SBM), 3) 11.50% DM CDDGS replaced for concentrate (DDGS), and 4) 3.58% DM roasted SBM plus 5.75% DM CDDGS replaced for concentrate (SB-DG). Dry matter intake (DMI) was not affected by feeding high rumen undegradable protein (RUP) sources, but the replacements of roasted SBM and CDDGS for concentrate improved ($P < 0.001$) RUP intake (0.90, 0.86, and 0.88 kg/d corresponding to R-SBM, DDGS, and SB-DG) compared to the control (0.61 kg/d). Feeding roasted SBM and CDDGS had no effect ($P > 0.05$) on milk composition while milk yield was increased ($P < 0.01$) by 3.08 kg/d in the SB-DG group relative to the control group. Net income over feed costs was meaningfully increased ($P < 0.05$) from 5th week post feeding, the SB-DG group reached the greatest net income ($3.48/head/d) while the control group got the lowest value

($2.60/d). In conclusion, the use of CDDGS alone or in combination with medium-roasted SBM as substitute for concentrate in lactating dairy cattle diet improved milk production and net income over feed costs without affecting total DMI and milk composition; however, feeding medium-roasted SBM showed intermediate values in almost parameters.

5.2 Introduction

Milk consumption has rapidly increased, and the annual per capita consumption of milk in developing countries is projected to increase from 52 kg in 2005-2007 to 66 kg in 2030 and 76 kg in 2050 (Alexandratos and Bruinsma, 2012); however, milk production of dairy cows in developing countries in the tropical region is still very low, less than 15 kg/cow/d in average (Aguilar-Pérez et al., 2009; Chingala et al., 2013; Wanapat et al., 2013). Although feeding strategy to improve milk production in dairy cows through protein supplement has been extensively studied, feeding of RDP in excess of microbial utilization contributes to pre-duodenal N losses environmental emission of N which is one of the pollutants contributing to ground water nitrate, surface water eutrophication, and atmospheric ammonia and nitrous oxide emissions (US EPA, 2011). Therefore, manipulation of dietary protein is a critical strategy that will not only abate N emissions but can also be economically feasible for the producer (Hristov et al., 2011). The use of high quality protein sources like SBM may be more efficient if their proteins are mainly protected from ruminal degradation to directly supply AA for host animals' production. Roasting treatment, one of protection methods of SBM protein, which have been showed to be feasible, environmentally friendly, and relatively cheap compared to other methods (such as

plant breeding and chemical treatments) and does not cause any health hazard to animals and consumers (Goelema, 1999). This method can even improve nutritional quality of SBM by destruction of some heat labile anti-metabolites (Masoero et al., 2005) and increase its RUP.

In recent years, due to steadily increasing crude oil prices, the corn ethanol production industry has been expanding rapidly and increased production of CDDGS available for feeding to dairy cattle. In comparison with SBM, CDDGS has more RUP, and their intestinal digestibility is lower (Kleinschmit et al., 2007). Moreover, NRC (2001) indicates that milk yield increase linearly to RUP at the rate of 1.85 kg for each percentage unit increase in RUP, indicating increased efficiency of N utilization use for milk production. Therefore, the aims of this study were to determine milk production, milk composition, feed efficiency, and net income over feed costs in dairy cows fed medium-roasted SBM and CDDGS.

5.3 Materials and methods

5.3.1 Animals, experimental design, and diets

All experimental procedures were conducted following the Ethical Principles and Guidelines for the Use of Animals issued by National Research Council of Thailand. Twenty four crossbred multiparous Holstein Friesian lactating dairy cows in early- (12 cows, 47.67 ± 10.88 days in milk) and mid-lactation (12 cows, 134.67 ± 17.57 days in milk) averaging 10.41 ± 1.27 kg of milk, and 346.54 ± 27.30 kg of body weight, were housed in individual tie stalls and offered daily rations as equal meals at 06:00 and 17:00 h. The animals had free access to water and mineral block, and they had enough space to walk. The animals were assigned to a randomized complete

block design experiment, which lasted for 6 weeks consisting a former 2-week for adjustment, followed by a latter 4-week for sample collection. Cows were blocked by lactating stage and were then randomly assigned to 1 of 4 treatments. The cows were fed a basal diet based on corn silage and 21% CP concentrate (60 : 40, DM basis) at 27.17% RUP in dietary CP. The dietary treatments included : 1) basal diet without feed substitute (Control), 2) 7.17% DM roasted SBM replaced for concentrate (R-SBM), 3) 11.50% DM CDDGS replaced for concentrate (DDGS), and 4) 3.58% DM roasted SBM plus 5.75% DM CDDGS replaced for concentrate (SB-DG). The roasted SBM and CDDGS were substituted for concentrate to meet 35.00% RUP in dietary CP. The daily amounts of roasted SBM and CDDGS were offered together with a 21% CP concentrate before feeding corn silage. The concentrate was formulated to meet nutrient requirements of dairy cows (NRC, 2001) while corn silage was offered *ad libitum* as a main roughage source. Chemical composition of experimental feeds and diets is showed in Tables 5.1 and 5.2. The medium-roasting process to achieve protected SBM was handled as follow : 1) The raw SBM was procured in Thailand and was medium-roasted using a temperature-controlled mixer with a roasting temperature around 100 ± 5°C for 180 min or high-roasted using a tray drying oven with a roasting temperature around 130°C for 30 min. 2) After heating, the roasted SBM was cooled down to room temperature, re-bagged, and kept in the storeroom close the cows' shed for further use. Representative samples (200 g each) of raw SBM, roasted SBM, and other feeds were collected for an *in situ* nylon bag technique to evaluate protein quality.

In situ technology : Three multiparous crossbred Holstein Friesian non-lactating cows fitted with ruminal cannulas were used in the *in situ* technique. The

cows were kept in individual stalls and had free access to water and mineral block. All animals were daily fed 3 kg of 21% CP concentrate while corn silage was offered *ad libitum*. Diet was offered in equal amounts twice daily at 08:00 and 16:00 h for *ad libitum* intake (at least 10% refusals). *In situ* bags made from Dacron cloth (18 × 9 cm, 52 µ pore size) were prepared as described by Shaver et al. (1986). Pre-weighed nylon bags containing approximately 5 g of raw SBM, roasted SBM, CDDGS, and concentrate (ground to pass a 2.5-mm sieve) were immersed in duplicate at each time point in each cow in reverse order at 2, 4, 8, 16, 24, and 48 h while *in situ* bags of corn silage were carried out at 4, 8, 16, 24, 48, and 72. The *in situ* bags were tied to a weighted chain and located in the ventral rumen. Six measurements were made for each test feed at each time point (3 cows × 2 replicates). After incubation, all bags were removed at the same time and hand washed until rinsing is clear. Zero hour bags were soaked in tepid tap water for 30 min prior to hand washing to estimate the rapid disappearance. Bags were then dried at 60°C for 48 h in a forced-air oven. Residual DM of each bag was corrected for DM entry into their corresponding blank bags. Dried residues from six bags at each time point was composited for further analysis of N content to estimate protein degradability. The degradability values were obtained by subjecting nutrient losses at arbitrary of time using NEWAY EXCEL (Chen, 1996).

5.3.2 Sampling, measurements, and chemical analysis

Feeds offered and residual were daily recorded, and feed samples were collected for two consecutive days weekly to calculate daily feed intake. Feed samples were taken and dried at 60°C for 48 h. Feed samples were ground through a 1-mm screen and subjected to proximate analysis. Crude protein was determined by

Kjeldahl method, procedure 928.08 of AOAC (1998). Ether extract was determined by using petroleum ether in a Soxtec System, procedure 948.15 of AOAC (1998). Neutral detergent fiber and ADF were determined using the method described by Van Soest et al. (1991). The NDF analysis used sodium sulfite in the neutral detergent solution. Both NDF and ADF are expressed inclusive of residual ash. Net energy for lactation (NE_L; Mcal/kg) of feeds was calculated according to the equations of NRC (2001). Chemical analysis was expressed on the basis of final DM. Live weights were recorded at the initial and end of the experiment to calculate live weight change. The cows were milked daily at 05:00 and 16:00 h, and milk yields were recorded at each milking time. Milk from both morning and afternoon milking were sampled weekly in 2 consecutive milking days. The morning milk samples were pooled to one composite sample, afternoon samples were also pooled for the meanwhile. The composite milk samples of both milking times were analyzed for milk composition including fat, protein, casein, lactose, solid not fat, total solid, urea, free fatty acid, citric acid, and acidity using MilkoScan™ FT2 infrared automatic analyzer (Foss, Hillerød, Denmark).

5.3.3 Statistical analysis

In situ degradation kinetics

From the *in situ* degradability data at different hours with respect to DM and CP degradability in the rumen, the effective degradability of DM and CP was calculated as described by Woods et al. (2002) using an exponential model of Ørskov and McDonald (1979) described as $p = a + b\ (1 - e^{-ct})$, where p = rumen disappearance (%) at time t (h); a = water soluble fraction (%); b = water insoluble but potentially degradable fraction (%); c = rate at which b is degraded (rate constant)

(% / h); t = incubation time (h); and e = 2.7182 (natural logarithm base). The *ED* of feeds = $a + (b \times c) / (c + k)$ (Ørskov and McDonald, 1979), where *ED* = effective degradability; a, b, and c = constant parameters as described above; and k = rate constant of passage. When calculating effective degradability, rate constant of passage was assumed to be 0.05 per hour which feed flows from rumen to small intestine.

In vivo data

Data for body weight were analyzed using ANOVA procedure of SAS (2002) for a randomized complete block design with the statistical model $Y_{ijk} = \mu + B_i + T_j + \varepsilon_{ijk}$, where Y_{ijk} = the dependent variable, μ = the overall mean, B_i = the block effect, T_j = the treatment effect, and ε_{ijk} = the random residual error.

The ANOVA for averages of feed intakes, milk yield, milk composition, feed efficiency, and net income were analyzed according to a randomized complete block design with the repeated measures (weeks) using PROC MIXED procedure of SAS (2002) with the statistical model $Y_{ijkm} = \mu + B_i + T_j + W_k + (T \times W)_{jk} + \varepsilon_{ijkm}$, where Y_{ijkm} = the dependent variable, μ = the overall mean, B_i = the effect of block, T_j = the effect of treatment, W_k = the effect of week, $(T \times W)_{jk}$ = the effect of interaction between treatment and week, and ε_{ijkm} = the random residual error. The block, treatment, week, and interaction between treatment and week were considered fixed effects, whereas cow within treatment was included as a random effect. Significant differences among treatment means were assessed by Tukey's multiple comparison tests after a significant F-test. Overall differences between treatment means were considered to be significant as $P < 0.05$, meanwhile a tendency toward was declared at $0.10 > P \geq 0.05$. Data are expressed as mean ± SEM, which represents the pooled SEM for the model.

5.3.4 Site and period of the study

The experiment was conducted at Dairy cattle farm and The Center of Scientific and Technological Equipment of Suranaree University of Technology from 26 December 2013 to 05 February 2014. The daily temperature and relative humidity at the farm during the study were 23.90 ± 3.33 and 65.71 ± 5.37, respectively (n = 42).

5.4 Results

5.4.1 Chemical compositions of experimental feeds and diets

The chemical composition of the individual and experimental diets used in this study is presented in the Tables 5.1 and 5.2. Concentrate, MR-SBM, and CDDGS were used as the main sources of protein (> 20% CP; Table 5.1) while corn silage was the main source of fiber because of its great NDF content (63.47%). The substitute of medium roasted SBM and CDDGS for concentrate improved RUP from 3.85% DM in the control diet to 5.23-5.56% DM in the treatment diets (Table 5.2). The proportion of RUP was improved from 27.17% CP in the control diet to 35.00% CP in the treatment diets.

Table 5.1 Chemical composition of individual feeds

Composition (% of DM unless otherwise noted)	**Experimental feeds[1]**			
	Concentrate	**MR-SBM**	**CDDGS**	**Corn silage**
DM (%)	90.49	91.49	88.03	24.02
OM	90.40	92.40	95.07	92.19
CP	21.22	45.34	28.07	9.45
EE	4.13	1.24	8.88	1.49

Table 5.1 Chemical composition of individual feeds (conc.)

Composition (% of DM unless otherwise noted)	Experimental feeds[1]			
	Concentrate	**MR-SBM**	**CDDGS**	**Corn silage**
Ash	9.60	7.60	4.93	7.81
NFC[2]	23.53	24.98	17.94	17.78
NDF	41.52	20.84	40.18	63.47
ADF	25.35	10.89	20.49	42.21
Lignin (sa)	3.68	1.19	4.13	4.53
NDICP	7.04	1.25	7.52	1.59
ADICP	2.47	0.78	5.34	1.23
NE_L (Mcal/kg)[3]	1.80	2.28	2.18	1.37

[1] MR-SBM : medium-roasted soybean meal; CDDGS : corn dried distillers grains with solubles.

[2] Calculated as 100 − (CP + NDF + EE + ash).

[3] Calculated using published formulas of NRC (2001).

Table 5.2 Chemical composition of experimental treatments

Composition (% of DM unless otherwise noted)	Treatment[1]			
	Control	**R-SBM**	**DDGS**	**SB-DG**
DM (%)	50.61	50.68	50.33	50.50
OM	91.47	91.62	92.01	91.81
CP	14.16	15.89	14.94	15.42
RDP[2]	10.31	10.33	9.71	10.02
RUP[2]	3.85	5.56	5.23	5.40
RUP/CP (%)	27.17	35.00	35.00	35.00

Table 5.2 Chemical composition of experimental treatments (conc.)

Composition (% of DM unless otherwise noted)	Treatment[1]			
	Control	**R-SBM**	**DDGS**	**SB-DG**
EE	2.55	2.34	3.09	2.72
Ash	8.53	8.38	7.99	8.19
NFC	20.08	20.18	19.44	19.81
NDF	54.69	53.21	54.54	53.87
ADF	35.47	34.43	34.91	34.67
Lignin (sa)	4.19	4.01	4.24	4.13
NDICP	3.77	3.35	3.83	3.59
ADICP	1.73	1.60	2.06	1.83
NE_L (Mcal/kg)	1.56	1.59	1.58	1.59

[1] Control : basal diet at 27.17% RUP; R-SBM, DDGS, and SB-DG : medium-roasted SBM, CDDGS, and medium-roasted SBM + CDDGS replaced for concentrate to meet 35.00% RUP in total CP.

[2] Calculated from *in situ* bag technique using three fistulated cows (Table 5.3).

5.4.2 Degradation kenetics

The degradation kenetics of DM and CP are given in the Table 5.3. Concentrate, raw SBM, and corn silage were high in effective degradability of DM and CP that resulted in the obviously lower RUP in these feeds (22.60, 24.60, and 34.00, respectively) while RUP was predominant in CDDGS (60.00%). The roasted treatments strongly improved RUP of raw SBM, 63.30 and 70.50% corresponding to medium- and high roasting methods. The readily and potentially degradable fractions ($a + b$) were higher in raw SBM than those in roasted SBM products, this indicated

that roasting treatments might have effects on some physical or chemical characteristics of raw SBM, resulting in reduction of these fractions. The heating methods reduced the readily soluble fraction (*a*) and increased the potentially degradable fraction (*b*), the numerous changes were observed in the HR-SBM than those in the MR-SBM and raw SBM.

Table 5.3 *In situ* degradation kinetics of feeds

Item	Individual feeds[1]					
	Concentrate	**Raw SBM**	**MR-SBM**	**HR-SBM**	**CDDGS**	**Corn silage**
Dry matter						
a	47.34	36.54	28.16	24.84	35.57	28.85
b	44.99	58.93	54.43	54.79	45.22	39.46
c, per h	0.05	0.07	0.03	0.04	0.04	0.04
a + *b*	92.33	95.47	82.59	79.63	80.79	68.31
ED, 0.05/h	70.70	70.50	47.70	47.40	54.50	45.10
RUP (%)	29.30	29.50	52.30	52.60	45.50	44.90
Crude protein						
a	60.08	31.96	20.89	3.97	21.95	56.40
b	36.15	66.55	68.51	83.55	65.55	23.72
c, per h	0.05	0.09	0.02	0.02	0.02	0.03
a + *b*	96.23	98.51	89.40	87.52	87.50	80.12
ED, 0.05/h	77.40	75.40	36.70	29.50	40.00	66.00
RUP (%)	22.60	24.60	63.30	70.50	60.00	34.00

[2] SBM : soybean meal; MR-SBM : medium-roasted SBM; HR-SBM : high-roasted SBM; CDDGS : corn dried distillers grains with solubles.

5.4.3 Intakes and live weight change

The different intakes of concentrate, roasted SBM, and CDDGS were results of roasted SBM and CDDGS replacements for concentrate in the study; however, DMI was non significantly higher in the treatment animals over the control animals with respect to corn silage and total DMI which expressed as kg/d, g/kg LW, and g/kg $LW^{0.75}$ (Table 5.4 and Figure 5.1).

Table 5.4 Intakes and live weight change

Item	Treatment[1]				SEM	*P*-value
	Control	R-SBM	DDGS	SB-DG		
LW (kg)	392.00	404.42	403.08	404.83	25.39	0.792
$LW^{0.75}$ (kg)	88.09	90.10	89.94	90.22	4.23	0.797
DM intake (kg/d)						
Total	16.47	16.82	16.98	17.04	0.69	0.272
Concentrate	6.15^{a}	5.16^{b}	4.53^{d}	4.87^{c}	0.27	<0.001
SBM	0.00^{c}	1.15^{a}	0.00^{c}	0.56^{b}	0.05	<0.001
CDDGS	0.00^{c}	0.00^{c}	1.86^{a}	0.96^{b}	0.05	<0.001
Silage	10.32	10.51	10.59	10.65	0.40	0.208
Concentrate[2] (%)	37.34	37.47	37.62	37.47	0.68	0.744
Roughage[2] (%)	62.66	62.53	62.38	62.53	0.68	0.744
DMI/LW (g/kg)	42.02	41.80	42.19	42.14	2.35	0.991
$DMI/LW^{0.75}$ (g/kg)	186.95	187.04	188.94	188.88	8.57	0.954
CP intake (kg/d)						
Total	2.22^{b}	2.55^{a}	2.44^{a}	2.52^{a}	0.12	<0.001

Table 5.4 Intakes and live weight change (conc.)

Item	Treatment[1]				SEM	*P*-value
	Control	R-SBM	DDGS	SB-DG		
Concentrate	1.30[a]	1.09[b]	0.95[d]	1.02[c]	0.06	<0.001
SBM	0.00[c]	0.52[a]	0.00[c]	0.26[b]	0.02	<0.001
CDDGS	0.00[c]	0.00[c]	0.53[a]	0.27[b]	0.01	<0.001
Silage	0.93	0.94	0.96	0.97	0.05	0.343
CPI/DMI (%)	13.48[d]	15.15[a]	14.40[c]	14.76[b]	0.27	<0.001
RUP intake (kg/d)						
Total	0.61[b]	0.90[a]	0.89[a]	0.88[a]	0.04	<0.001
Concentrate	0.29[a]	0.25[b]	0.22[d]	0.23[c]	0.01	<0.001
SBM	0.00[c]	0.33[a]	0.00[c]	0.16[b]	0.01	<0.001
CDDGS	0.00[c]	0.00[c]	0.31[a]	0.16[b]	0.01	<0.001
Silage	0.31	0.32	0.33	0.33	0.02	0.352
RUPI/CPI (%)	27.35[c]	35.12[b]	35.15[a]	35.13[ab]	0.06	<0.001
RUPI/DMI (g/kg)	36.88[d]	53.21[a]	50.62[c]	51.85[b]	0.93	<0.001
RDPI/DMI (g/kg)	109.37	115.51	110.27	113.04	13.34	0.610
NE_L intake						
Mcal/d	25.66[b]	26.74[a]	26.90[a]	27.04[a]	1.09	0.041
Mcal/kg $LW^{0.75}$	0.29	0.30	0.30	0.30	0.01	0.644
LW change (g/d)	761.90	839.30	815.50	1011.90	313.39	0.556

[1] Control : basal diet at 27.17% RUP; R-SBM, DDGS, and SB-DG : medium-roasted SBM, CDDGS, and medium-roasted SBM + CDDGS replaced for concentrate to meet 35.00% RUP in total CP.

[2] Concentrate and roughage were calculated as percent to total dry matter intake.

[a-d] Means within a row with different superscripts are significantly different at $P < 0.05$, $n = 6$.

The roughage and concentrate proportions were similar ($P > 0.05$) among groups. The replacements of roasted SBM and CDDGS for concentrate in the diet improved ($P < 0.001$) CP intake, accompanied by an increase ($P < 0.001$) of RUP intake, the higher values were in the treatment groups (0.90, 0.89, and 0.88 kg/d corresponding to R-SBM, DDGS, and SB-DG) compared to 0.61 kg/d in the control group. Substitution of either roasted SBM or CDDGS or both for concentrate improved ($P = 0.041$) NE_L intake, accounting for 26.74-27.04 Mcal/d compared to 25.66 Mcal/d in the control diet. No difference ($P > 0.05$) was measured for live weight change among the diets.

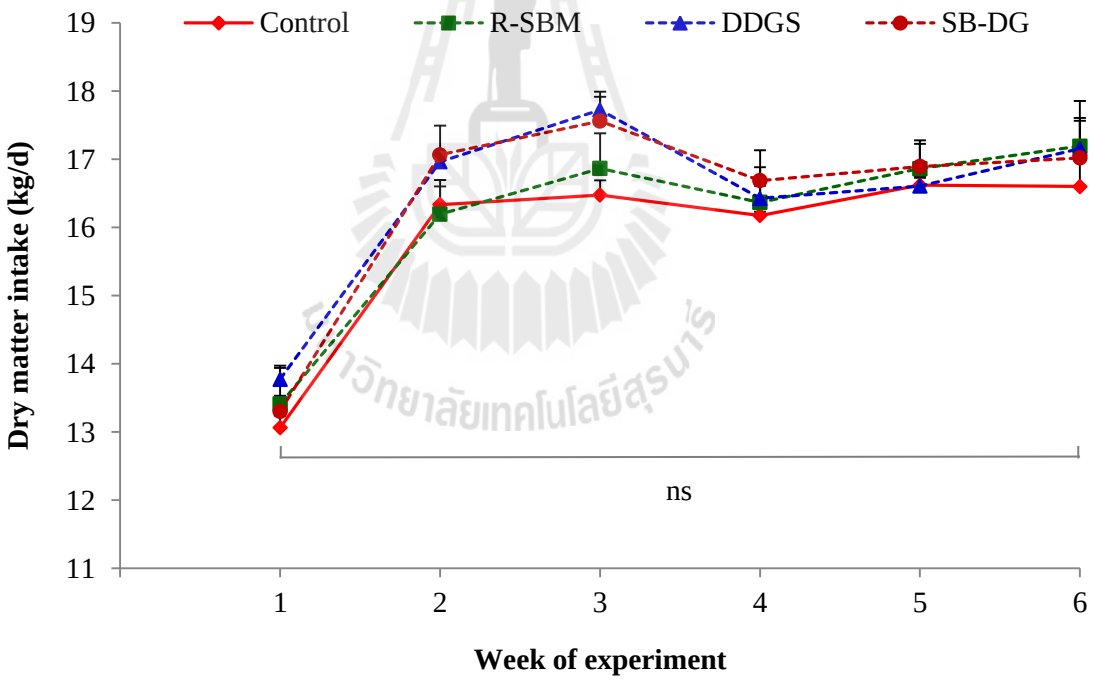

Figure 5.1 Dry matter intake changes during the experiment. The standard error of the mean is indicated by the error bars over each point. ns : not significance ($n = 6$).

5.4.4 Milk yield and composition

The results in the Table 5.5 show that feeding roasted SBM and CDDGS had no effect ($P > 0.05$) on milk composition of dairy cows. However, the significantly higher yields of milk and composition in the treatment groups resulted in improved feed efficiency. Particularly, the yields of milk, 3.5% FCM, and ECM were greater ($P < 0.01$) in the animals fed diet containing CDDGS compared to the animals fed control diet, particular milk yield in average was increased by 3.08 kg/d in the SB-DG group relative to the control group.

Table 5.5 Milk yield and composition

Item	Treatment[1]				SEM	*P*-value
	Control	R-SBM	DDGS	SB-DG		
Yield						
Milk (kg/d)	13.47^{b}	14.73^{ab}	16.21^{a}	16.55^{a}	1.56	0.008
3.5% FCM[2] (kg/d)	13.94^{c}	15.02^{bc}	16.73^{a}	16.26^{ab}	1.70	0.006
ECM[3] (Mcal/d)	13.86^{c}	15.01^{bc}	16.61^{a}	16.35^{ab}	1.54	0.004
Fat (kg/d)	0.51^{c}	0.53^{bc}	0.60^{a}	0.56^{ab}	0.08	0.026
Protein (kg/d)	0.39^{c}	0.44^{bc}	0.47^{ab}	0.49^{a}	0.04	0.003
Casein (kg/d)	0.34^{b}	0.37^{a}	0.40^{a}	0.41^{a}	0.03	0.003
Lactose (kg/d)	0.60^{b}	0.65^{ab}	0.74^{a}	0.75^{a}	0.08	0.019
Solid-not-fat (kg/d)	1.15^{b}	1.28^{ab}	1.42^{a}	1.43^{a}	0.13	0.005
Total solid (kg/d)	1.66^{c}	1.81^{bc}	2.02^{a}	1.99^{ab}	0.18	0.003
Composition						
Fat (%)	3.73	3.66	3.72	3.41	0.56	0.434

Table 5.5 Milk yield and composition (conc.)

Item	Treatment[1]				SEM	*P*-value
	Control	R-SBM	DDGS	SB-DG		
Protein (%)	2.93	2.96	2.90	2.93	0.09	0.584
Casein (%)	2.50	2.55	2.49	2.46	0.16	0.756
Lactose (%)	4.42	4.44	4.54	4.51	0.17	0.380
Solid-not-fat (%)	8.57	8.66	8.76	8.63	0.23	0.416
Total solid (%)	12.30	12.32	12.48	12.04	0.61	0.422
Urea N (mg/dl)	18.62	19.59	16.85	18.69	2.59	0.230
Citric acid (%)	0.16	0.16	0.17	0.16	0.02	0.399
FFA (mekv/l)	0.58	0.49	0.43	0.43	0.18	0.192
Acidity (^{o}TH)	16.94	17.84	17.62	17.23	1.19	0.521
Feed efficiency						
Milk/DMI	0.82^{b}	0.87^{ab}	0.96^{a}	0.98^{a}	0.10	0.036
3.5% FCM/DMI	0.85^{c}	0.89^{bc}	0.99^{a}	0.96^{ab}	0.10	0.021
Milk ECM/DMI	0.84^{c}	0.89^{bc}	0.98^{a}	0.97^{ab}	0.09	0.013
Milk ECM/NE_L intake	0.54^{c}	0.56^{bc}	0.62^{a}	0.61^{ab}	0.07	0.026
Milk N[4]/NI	0.17^{ab}	0.17^{b}	0.19^{a}	0.19^{a}	0.02	0.039

[1] Control : basal diet at 27.17% RUP; R-SBM, DDGS, and SB-DG : medium-roasted SBM, CDDGS, and medium-roasted SBM + CDDGS replaced for concentrate to meet 35.00% RUP in total CP

[2] 3.5% FCM = [0.432 × milk (kg)] + [16.216 × fat (kg)] (Dairy Records Management Systems, 2014).

[3] ECM = [0.327 × milk (kg/d)] + [12.86 × fat (kg/d)] + [7.65 × protein (kg/d)] (Peterson et al., 2012).

[4] Milk N = milk true protein yield/6.38.

[a-c] Means within a row with different superscripts are significantly different at $P < 0.05$, $n = 6$.

Figure 5.2 represents weekly tendency of milk production throughout the experimental period. Substitutes of roasted SBM and CDDGS for concentrate had positive effect ($P < 0.05$) on milk yield after 1st week feeding, the highest milk yield was in the SB-DG contrary to the lowest value in the control, and this trend was remained until the end of the experiment. The meaningfully highest ($P < 0.01$) yields of protein, casein, lactose, and total solid were found in the SB-DG while milk fat yield was greatest ($P < 0.05$) in the DDGS compared to the control.

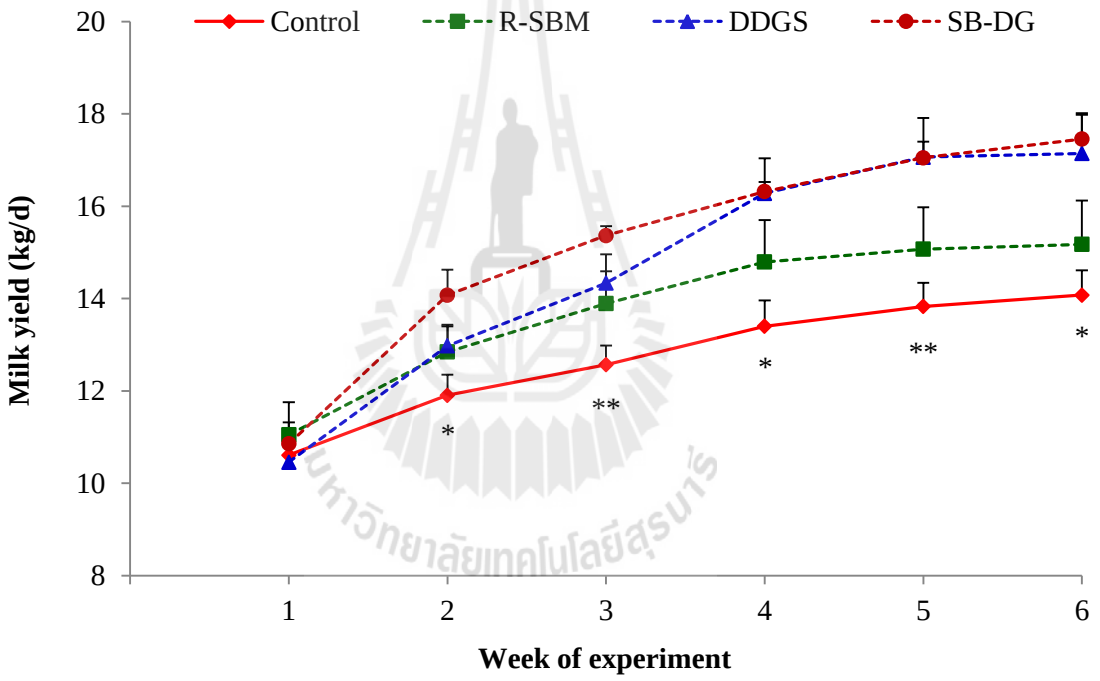

Figure 5.2 Milk yield changes during the experiment. The standard error of the mean is indicated by the error bars over each point. * : $P < 0.05$ and ** : $P < 0.01$ ($n = 6$).

While DMI was remained unchanged over the entire feeding (Figure 5.1) the increased milk yield in the animals fed DDGS and SB-DG diets improved ($P < 0.05$) feed efficiency in terms of milk/DMI, 3.5% FCM/DMI, ECM/DMI, ECM/NE_L intake,

and milk N/N intake compared to those fed the control diet. The effect of feeding roasted SBM and CDDGS on feed efficiency was exposed ($P < 0.05$) after 3rd week feeding, where the SB-DG group reached the greater peak while control group was at the bottom until the end of the experiment (Figure 5.3). In this study, the animals fed R-SBM diet showed the intermediate values in almost parameters.

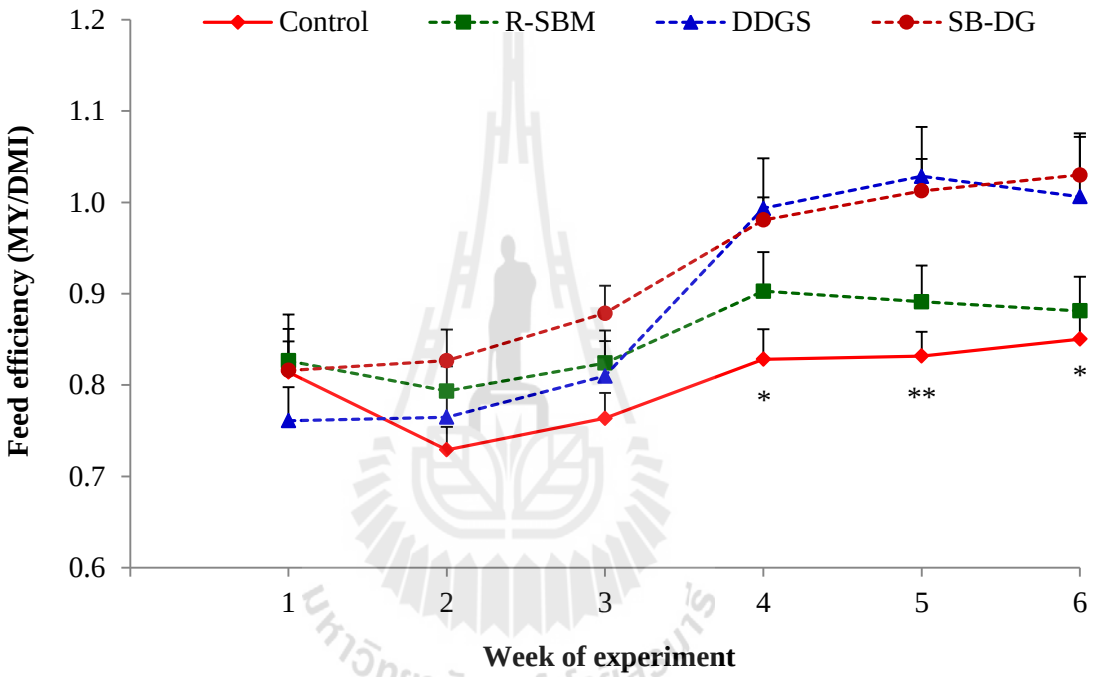

Figure 5.3 Feed efficiency changes during the experiment. The standard error of the mean is indicated by the error bars over each point. * : $P < 0.05$ and ** : $P < 0.01$ ($n = 6$).

5.4.5 Net income

The most important thing of feeding strategy to improve animal performance is how it is beneficial for economic returns. The great finding in this study was that although feed cost was higher ($P < 0.05$) in the animals fed diet containing roasted SBM and CDDGS for the whole feeding period compared to

the control animals, net income over feed costs was still remarkably increased (P < 0.05) in the DDGS and SB-DG groups due to milk sale money was dramatically increased (P < 0.05) in these groups. In particular, the average feed cost (Figure 5.4) of the control group in the whole study was at the bottom ($4.46/head/d) while these were 4.90, 4.90, and $4.95/head/d corresponding to R-SBM, DDGS, and SB-DG groups.

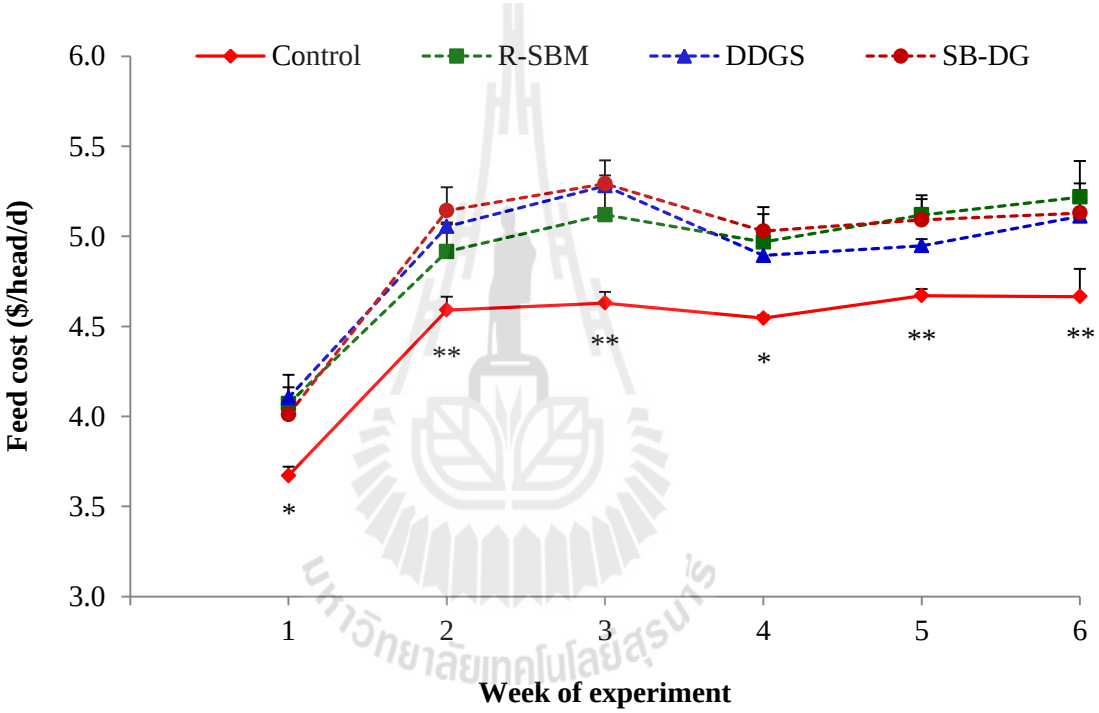

Figure 5.4 Feed cost changes during the experiment. The costs of raw materials as fresh basis were 0.32, 0.62, 0.47, and $0.05/kg corresponding to concentrate, roasted SBM, CDDGS, and corn silage (exchange rate as of 01 January 2014 was 32.45 THB/1 USD). The standard error of the mean is indicated by the error bars over each point. * : P < 0.05 and ** : P < 0.01 (n = 6).

As the result of increased milk yield (Figure 5.2), milk sale money (Figure 5.5) was remarkably improved after 1st week feeding high RUP sources, the highest value in average was observed in the SB-DG group ($8.43/head/d) contrary to the lower value in the control group ($7.06/head/d).

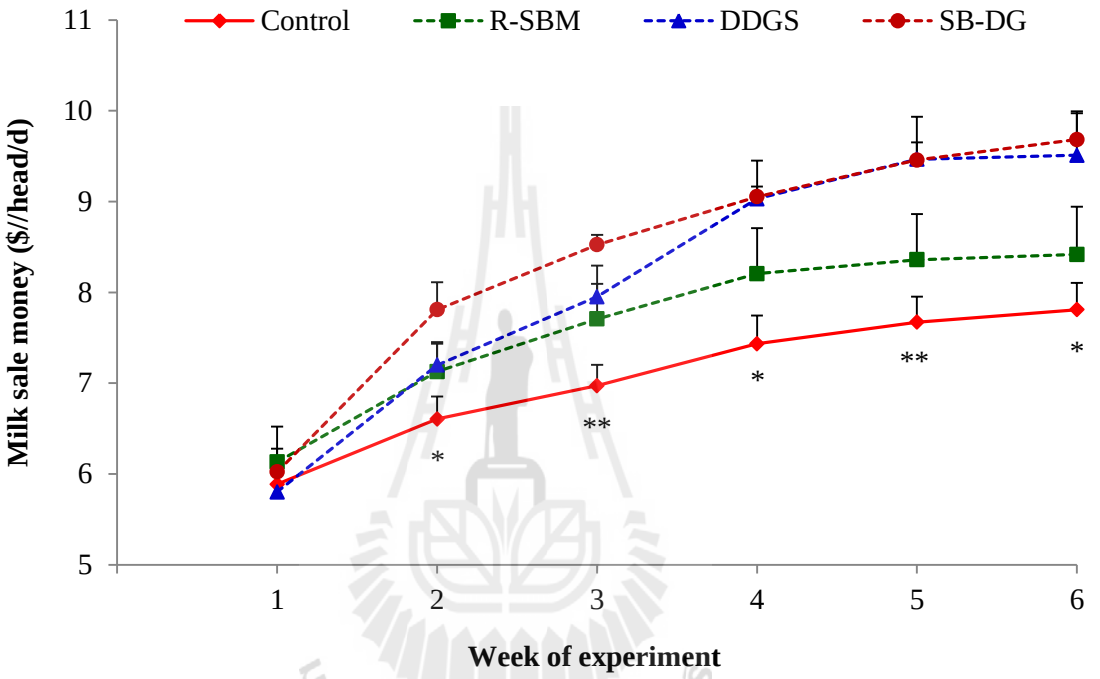

Figure 5.5 Milk sale money changes during the experiment. Milk price was $0.55/kg (exchange rate as of 01 January 2014 was 32.45 THB/1 USD). The standard error of the mean is indicated by the error bars over each point. * : $P < 0.05$ and ** : $P < 0.01$ ($n = 6$).

The final calculation for net income over the feed cost (Figure 5.6) shows that net income was meaningfully increased from 5th week post feeding, the SB-DG group reached the greatest net income in average ($3.48/head/d) while the control group got the lowest value ($2.60/head/d).

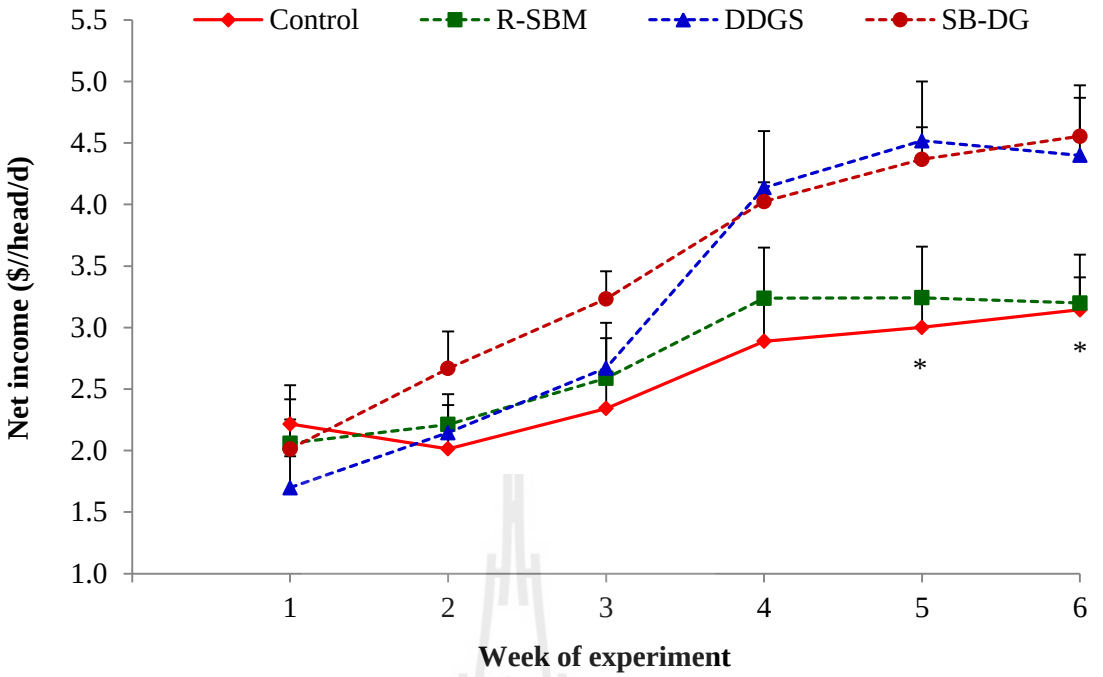

Figure 5.6 Net income changes during the experiment. The exchange rate as of 01 January 2014 was 32.45 THB/1 USD. The standard error of the mean is indicated by the error bars over each point. * : $P < 0.05$ ($n = 6$).

5.5 Discussion

5.5.1 Rumen undegradable protein of roasted SBM and CDDGS

It clearly shows from this study that roasting treatments had strong effect on RUP of SBM. The remarkably increase in RUP in roasted SBM could be attributed to suitable time and temperatures of both heat treatments, 100°C for 180 min (medium-roasted method) and 130°C for 30 min (high-roasted method). Soy protein could be protected to the desired level at these time and temperature points. Fathi Nasri et al. (2008) even suggested the higher temperature between 140°C and 150°C to protect soybean protein, but these high temperatures may cause an unfeasible application in farm or even small factory, more expensive, and over-protection of

protein to compare with lower temperature. In this study, the heat transferred from tray drying oven heater (high-roasted method) to raw soybean materials recorded during heat processing was very variable between the samples located at the top and bottom of feed trays, approximately 20°C variation. Meanwhile, SBM product obtaining from medium-roasted method was homogenized due to this process was heating controlled, and raw SBM was well mixed during the treatment. Therefore, only SBM product achieving from medium-roasted method was chosen to use for the feeding trial. It is clear that roasting method should have an appropriate time and temperature combination for effective bypassing rumen. Roasting of soybean led to increase its bypass protein as reflected by increasing in RUP value and a corresponding decrease in effective protein degradability (Fathi Nasri et al., 2008; Sirohi et al., 2011). The increase in protein bypass ability was also caused the changes in protein fractions due to heat transfer during roasting (Fathi Nasri et al., 2008). Sirohi et al. (2011) reported that roasting method also led to increase undegradable protein fractions for the whole digestive tract, which may be corresponding to an increase in the formation of indigestible N containing compounds (Maillard products) resulting from heat treatment and also indicates decreases in nutritive value, especially protein.

The estimating RUP of CDDGS in the current study was 60.0%. This is greater than the values reported by Schingoethe et al. (2009) and Kelzer et al. (2010), who estimated that the DDGS RUP was 55.0 and 56.3%, respectively. However, the calculated result of CDDGS RUP in this study is still less in comparison with 63.0% in the research of Castillo-Lopez et al. (2013) and 70.3% reported by NRC (2001). These variable results may be due to the different processes of corn ethanol

production industry. Kleinschmit et al. (2007) concluded that the heat applied during the drying process reduces the availability of protein to ruminal degradation, resulting in improved RUP.

5.5.2 Intakes, milk yield, milk composition, and net income

In this study, that no different effect on DMI by dietary RUP supplement was in agreement with some previous studies (Anderson et al., 2006; Sirohi et al., 2011). Experimental diets were formulated to be dietary RDP levels at 103.12, 103.27, 97.14, and 100.21 g/kg DM corresponding to the control, R-SBM, DDGS, and SB-DG diets to meet or exceed the nutrient requirements of the lactating cows based on NRC (2001). According to NRC (2001), dietary RDP drops below 95–105 g/kg DM may depress microbial protein (MCP) production, which may accompany by low DMI, digestibility, and milk production. Moreover, Boucher et al. (2007) reported a maximum response of MCP production when RDP was 100-108 g/kg DM, but MCP production decreased at 116 g/kg DM for the meanwhile, probably due to over-production of ammonia. The current experiment was conducted in winter season with average daily temperature at 23.90 ± 3.33°C. This housing climate might be comportable for lactating dairy cows, which led to improve feed intake (Figure 5.1) and therefore live weight gain.

No significant difference in milk protein concentration among the treatments revealed that dietary treatments had no effect on microbial protein synthesis. The improved yields of milk and composition in this study were in agreement with other researches evaluating either wet or dry distillers grains with solubles (Anderson et al., 2006; Gehman and Kononoff, 2010; Janicek et al., 2008). Recently, Sirohi et al. (2011) reported that roasted SBM inclusion in dairy cattle diet led to improve milk

production without affecting DMI. The increased milk production was strongly observed when roasted SBM and CDDGS mixture added in the diet of dairy cows, but this would depend on the protein quality of feed currently used in the current study. The concentrate for dairy cattle was high in degradable nutrients as shown by very high effective degradability of CP (Table 5.3). Meanwhile, roasted SBM and CDDGS which contain high protein, particularly protected protein would be an ideal ingredient for dairy cattle feeding. The current results showed that while DMI was constant over the whole period of feeding milk yield increase could be attributed to the increase in CP and RUP intakes and improved energy status (Table 5.4). Milk protein yield and milk N : N intake ratio were numerical improved in the current study, perhaps due to the higher protein level supplied for milk protein synthesis in the animals fed higher RUP diets containing CDDGS alone or a mixture of roasted SBM and CDDGS.

The economic effect of feeding strategy to improve animal performance is necessary to determine whether the strategy has the potential to be used in practice. Farmers are more willing to apply strategies when the economic effects are remarkably positive. Further calculation showed that although feed cost was increased up to 10.99% in the SB-DG diet (Figure 5.4), net income over the feed cost was improved by 33.85% in this diet as compared to the control diet (Figure 5.6). The greater net income in the animals fed a mixture of roasted SBM and CDDGS substituted for concentrate might relate to the higher milk production compared to the control animals. Based on the net income over the feed cost, the price which could have paid for roasted SBM and CDDGS was still made as much profit as with commercial concentrate.

5.6 Conclusions

The use of CDDGS alone or in combination with medium-roasted SBM as substitute for concentrate in lactating dairy cattle diet led to improve milk production and net income over feed costs without affecting total DMI and milk composition. However, feeding medium-roasted SBM alone seemed to show intermediate values in almost parameters.

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CHAPTER VI

EFFECTS OF RUMEN UNDEGRADABLE PROTEIN SOURCES ON *IN VITRO* RUMEN FERMENTATION AND GAS PRODUCTION

6.1 Abstract

The objective of this study was to examine the effects of including medium-roasted SBM and CDDGS alone or in combination at the expense of concentrate in diet on *in vitro* gas and CH_4 production, ruminal fermentation patterns, and digestibility. An *in vitro* incubation was conducted as a completely randomized design using rumen fluid obtained from three non-lactating Holstein Friesian dairy cows. The dietary treatments included : 1) basal ration based corn silage and 21% CP concentrate (60 : 40, DM basis) at 27.13% RUP (Control), 2) 7.27% medium-roasted SBM replaced for concentrate (R-SBM), 3) 11.26% CDDGS replaced for concentrate (DDGS), and 4) 3.53% medium roasted SBM plus 5.82% CDDGS replaced for concentrate (SB-DG). Medium roasted-SBM and CDDGS were substituted for concentrate to meet 35.00% RUP in total CP. Cumulative gas production was recorded at 2, 4, 6, 8, 10, 12, 18, 24, 36, and 48 h incubation. *In vitro* digestibilities were determined after 48 h incubation. Ruminal pH, NH_3-N, VFA, and CH_4 values were measured at 0, 2, 4, 6, and 24 h post incubation. The DDGS and SB-DG treatments could reduce ($P < 0.05$) gas production from 8 to 24 h incubation. The R-

SBM, DDGS, and SB-DG reduced ($P < 0.001$) CH_4 production compared with the control. Replacement of roasted SBM and CDDGS alone or both for concentrate didn't modulate mean ruminal pH and concentration of NH_3-N, whereas R-SBM and DDGS reduced ($P < 0.001$) mean total VFA concentration. Compared to the control, DDGS decreased ($P < 0.05$) molar proportion of acetate but increased ($P < 0.05$) molar proportion of propionate, which resulted in declined ($P < 0.05$) ruminal C2/C3 ratio. The DDGS reduced ($P < 0.05$) MCP and IVOMD compared to the control. Results from this study show that partially replacing concentrate in ruminant diet with a mixture of both medium-roasted SBM and CDDGS can help to reduce CH_4 production without negatively affecting rumen fermentation and digestion.

6.2 Introduction

Substitution of dietary protein with high RUP sources to reduce its solubility and therefore its hydrolysis to ruminal ammonia could improve the amount of dietary protein which escapes from ruminal degradation without sacrificing N supplied for growing of ruminal microbes (Nishimuta et al., 1974). The greater amount of protected dietary protein from microbial degradation in the rumen enhances the direct supply of amino acids to the host small intestine and thus efficiency of protein utilization and animal's performance. Roasted SBM at around 130°C for 20-25 min has been used as a good source of RUP in the ruminant (Sirohi et al., 2011). Although this roasting method is environmentally safe and relatively cheap compared to other methods, roasting at high temperature may be unavailability at local feed factories. Therefore, study on a roasting method which is feasible with available instruments at the farms and local feed mills is advisable. Thanh and Suksombat (2015) suggested

that medium-roasted SBM at 100°C for 3 h is a feasible method to improve RUP content in raw SBM. Concerning RUP sources from by-products, incorporation of CDDGS, a by-product of ethanol production by yeast fermentation of grain starch, as an alternative to SBM or concentrate in ruminant diet has been common used as an economically and environmentally viable practice for livestock production (Khiaosa-Ard et al., 2015; Schingoethe et al., 2009; Thanh and Suksombat, 2015). Addition of CDDGS has been showed to improve milk production in dairy cows (Benchaar et al., 2013; Hubbard et al., 2009), whereas other studies (Hünerberg et al., 2014; Hünerberg et al., 2013; Khiaosa-Ard et al., 2015) reported that CDDGS inclusion in cattle diets decreased enteric CH_4 emissions. The effects of RUP on gas and CH_4 production, proportion of nutrient digested by rumen microbes and fermentation patterns may depend on not only RUP source but also fat removal in the products. The aims of this study were to measure the effects of partially replacing concentrate with roasted SBM and CDDGS alone or both on gas and CH_4 production, ruminal fermentation patterns, and digestibility.

6.3 Materials and methods

6.3.1 Experimental design and treatments

This experiment was carried out using a syringe gas production technique at various incubation time intervals. The experimental model was a completely randomized design with the treatments included : 1) basal ration based corn silage and 21% CP concentrate (60 : 40 ratio, DM basis) at 27.13% RUP (Control), 2) 7.27% medium-roasted SBM replaced for concentrate (R-SBM), 3) 11.26% CDDGS replaced for concentrate (DDGS), and 4) 3.53% medium roasted SBM plus 5.82%

CDDGS replaced for concentrate (SB-DG). Medium-roasted SBM and CDDGS were substituted for concentrate to meet 35.00% RUP in total CP.

6.3.2 Substrates and rumen inoculum

Concentrate was produced by SUT feed mill, corn silage and full-fat CDDGS were bought from commercial sources in Thailand, whereas medium-roasted SBM was achieved from a roasting method of raw SBM at 100°C for 180 min using a temperature-controlled mixer at SUT farm. Percentages of RUP of current substrates are presented in Chapter 5 (heading 5.4.2). Corn silage, 21% CP concentrate, roasted SBM, and CDDGS were ground in a Retsch mill (SR200 model, Retsch, Haan, Germany) to pass a 1-mm mesh prior to analyze for chemical composition and *in vitro* gas production measurements. The incubation substrates were mixed at above ratios (w/w, DM basis) and stored until incubation. Chemical characteristics of feeds used in this study are presented in the Table 6.1.

Table 6.1 Ingredients and chemical composition of feeds used in the experiment

Item	Concentrate	Corn silage	Roasted-SBM	CDDGS
Ingredient (% DM)				
Cassava distillers dried meal	32.00	-	-	-
Soybean meal	20.00	-	-	-
CDDGS	17.50	-	-	-
Rice bran	10.00	-	-	-
Wheat bran	10.00	-	-	-
Molasses	8.00	-	-	-
Mineral and vitamin mix	2.50	-	-	-

Table 6.1 Ingredients and chemical composition of feeds used in the experiment (conc.)

Item	Concentrate	Corn silage	Roasted-SBM	CDDGS
Chemical composition (% DM)				
DM (%)	90.53	24.03	91.50	88.08
OM	90.35	92.30	92.43	93.55
CP	21.15	9.46	44.51	28.45
EE	4.03	1.52	1.26	9.01
Ash	9.65	7.70	7.57	6.45
NFC[1]	24.32	16.99	25.97	16.08
NDF	40.85	64.33	20.69	40.01
ADF	25.34	43.01	10.76	20.12
Lignin	3.72	4.64	1.22	4.15

[1] Calculated as 100 − (CP + NDF + EE + ash).

Rumen contents were obtained before the morning feeding from three fistulated non-lactating Holstein Friesian dairy cows (approximately 500 kg) fed at maintenance diet based on corn silage and 21% CP concentrate (R : C 70 : 30, w/w on DM basis). The animals were fed twice daily at 08:30 and 17:00 for 1-week period before taking the rumen contents. The 1,000 ml rumen liquor obtaining from donor cows were transported in three thermos flasks to the *in vitro* laboratory within 10 min. The rumen fluid was filtered through 2 layers of cheesecloth into pre-warmed thermos flasks to retain small particles.

6.3.3 Medium preparation

Medium preparation was similar to which in the Chapter 4 (heading 4.3.3).

6.3.4 *In vitro* fermentation

Substrates were weighed to 500 mg of DM into 100-ml glass test syringes whereas three blank syringes for gas production were not added any substrate. Under continuous CO_2 flushing, the filtrated rumen fluid was mixed (1 : 4, v/v) with pre-warmed (39°C) medium and then introduced (50 ml of rumen fluid and medium mixture) into gastight glass syringes. The lower end of syringes was closed afterward, and the syringes were incubated in a water bath at 39°C for 48 h. Gas volume produced was recorded at 2, 4, 6, 8, 10, 12, 18, 24, 36, and 48 h incubation.

6.3.5 Sampling, measurements, and chemical analysis

Sampling, measurements, and chemical analysis procedures were similar to Chapter 4 (heading 4.3.5).

6.3.6 Calculations

The equations used to calculate experimental data were similar to Chapter 4 (heading 4.3.6).

6.3.7 Statistical analysis

Data on mean values of CH_4 production, protozoa population, pH, NH_3-N, and VFA were analyzed according to a completely randomized design with the repeated measures (hours) using PROC MIXED procedure of SAS (2002) with the statistical model $Y_{ijk} = \mu + T_i + H_j + (T \times H)_{ij} + \varepsilon_{ijk}$, where Y_{ijk} = the dependent variable, μ = the overall mean, T_i = the fixed effect of treatment, H_j = the fixed effect of incubation time (hour), $(T \times H)_{ij}$ = the fixed effect of interaction between treatment and time, and ε_{ijk} = the random residual error. The replicate within treatment was considered as a random effect.

Data on gas production, MCP, and digestibility were analyzed by ANOVA procedure of SAS (2002) for a completely randomized design with the statistical model $Y_{ij} = \mu + T_i + \varepsilon_{ij}$, where Y_{ij} = the dependent variable, μ = the overall mean, T_i = the treatment effect, and ε_{ij} = the random residual error. Overall differences between treatment means were considered to be significant as $P < 0.05$. Significant differences among treatment means were assessed by Tukey's multiple comparison tests after a significant F-test. Data are expressed as mean ± SEM, which represents the pooled SEM for the model.

6.4 Results

6.4.1 Gas production, methane production, and protozoa

Table 6.2 shows that there was no effect of feed substitution on cumulative gas production at 48 h incubation. However, DDGS and SB-DG treatments could reduce gas production from 8 to 24 h incubation. It ranged from 104.16 to 197.09 ml/g DM in the DDGS and ranged from 103.97 to 193.94 ml/g DM in the DB-DG, which were different ($P < 0.05$) from 111.62 to 201.91 ml/g DM in the control (Figure 6.1). In contrast to gas production, which similar values between the control and high RUP groups were measured, mean CH_4 production was different among the treatments (Table 6.2). The R-SBM, DDGS, and SB-DG reduced ($P < 0.001$) CH_4 production (mmol/g DM) compared with the control by 15.15, 21.97, and 8.33%, respectively. At 2 and 6 h incubations, the decrease ($P < 0.05$) of CH_4 production was only observed between DDGS (0.65 and 8.11 mmol/g DM) and control (0.79 and 1.16 mmol/g DM). However, CH_4 production at 24 h incubation was differed ($P < 0.01$) between RUP groups and control, the lowest value was in the DDGS while the highest value was in

the control (2.40 vs 3.22 mmol/g DM, respectively). Dietary substitution of either roasted SBM or CDDGS or both to concentration reduced ($P < 0.01$) ruminal populations of protozoa. Protozoa counts were lowest in the SB-DG (6.40×10^5 cfu/ml) versus the highest in the control (10.13×10^5 cfu/ml).

Table 6.2 Gas production, CH_4 production, and protozoa

Item	**Treatment**				**SEM**	***P*-value**
	Control	**R-SBM**	**DDGS**	**SB-DG**		
Gas (48 h)						
ml/g DM	239.06	241.56	236.73	238.47	6.88	0.397
mmol	5.34	5.39	5.27	5.32	0.15	0.253
mmol/g DM	10.67	10.78	10.57	10.65	0.31	0.397
Methane						
mmol	0.66^a	0.56^b	0.51^b	0.60^b	0.05	<0.001
mmol/g DM	1.32^a	1.12^b	1.03^b	1.21^b	0.09	<0.001
Protozoa ($\times 10^5$ cfu/ml)	10.13^a	7.13^b	6.87^b	6.40^b	1.64	0.002

[a-b] Means within a row with different superscripts are significantly different at $P < 0.05$ ($n = 12$ for gas production and $n = 3$ for methane production and protozoa).

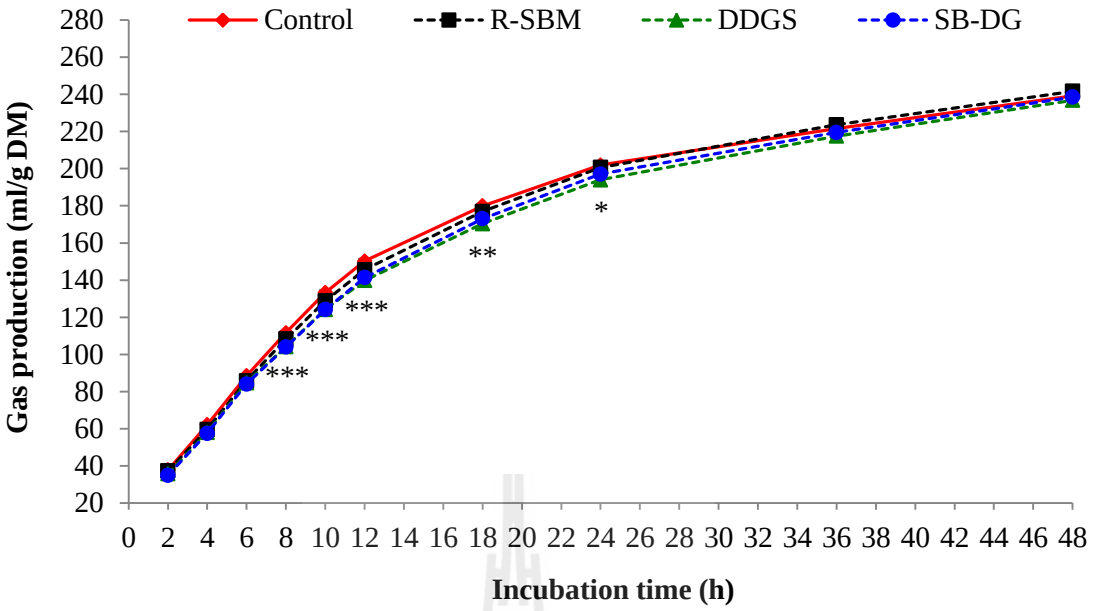

Figure 6.1 Cumulative gas production changes during the incubation. The standard error of the mean is indicated by the error bars over each point. * : $P < 0.05$, ** : $P < 0.01$, and *** : $P < 0.001$ ($n = 12$).

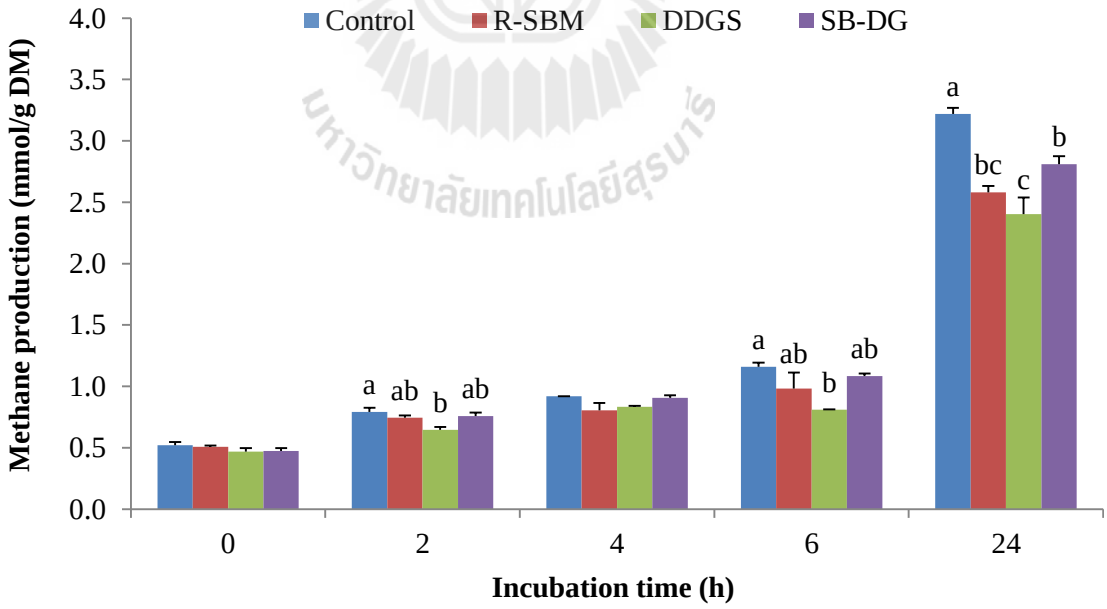

Figure 6.2 Methane production changes during the incubation. The standard error of the mean is indicated by the error bars over each point. The statistical significance is indicated by different letters over the columns ($P < 0.05$, $n = 3$).

6.4.2 Volatile fatty acids, nitrogen metabolism, and digestibility

Replacement of roasted SBM and CDDGS alone or both for concentrate didn't modulate mean ruminal pH and concentration of NH_3-N, whereas concentrate substituted with either roasted SBM or CDDGS affected ($P < 0.001$) mean total VFA concentration (Table 6.3). Mean total VFA concentrations in the R-SBM and DDGS (3.20 and 3.00 mmol/g DM, respectively) were lower than that in the control (3.64 mmol/g DM). The difference ($P < 0.05$) of the total VFA concentration was observed after 2 h incubation with the higher value in the control and lower value in the DDGS, and this different tendency was kept until 24 h incubation (except at 4 h). Concerning individual VFA proportion, compared to the control, DDGS decreased ($P < 0.05$) molar proportion of acetate but increased ($P < 0.05$) molar proportion of propionate, which resulted in declined ($P < 0.05$) ruminal C2/C3 ratio. The molar proportion of butyrate tended ($P = 0.095$) to increase in the DDGS (11.79%) compared to the control (11.07%). That reduced total VFA concentration reflecting in decreased ruminal CH_4 proportion in the DDGS seemed beneficial for environmental aspect; however, it also caused negative effects on microbial protein synthesis in the rumen as well as *in vitro* digestibility of OM. In particular, DDGS reduced ($P < 0.05$) protein synthesis of rumen microbes to 0.28 g/kg OM while reduced *in vitro* digestibility of OM to 1.44% relative to the control. In this study, the replacement of either roasted SBM or CDDGS or both to concentrate didn't show any effect ($P < 0.05$) on *in vitro* digestibilities of true DM and NDF.

Table 6.3 Volatile fatty acid production, nitrogen metabolism, and digestibility

Item	Treatment				SEM	*P*-value
	Control	R-SBM	DDGS	SB-DG		
pH	6.64	6.62	6.61	6.63	0.03	0.381
Volatile fatty acid						
Total (mmol)	1.82[a]	1.60[b]	1.50[b]	1.70[ab]	0.11	<0.001
Total (mmol/g DM)	3.64[a]	3.20[b]	3.00[b]	3.41[ab]	0.22	<0.001
Acetate, C2 (%)	69.61[a]	68.50[a]	67.11[b]	69.03[a]	1.56	0.040
Propionate, C3 (%)	19.32[b]	20.21[b]	21.10[a]	19.94[b]	0.99	0.021
Butyrate (%)	11.07	11.29	11.79	11.03	0.59	0.095
C2/C3 ratio	3.67[a]	3.44[a]	3.24[b]	3.50[a]	0.20	0.010
Nitrogen metabolism						
NH_3-N (mg N/dl)	25.15	24.93	24.25	24.36	1.43	0.498
MCP (g/kg OM)	11.29[a]	11.25[ab]	11.01[b]	11.13[ab]	0.24	0.026
Digestibility (%)						
IVTD	59.11	61.63	59.88	61.48	1.62	0.236
IVOMD	58.48[a]	58.30[ab]	57.04[b]	57.67[ab]	1.23	0.026
IVNDFD	25.56	28.92	26.91	28.23	2.97	0.552

[a-b] Means within a row with different superscripts are significantly different at $P < 0.05$ ($n = 12$ for MCP and IVOMD and $n = 3$ for other parameters).

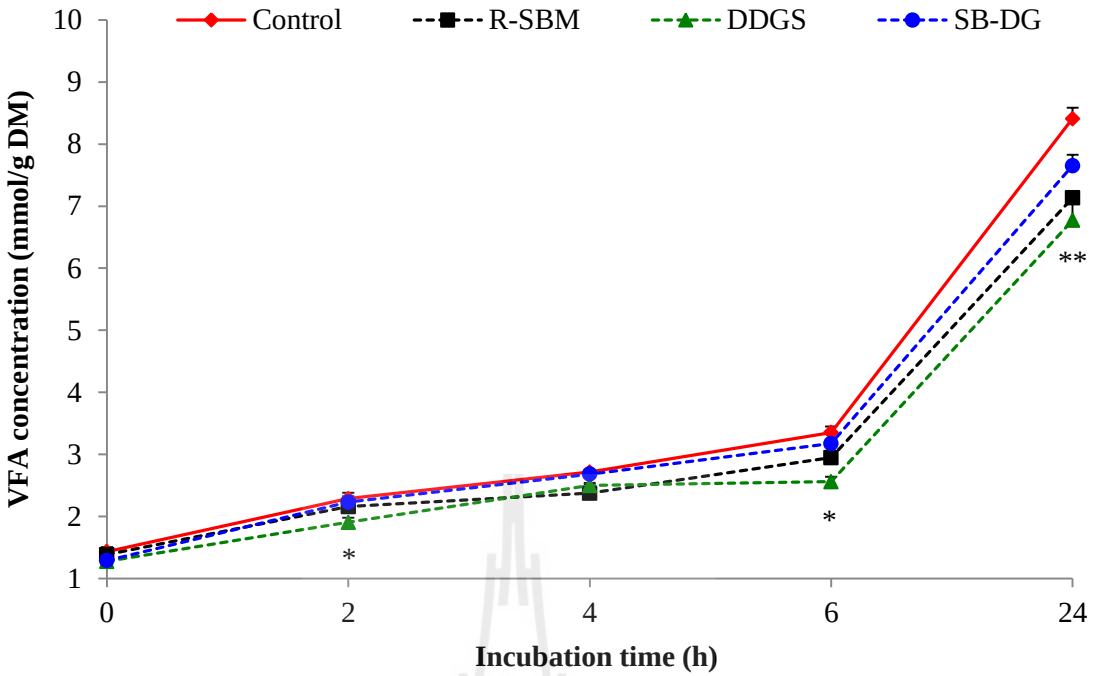

Figure 6.3 Volatile fatty acid concentration changes during the incubation. The standard error of the mean is indicated by the error bars over each point. * : $P < 0.05$ and ** : $P < 0.01$ ($n = 3$).

6.5 Discussion

6.5.1 Gas and methane production

Even though the total gas production after 48 h incubation was similar among the treatments, the lower gas production in the DDGS and SB-DG from 8 to 24 h incubations suggested that nutrient contents in these feeds were fermented at lower rates than those in the control. That unchanged cumulative gas production in this study was in line with Williams et al. (2010), where intact or full fat CDDGS had longer lag time than defatted forms. The delayed time of fermentation in the DDGS in this study was likely due to residue of free fatty acids inhibiting the growth of rumen microbes. Maczulak et al. (1981) showed detrimental effects of some LCFA on the

growth of 7 rumen bacteria involving fiber degradation. Moreover, substrates could be subjected to lipid coating along with the bacterial hydrolytic enzymes (Jenkins, 1993). Substitute of CDDGS to concentrate or protein feeds in the diet of ruminants often decreases CH_4 production (Benchaar et al., 2013; Hünerberg et al., 2014; Hünerberg et al., 2013). Similar result was observed in the recent research of Khiaosa-Ard et al. (2015). The high effect on CH_4 mitigation in the DDGS and SB-DG as compared to the R-SBM and control might relate to the higher unprotected fat content in CDDGS (9.01%) than those in the roasted SBM and concentrate (4.03 and 1.26%, respectively). The unprotected fat from ruminal fermentation depresses CH_4 production primarily by lowering the quantity of organic matter fermented in the rumen (Beauchemin et al., 2008; Johnson and Johnson, 1995). The reduced *in vitro* digestibility of OM might contribute to explain for lower CH_4 emissions from DDGS compared to the control. In addition, fat exerts detrimental effects on rumen protozoa as well as methanogens (Johnson and Johnson, 1995). Protozoa and methanogens exist in a synergistic relationship involving inter-species hydrogen transfer (Finlay et al., 1994). Consequently, a reduction in rumen protozoa counts or activity is usually resulted in a decrease of methanogens, which accompanied by a reduced CH_4 production (Martin et al., 2010). The lower rumen protozoa population in the treatments containing either roasted SBM or CDDGS or both might account for lower CH_4 production in these treatments compared to the control. Similar result on protozoa depression by supplementing protected SBM was also reported by Jolazadeh et al. (2015). The use of full-fat CDDGS in the current study could also increase ruminal propionate, and formation pathway of propionate requires reducing

equivalents, it therefore declined the quantity of free hydrogen available to reduce CO_2 to CH_4 (Janssen, 2010).

6.5.2 Ruminal fermentation and digestibility

Decreasing the starch content of the cattle diet by replacing rapidly fermentable, such as cereal grains or concentrate with less or slower rapidly fermentable feed sources has been proposed as a suitable method to modulate ruminal pH and hence reduce the incidence of sub-acute and acute ruminal acidosis in cattle (Klopfenstein et al., 2008). However, The rumimal pH in this study always remained within a physiological range (6.40-6.91), and that uninfluenced pH by RUP sources was in agreement with some previous studies using different sources and levels of feeds high in RUP (Jolazadeh et al., 2015; Khiaosa-Ard et al., 2015; McCormick et al., 2015). The unchanged pH suggested that rumen microbes were not much turbulent by replacement of RUP sources to concentrate, and they could digest normally the feed nutrients. The higher total VFA concentration in the control as compared to those in the R-SBM and DDGS suggesting that roasted SBM and CDDGS were not rapidly fermented in the rumen. Similar resulted were found in previous studies (Benchaar et al., 2013; Hünerberg et al., 2013), where CDDGS supplementation reduced total VFA concentration as compared to the control. However, recent published studies of other authors (Jolazadeh et al., 2015; Khiaosa-Ard et al., 2015; McCormick et al., 2015) showed that addition of RUP sources from SBM treated with tannins and CDDGS to ruminant diets didn't change total VFA concentration in the rumen. Replacement of CDDGS alone for concentrate reduced formation of acetate and increased formation of propionate, but substitutes of roasted SBM alone or in combination with CDDGS for concentrate did not. Benchaar et al.

(2013) noticed the linear decrease in acetate proportion and increase in propionate proportion in the rumen as the amount of DDGS increased in the diet, whereas Jolazadeh et al. (2015) found no effect on ruminal acetate and propionate by increasing proportion of tannin-treated SBM in the diet of Holstein bulls. The different effect of roasted SBM and full-fat CDDGS on ruminal acetate and propionate proportion might be partially due to the higher fat residue in full-fat CDDGS compared to roasted SBM. The reduction in molar proportion of ruminal acetate in the DDGS could relate to the decrease in ruminal fiber digestion. Fats negatively affected not only rumen protozoa but also various bacteria including fibrolytic bacterial populations and activities of fiber degrading enzymes (Maia et al., 2007; Yang et al., 2009; Zhang et al., 2008). The increase in molar proportion of ruminal propionate in the DDGS treatment is a consequence of a decrease in acetate molar proportion rather than an increase in propionate concentration. The lower IVOMD in the DDGS treatment might be attributable to lower starch content in CDDGS as compared with concentrate. Similarly, Hünerberg et al. (2013) found the lower DMD and OMD in the heifers fed CDDGS diets compared with the control diet. Several experiments reported a decrease in NDF digestibility coupled with a depression in CH_4 production as unprotected lipid was supplemented to ruminant diets (Beauchemin et al., 2009; Chung et al., 2011; Martin et al., 2008). However, replacements of concentrate by CDDGS alone or in combination with roasted SBM in this study did not change *in vitro* NDFD. This could be due to the lower amount of added CDDGS in the current study and hence lower fat content in the diet, or it could be also due to the short duration of the incubation.

6.6 Conclusions

Methane production was reduced when only or both roasted SBM and CDDGS were incorporated in the diet at the expense of concentrate. The current results show that CH_4 production and ruminal fermentation patterns in response to added RUP sources are dependent on not only RUP content but also fat content of RUP-high feed sources. Therefore, method to improve fat extraction from CDDGS could decrease its capacity to depress CH_4 emissions in cattle feeding. However, feeding full-fat CDDGS could cause shift in ruminal fermentation patterns and digestibility. In order to reduce environmental impact and to improve animal performance, replacement of concentrate in cattle diet with a mixture of both CDDGS and roasted SBM should be an ideal approach.

6.7 References

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CHAPTER VII

MILK RESPONSES AND FATTY ACID PROFILES IN DAIRY COWS FED RUMEN UNDEGRADABLE PROTEIN AND OIL MIXTURE

7.1 Abstract

This study aimed to determine the effects of feeding oil mixture rich in PUFA along with RUP on feed efficiency, milk responses, and milk FA profiles in dairy cattle. Twelve crossbred multiparous Holstein Friesian lactating dairy cows at 105.83 ± 18.60 days in milk, 13.06 ± 1.67 kg of milk, and 409.08 ± 23.93 kg body weight were assigned to a completely randomized design. The cows were fed *ad libitum* a basal diet, mainly based on corn silage and concentrate, containing 27.16% RUP in dietary CP. The experimental diets composed of only basal diet (Control) or 3.58% DM roasted SBM plus 5.75% DM CDDGS substituted for concentrate then supplemented with 3% mixture (1 : 1 : 1, w/w) of linseed, sunflower, and fish oils (Treatment). The roasted SBM and CDDGS were substituted for concentrate to meet 35.00% RUP in the diet. Total DMI tended to decrease (P = 0.056) by the treatment, whereas daily intake of RUP was 0.11 kg/d greater (P < 0.05) in the animals fed treatment diet. Milk yield was not affected by treatment (P = 0.651); however, feed efficiency (Milk/DMI) showed a trend for 9.09% improvement (P = 0.06) compared with the control diet. Milk fat yield was 0.14 kg/d decreased (P < 0.001) in the

treatment that resulted in reduced ($P < 0.05$) yield of total solid. The treatment declined ($P < 0.05$) proportions and yields of *de novo* synthesized milk FA, mixed FA, and C18:0. On the contrary, treatment diet improved ($P < 0.001$) proportions and yields of milk preformed FA mainly including *cis*-9,*trans*-11 CLA, ALA, EPA, and DHA. Dietary inclusion of oil mixture increased ($P < 0.001$) the ratios of MUFA to SFA and PUFA to SFA but decreased n-6 to n-3 ratio. In conclusion, feeding oil mixture rich PUFA along with RUP in dairy cows effectively enhanced proportions and yields of milk healthy FA, such as *cis*-9,*trans*-11 CLA, ALA, EPA, and DHA, and improved feed efficiency use for milk production without affecting milk yield.

7.2 Introduction

Bovine milk contains a variety of PUFA, some of which may be beneficial for human health, including *cis*-9,*trans*-11 CLA, ALA, EPA, and DHA (Bhattacharya et al., 2006; Jacobsen et al., 2013). The *cis*-9,*trans*-11 CLA has been showed to inhibit the growth of several human cancer cell lines, decrease the rate of chemically induced tumor development, modify lipoprotein metabolism, and alter immunity in animal models (Shingfield et al., 2008), and keep an role in the prevention or treatment of diseases ranging from cancer to cardiovascular disease in human (Gebauer et al., 2011). The ALA reduces serum low-density lipoprotein cholesterol and incidence of human cardiovascular disease risk (Shingfield et al., 2008). The EPA would exert protective effects against neuropsychiatric disorders, hypertension, and autoimmune diseases (Narayan et al., 2006). In contrast, dietary consumption of SFA induces to increase concentration of low-density lipoprotein cholesterol (Givens, 2010).

Therefore, increasing interest exists in enhancing proportion of above beneficial FA and reducing SFA in milk fat.

Inclusion of SO in dairy animals' diet leads to improve *cis*-9,*trans*-11 CLA (Gómez-Cortés et al., 2011; Mohammed et al., 2011). Linseed oil is the one of common oilseed which provides very high content of ALA, and supplementation of cow diets with linseed resulted in an increase in the milk ALA concentration (Ferlay et al., 2013; Neveu et al., 2014). In the rumen, however, most dietary PUFA are isomerized and then hydrogenated by actions of mixed populations of solid-adherent bacteria, resulting in the large formation of SFA and further accumulation of *cis* and *trans* isomers (Fievez et al., 2007; Jenkins et al., 2008) that reflects to lost the healthy benefit of these PUFA for human. For that reason, minimizing ruminal BH of PUFA is the major challenge in formulating dietary supplements which could improve the postruminal supply of PUFA and therefore in animals' products. An *in vitro* study of Chow et al. (2004) showed that FO could inhibit the final step of BH of LA and ALA, resulting in increased *trans*-11 C18:1, a precursor to synthesize *cis*-9,*trans*-11 CLA in mammary glands.

However, feeding oil sources rich in PUFA in the ruminant diets could cause adverse effects on feed intake and therefore animal performance (Chilliard et al., 2009; Toral et al., 2010). One of among resolutions to enhance ruminant performance is to supply RUP. Feeding RUP to dairy animals has been known to result in a proportionate increase of AA supply to the host animals for production. Some previous studies on dairy cattle and buffaloes reported that feeding RUP increased milk yield by 10-15% (Ghorbani et al., 2007; Shelke and Thakur, 2011; Shelke et al.,

2012). Up to now, there is still lack of information on feeding dairy cows a RUP-rich diet supplemented with oil mixture rich in PUFA.

This study aimed to evaluate the effects of supplementing oil mixture (LO, SO, and FO) in dairy cattle diet high in RUP (roasted SBM and CDDGS) on milk responses, feed efficiency, and milk FA proflies. It was hypothesized that the combined supplement of LO, SO, and FO would result in an increase of healthier milk FA composition, the potential enhancement of CLA and n-3 PUFA, whereas RUP-high diet could alleviate milk yield depression, which causes by supplementing oil mixture.

7.3 Materials and methods

7.3.1 Animals, experimental design, and diets

All experimental procedures were conducted following the Ethical Principles and Guidelines for the Use of Animals issued by National Research Council of Thailand. Twelve crossbred multiparous Holstein Friesian lactating dairy cows in mid-lactation averaging 105.83 ± 18.60 days in milk, 13.06 ± 1.67 kg of milk, and 409.08 ± 23.93 kg body weight, housed in individual tie stalls and offered daily rations as equal meals at 06:00 and 17:00 h. The animals had free access to water and mineral block, and they had enough space to walk. The animals were assigned to a completely randomized design with six replicates per each treatment. The experiment lasted for 6 weeks consisting a former 2-week for adjustment, followed by a latter 4-week for sample collection. The cows were fed *ad libitum* a basal diet, mainly based on corn silage and concentrate, containing 27.16% RUP in dietary CP. The experimental diets composed of only basal diet (Control) or 3.58% DM

medium-roasted SBM plus 5.75% DM CDDGS substituted for concentrate then supplemented with 3% mixture (1 : 1 : 1, w/w) of linseed, sunflower, and fish oils (Treatment). The F : C ratio of total diets was weekly adjusted to achieve 60 : 40 on a DM basis. The roasted SBM and CDDGS were substituted for concentrate to meet 35.00% RUP in the diet. The added oil mixture was daily mixed with roasted SBM, CDDGS, and 21% CP concentrate before feeding to the animals. The concentrate was formulated to meet nutrient requirements of dairy cows (NRC, 2001) while corn silage was offered *ad libitum* as a main roughage source. The chemical compositions of experimental feeds and diets used in the current study are presented in Table 7.1.

7.3.2 Sampling, measurements, and chemical analysis

Feeds offered and residual were daily recorded, and feed samples were collected for two consecutive days weekly to calculate daily feed intake. Feed samples were taken and dried at 60°C for 48 h. At the end of the experimental period, feed and oil samples were pooled and representative samples were taken for further chemical analysis. Samples were ground through a 1-mm screen and subjected to proximate analysis. Crude protein was determined by Kjeldahl mothod, procedure 928.08 of AOAC (1998). Ether extract was determined by petroleum ether in a Soxtec System, procedure 948.15 of AOAC (1998). Neutral detergent fiber and ADF were determined using the method described by Van Soest et al. (1991), adapted for Fiber Analyzer. The NDF analysis used sodium sulfite in the neutral detergent solution. Both NDF and ADF are expressed inclusive of residual ash. The net energy for lactation (NE_L; Mcal/kg) of feeds and oils was calculated according to the equations of NRC (2001). Chemical analysis was

expressed on the basis of final DM. Live weights were recorded at the initial and end of the experiment.

The dairy cows were milked daily at 5:00 and 16:00 h, and milk yields were recorded at each milking time. Milk from both morning and afternoon milking were sampled in 2 consecutive milking days on the last day of each sampling week. The morning milk samples were pooled to one composite sample, whereas afternoon samples were pooled to another composite sample. The different composite milk samples of both milking times were split into 2 portions for further analysis. The former portion was analyzed for milk composition including fat, protein, casein, lactose, solid not fat, total solid, urea, free FA, citric acid, and acidity using MilkoScan™ FT2 infrared automatic analyser (Foss, Hillerød, Denmark). Meanwhile, the latter portion was frozen at –20°C for further analysis of FA using gas chromatography. Fatty acid compositions of milk and feed samples were analyzed following the method described in Chapter 3 (heading 3.3.2).

7.3.3 Calculations

The formulas to calculate dietary FA, FA intake, milk FA, AI, TI, and apparent transfer of FA from diet into milk were same with those in the Chapter 3 (heading 3.3.3).

7.3.4 Statistical analysis

Data for average body weight was analyzed using ANOVA procedure of SAS (2002) for a completely randomized design with the statistical model $Y_{ij} = \mu + T_i + \varepsilon_{ij}$, where Y_{ij} = the dependent variable, μ = the overall mean, T_i = the treatment effect, and ε_{ij} = the random residual error.

Data for averages of feed intakes, milk yield, milk composition, feed efficiency, milk FA proportion and yield, and apparent transfer of FA were conducted according to a completely randomized design with the repeated measures (weeks) using PROC MIXED procedure of SAS (2002) with the statistical model $Y_{ijk} = \mu + T_i + W_j + (T \times W)_{ij} + \varepsilon_{ijk}$, where Y_{ijk} = the dependent variable, μ = the overall mean, T_i = the effect of treatment, W_j = the effect of week, $(T \times W)_{ij}$ = the effect of interaction between treatment and week, and ε_{ijk} = the random residual error. The treatment, week, and interaction between treatment and week were considered as fixed effects, whereas cow within treatment was included as a random effect. Overall statistical differences between treatment means were considered to be significant as $P < 0.05$, meanwhile a tendency toward was declared at $0.10 > P \geq 0.05$. Significant differences among treatment means were assessed by Tukey's studentized range test after a significant F-test. Data are expressed as mean ± SEM, which represents the pooled SEM for the model.

7.3.5 Site and period of the study

The experiment was conducted at Dairy cattle farm and The Center of Scientific and Technological Equipment of Suranaree University of Technology from 16 February 2014 to 29 March 2014. The daily temperature and relative humidity at the farm during the study were 30.99 ± 1.15 and 66.92 ± 3.82, respectively (n = 42).

7.4 Results

7.4.1 Feed and dietary composition

Chemical components including CP, NDF, ADF, and ash were varied between experimental feeds (Table 7.1); however, these components were similar for both

diets (Table 7.2). Dietary EE was low (2.58%) in the control versus high level (5.71%) in the treatment. The treatment contained 35.00% RUP while this was only 27.16% RUP in the control. Measurements of FA composition indicated that concentrate, LO, and FO contained relatively high proportions of SFA, C18:3n-3, and C22:6n-3, whereas *cis*-9,*cis*-12 C18:2 predominated in R-SBM, CDDGS, and corn silage. Medium chain FA including C12:0 and C14:0 were high amounts in the concentrate, whereas trace or even devoid amounts of these FA were detected in other experimental feeds and pure oils. In the control diet, *cis*-9 C18:1 (oleic), *cis*-9,*cis*-12 C18:2 (linoleic), and SFA were the major FA, accounting for 0.59, 0.79, and 1.19 g/100 g DM, respectively. Beside oleic and linoleic acids were predominant corresponding to 1.19 and 1.74 g/100 g DM, total n-3 PUFA including C18:3n-3, C20:5n-3, and C22:6n-3 contributed to 1.09 g/100 g DM in the treatment diet contrary to trace amounts in the control diet (0.04 g/100 g DM).

Table 7.1 Chemical and major FA compositions of experimental feeds

Item	**Experimental feed**						
	Concentrate	**R-SBM**	**CDDGS**	**Corn silage**	**LO**	**SO**	**FO**
Chemical composition (% of DM unless otherwise noted)							
DM (%)	88.97	89.98	88.83	24.16	100	100	100
OM	89.58	91.18	92.14	95.21	-	-	-
CP	21.07	46.10	27.82	9.38	-	-	-
RDP[1]	77.40	36.70	40.00	66.00	-	-	-
RUP[1]	22.60	63.30	60.00	34.00	-	-	-
EE	3.98	1.40	9.05	1.64	100	100	100
Ash	9.27	7.07	4.26	8.33	-	-	-

Table 7.1 Chemical and major FA compositions of experimental feeds (conc.)

Item	Experimental feed						
	Concentrate	**R-SBM**	**CDDGS**	**Corn silage**	**LO**	**SO**	**FO**
NFC[2]	24.18	25.39	19.01	16.68	-	-	-
NDF	41.49	20.04	39.87	63.97	-	-	-
ADF	25.54	11.18	20.60	45.83	-	-	-
Lignin (sa)	3.64	1.30	4.46	5.09	-	-	-
NE_L(Mcal/kg)[3]	1.80	2.31	2.19	1.33	4.39	4.39	4.39
FA composition[4] (g/100 g FA)							
C12:0	19.45	0.00	0.00	0.00	0.01	0.03	0.07
C14:0	14.07	0.00	2.77	2.92	0.08	0.12	3.82
C16:0	14.55	10.57	16.04	16.91	5.61	6.63	21.93
C18:0	4.13	6.82	6.25	2.56	3.23	3.44	6.25
c-9 C18:1	23.31	21.32	23.46	22.00	17.07	27.66	12.59
c-9,*c*-12 C18:2	19.96	56.81	47.15	48.48	16.55	58.40	1.68
C18:3n-3	0.00	4.16	2.01	4.12	56.44	1.63	0.10
C20:5n-3	0.00	0.00	0.00	0.00	0.00	0.00	8.17
C22:6n-3	0.00	0.00	0.00	0.00	0.00	0.00	36.96
SFA	56.15	17.71	27.38	25.40	9.14	10.46	36.70
UFA	43.85	82.29	72.62	74.60	90.86	89.54	63.30
MUFA	23.89	21.32	23.46	22.00	17.21	27.76	13.74
PUFA	19.96	60.97	49.16	52.60	73.46	61.78	49.55
n-3 PUFA	0.00	4.16	2.01	4.12	56.72	1.83	45.40
n-6 PUFA	19.96	56.81	47.15	48.48	16.74	59.94	2.71

[1] Obtained from *in situ* bag technique using three fistulated cows (similar to Table 5.3).

[2] Calculated as 100 − (CP + NDF + EE + ash).

[3] Calculated using published formulas of NRC (2001).

[4] Zero values indicate FA in feed ingredients and diets were <0.01% total FA or undetectable.

Table 7.2 Chemical and major FA compositions of experimental diets

Item	Diet[1]	
	Control	**Treatment**
Chemical composition (% of DM unless otherwise noted)		
DM (%)	50.08	51.52
OM	92.96	93.38
CP	14.06	14.82
RDP	10.24	9.63
RUP	3.82	5.19
RUP/CP (%)	27.16	35.00
EE	2.58	5.71
Ash	8.71	8.08
NFC	19.67	18.82
NDF	54.98	52.57
ADF	37.71	35.86
Lignin (sa)	4.51	4.35
NE_L(Mcal/kg)	1.52	1.64
FA composition[2] (g/100 g DM)		
C12:0	0.31	0.23
C14:0	0.25	0.25
C16:0	0.40	0.76
C18:0	0.09	0.24
c-9 C18:1	0.59	1.19
c-9,*c*-12 C18:2	0.79	1.74

Table 7.2 Chemical and major FA compositions of experimental diets (conc.)

Item	Diet[1]	
	Control	**Treatment**
C18:3n-3	0.04	0.63
C20:5n-3	0.00	0.08
C22:6n-3	0.00	0.37
SFA	1.14	1.62
UFA	1.44	4.08
MUFA	0.60	1.21
PUFA	0.84	2.87
n-3 PUFA	0.04	1.09
n-6 PUFA	0.80	1.78

[1] Control : basal diet at 27.16% RUP in dietary CP; Treatment : 3.24% roasted SBM + 5.82% CDDGS replaced for concentrate to meet 35.00% RUP in dietary CP then supplemented with 3% oil mixture.

[2] Zero values indicate FA in feed ingredients and diets were <0.01% total FA or undetectable.

7.4.2 Intakes, milk yield, milk composition, and feed efficiency

Total DMI tended to decrease ($P = 0.056$) by the treatment. The treatment had no effect ($P > 0.05$) on DMI/LW, CPI, and NE_L intake (Table 7.3). On the other hand, daily intake of RUP was 0.11 kg/d greater ($P < 0.05$) in the animals fed the treatment diet. Oil supplementation increased ($P < 0.001$) intakes of almost FA (except C12:0 and C14:0). As expectably, added oil mixture rich in PUFA resulted in the higher ($P < 0.001$) consumption of several FA specific to theses oil sources including *cis*-9,*cis*-12 C18:2 and n-3 PUFA. Typically, respective intakes of C18:3n-3, C20:5n-3, and

C22:6n-3 were 83.88, 10.98, and 49.84 g/d in the treatment contrary to only 5.43 g/d (C18:3n-3) and even absent amounts of C20:5n-3 and C22:6n-3 in the control diet. Total FA intake was increased ($P < 0.001$) by 110.70% in the treatment.

Table 7.3 Intakes of main components and major fatty acids ($n = 6$)

Item	Diet[1]		SEM	*P*-value
	Control	Treatment		
Live weight (LW, kg)	412.33	405.92	24.39	0.658
Main components				
DMI (kg/d)	13.55	12.11	1.32	0.056
CPI (kg/d)	1.93	1.79	0.20	0.235
RDPI (kg/d)	1.41	1.16	0.14	0.009
RDPI/DMI (g/kg)	103.82	96.05	5.53	0.001
RUPI (kg/d)	0.52	0.63	0.06	0.012
RUPI/CPI (%)	27.00	35.00	0.15	<0.001
RUPI/DMI (g/kg)	38.40	51.71	2.87	<0.001
NE_L intake (Mcal/d)	20.57	19.89	2.06	0.525
Fatty acids[2] (g/d)				
C12:0	43.15	30.97	3.30	<0.001
C14:0	35.06	32.82	2.96	0.136
C16:0	54.57	99.18	6.88	<0.001
C18:0	12.54	31.38	1.93	<0.001
c-9 C18:1	80.72	155.41	10.42	<0.001
c-9,*c*-12 C18:2	108.20	223.81	15.49	<0.001

Table 7.3 Intakes of main components and major fatty acids (n = 6) (conc.)

Item	Diet[1]		SEM	*P*-value
	Control	Treatment		
C18:3n-3	5.43	83.88	4.67	<0.001
C20:5n-3	0.00	10.98	0.62	<0.001
C22:6n-3	0.00	49.84	2.81	<0.001
SFA	158.06	212.34	16.01	<0.001
UFA	195.64	532.89	33.12	<0.001
MUFA	82.01	158.18	10.59	<0.001
PUFA	113.63	374.71	22.68	<0.001
n-3 PUFA	5.43	145.01	8.09	<0.001
n-6 PUFA	108.20	227.49	15.64	<0.001
Total FA	353.70	745.23	48.64	<0.001

[1] Control : basal diet at 27.16% RUP in dietary CP; Treatment : 3.24% roasted SBM + 5.82% CDDGS replaced for concentrate to meet 35.00% RUP in dietary CP then supplemented with 3% oil mixture.

[2] Zero values indicate proportions of FA in feed ingredients were <0.01% total FA or undetectable.

Milk yield was not affected by the treatment (P = 0.651); however, milk fat yield was 0.14 kg/d decreased (P < 0.001) by the treatment that resulted in reduced (P < 0.05) yield of total solid (Table 7.4). In the current study, No influence (P > 0.05) of treatment diet was observed on yields of milk protein, casein, lactose, and solid not fat, but yield of total milk FA was 129.87 g/d lower (P < 0.001) than those in the control diet. Treatment diet depressed (P < 0.001) milk fat concentration by 25.98% and tended to decrease (P = 0.088) milk lactose concentration without affecting milk concentrations of protein, casein, solid not fat, and urea N.

Table 7.4 Milk yield, milk composition, and feed efficiency (n = 6)

Item	Diet[1]		SEM	*P*-value
	Control	Treatment		
Yield				
Milk (kg/d)	13.33	13.03	1.21	0.651
3.5% FCM[2] (kg/d)	13.98	11.60	1.40	0.004
ECM[3] (Mcal/d)	13.77	11.88	1.31	0.013
Fat (kg/d)	0.51	0.37	0.06	<0.001
Protein (kg/d)	0.38	0.38	0.03	0.948
Casein (kg/d)	0.29	0.28	0.03	0.928
Lactose (kg/d)	0.60	0.57	0.06	0.356
Solid-not-fat (kg/d)	1.15	1.07	0.13	0.195
Total solid (kg/d)	1.66	1.44	0.16	0.017
Total fatty acids (g/d)	473.29	343.42	42.09	<0.001
Composition				
Fat (%)	3.81	2.82	0.41	<0.001
Protein (%)	2.84	2.89	0.07	0.145
Casein (%)	2.14	2.18	0.13	0.562
Lactose (%)	4.53	4.40	0.14	0.088
Solid-not-fat (%)	8.67	8.23	0.61	0.111
Total solid (%)	12.48	11.05	0.69	<0.001
Urea N (mg/dl)	16.47	15.69	2.49	0.461
FFA (mekv/l)	0.646	0.859	0.15	0.003
Citric acid (%)	0.165	0.203	0.02	0.003
Acidity (^{o}TH)	16.64	14.30	1.50	0.009

Table 7.4 Milk yield, milk composition, and feed efficiency (n = 6) (conc.)

Item	Diet[1]		SEM	*P*-value
	Control	Treatment		
Fatty acid (%)	3.56	2.63	0.25	<0.001
Feed efficiency				
Milk/DMI	0.99	1.08	0.10	0.060
3.5% FCM/DMI	1.04	0.97	0.12	0.219
Milk ECM/DMI	1.03	0.99	0.12	0.559
Milk N[4]/N intake	0.19	0.21	0.02	0.169

[1] Control : basal diet at 27.16% RUP in dietary CP; Treatment : 3.24% roasted SBM + 5.82% CDDGS replaced for concentrate to meet 35.00% RUP in dietary CP then supplemented with 3% oil mixture.

[2] 3.5% FCM = [0.432 × milk (kg)] + [16.216 × fat (kg)] (Dairy Records Management Systems, 2014).

[3] ECM = [0.327 × milk (kg/d)] + [12.86 × fat (kg/d)] + [7.65 × protein (kg/d)] (Peterson et al., 2012).

[4] Milk N = milk protein yield/6.38.

Further analysis of milk fat concentration and yield over week of feeding (Figure 7.1) showed that depression (P < 0.05) of milk fat concentration in the treatment group occurred after 1-week feeding while this was 2-week post feeding for milk fat yield (P < 0.01). As the result of MFD, milk total solid concentration was declined (P < 0.001) to 11.05% in the treatment contrary to 12.48% in the control. Milk free FA and citric acid concentrations were improved by the treatment which reflected on decreased milk acidity (P < 0.01). Because of the decreased tendency in total DMI (Table 7.3) without reducing milk yield in the treatment diet, feed efficiency use for milk production (Milk/DMI) showed a trend for 9.09% improvement (P = 0.06) compared with the control diet (Table 7.4).

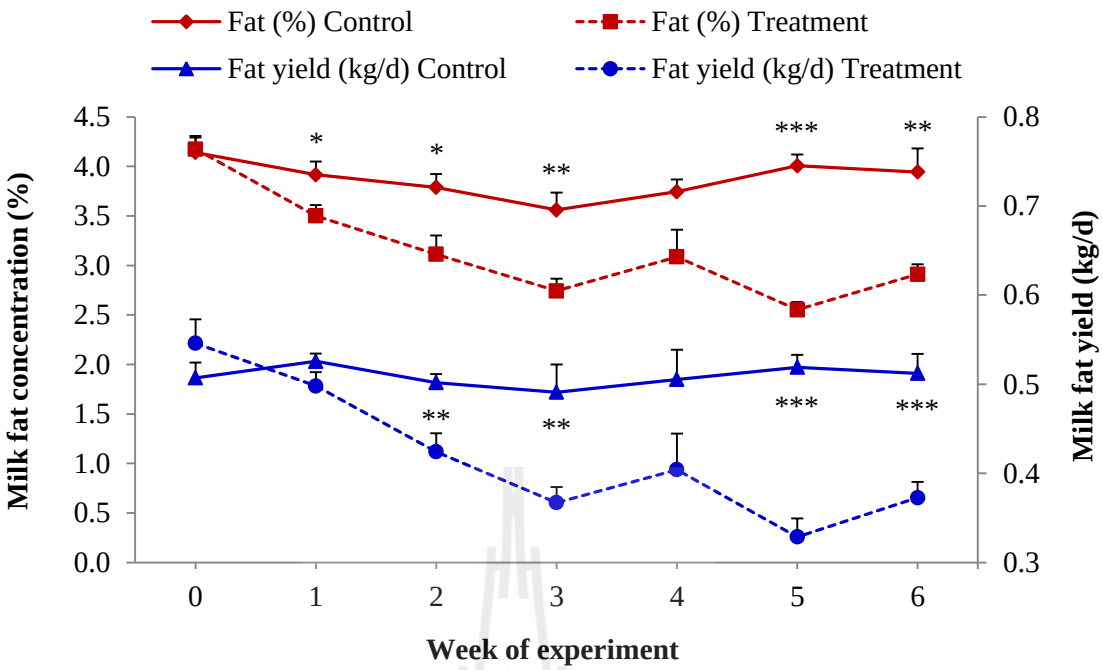

Figure 7.1 Milk fat concentration and yield changes during the experiment. The standard error of the mean is indicated by the error bars over each point. * : $P < 0.05$, ** : $P < 0.01$, and *** : $P < 0.001$ ($n = 6$).

7.4.3 Milk fatty acids

Milk FA composition and yield were strongly modified by oil mixture inclusion (Table 7.5 and 7.6). Remarkably, milk proportions of beneficial FA including *cis*-9,*trans*-11 CLA, C18:3n-3, C20:5n-3, and C22:6n-3 were increased ($P < 0.001$) with the treatment (Table 7.5). The increased proportions of these FA were observed after two-week feeding (Figure 7.2). The oil addition showed significantly higher proportions of other CLA isomers in milk fat, particularly *trans*-10,*cis*-12 CLA (0.13%) versus to trace amount (0.01%) in the control (Table 7.5). The treatment diet improved ($P < 0.05$) yields of all C18 UFA (except *cis*-9 C18:1 and C18:3n-6), C20:5n-3, and C22:6n-3 (Table 7.5).

Table 7.5 Milk composition (g/100 g FA) and yield (g/d) of individual fatty acids (n = 6)

Fatty acid[1]	FA composition		SEM	*P*-value	FA yield		SEM	*P*-value
	Control[2]	Treatment[2]			Control	Treatment		
C4:0	2.63	2.00	0.50	0.010	12.48	6.86	2.58	<0.001
C6:0	1.90	1.03	0.34	<0.001	9.01	3.54	1.75	<0.001
C8:0	1.18	0.56	0.20	<0.001	5.58	1.92	0.99	<0.001
C10:0	2.62	1.23	0.42	<0.001	12.40	4.22	2.00	<0.001
C11:0	0.33	0.13	0.06	<0.001	1.55	0.43	0.28	<0.001
C12:0	5.69	3.47	0.41	<0.001	26.94	11.90	2.58	<0.001
C13:0	0.20	0.13	0.03	<0.001	0.96	0.44	0.13	<0.001
C14:0	13.40	9.56	0.77	<0.001	63.43	32.72	5.56	<0.001
c-9 C14:1	1.16	1.11	0.27	0.657	5.47	3.80	1.10	0.006
C15:0	1.00	0.98	0.07	0.448	4.73	3.36	0.47	<0.001
c-10 C15.1	0.27	0.21	0.04	0.004	1.27	0.71	0.21	<0.001
C16:0	38.23	31.50	2.43	<0.001	180.63	107.70	13.39	<0.001
c-9 C16:1	2.05	2.58	0.49	0.044	9.70	8.82	2.05	0.404
c-10 C17:1	0.20	0.24	0.04	0.048	0.94	0.82	0.20	0.282
C18:0	8.29	5.73	1.87	0.021	39.33	20.06	8.53	<0.001
C18:1n-9	18.97	34.08	2.59	<0.001	89.95	117.19	17.20	0.015
c-9 C18:1	16.93	16.77	4.15	0.937	80.43	58.72	19.54	0.065
t-9,*t*-12 C18:2	0.09	0.19	0.04	<0.001	0.42	0.65	0.16	0.013
c-9,*c*-12 C18:2	0.69	1.55	0.04	<0.001	3.24	6.36	1.80	0.007
c-9,*t*-11 CLA	0.34	1.88	0.55	<0.001	1.64	6.47	2.11	<0.001
t-10,*c*-12 CLA	0.01	0.13	0.05	<0.001	0.04	0.46	0.20	<0.001
c-9,*c*-11 CLA	0.02	0.05	0.01	<0.001	0.08	0.18	0.03	<0.001

Table 7.5 Milk composition (g/100 g FA) and yield (g/d) of individual fatty acids (n = 6) (conc.)

Fatty acid[1]	FA composition		SEM	*P*-value	FA yield		SEM	*P*-value
	Control[2]	Treatment[2]			Control	Treatment		
t-9,*t*-11 CLA	0.05	0.14	0.05	<0.001	0.25	0.47	0.22	0.021
C18:3n-6	0.01	0.00	0.00	<0.001	0.05	0.02	0.01	<0.001
C18:3n-3	0.12	0.30	0.03	<0.001	0.57	1.02	0.16	<0.001
C20:0	0.15	0.16	0.04	0.536	0.73	0.56	0.16	0.036
c-11 C20:1	0.05	0.19	0.04	<0.001	0.22	0.64	0.16	<0.001
c-11,*c*-14 C20:2	0.02	0.10	0.10	0.041	0.09	0.33	0.30	0.033
C20:3n-6	0.06	0.07	0.02	0.105	0.29	0.26	0.08	0.253
C20:3n-3	0.00	0.01	0.00	<0.001	0.00	0.03	0.01	<0.001
C20:4n-6	0.10	0.12	0.03	0.266	0.48	0.40	0.13	0.236
C22:0	0.06	0.07	0.04	0.584	0.28	0.23	0.13	0.303
c-13 C22:1	0.01	0.02	0.02	0.008	0.03	0.08	0.06	0.035
c-13,*c*-16 C22:2	0.01	0.03	0.02	0.019	0.04	0.11	0.09	0.045
C23:0	0.03	0.07	0.02	<0.001	0.13	0.24	0.07	0.009
C24:0	0.06	0.09	0.02	0.002	0.30	0.32	0.09	0.541
c-15 C24:1	0.01	0.04	0.01	<0.001	0.07	0.14	0.03	<0.001
C20:5n-3	0.00	0.10	0.05	<0.001	0.00	0.35	0.20	<0.001
C22:6n-3	0.00	0.17	0.04	<0.001	0.00	0.57	0.13	<0.001

[1] Zero values indicate proportions of FA in milk fat were <0.01% total FA or undetectable.

[2] Control : basal diet at 27.16% RUP in dietary CP; Treatment : 3.24% roasted SBM + 5.82% CDDGS replaced for concentrate to meet 35.00% RUP in dietary CP then supplemented with 3% oil mixture.

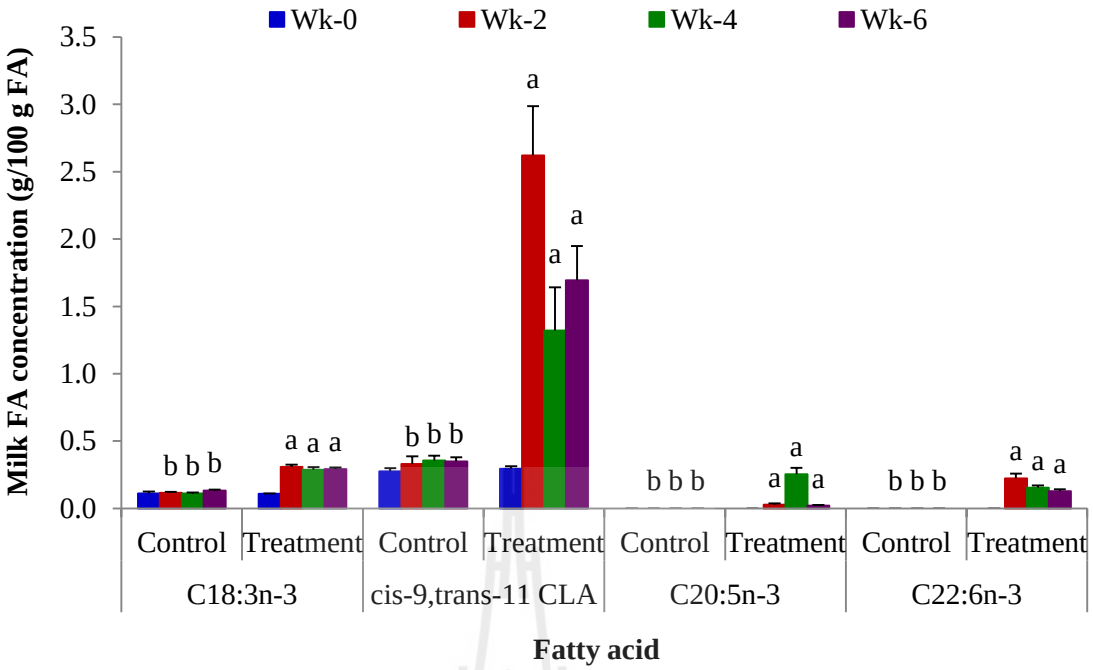

Figure 7.2 Changes of milk fatty acid concentration during the experiment. Standard error of the mean is indicated by error bars over each column. The statistical significance is indicated by letters over the columns (all $P < 0.01$, $n = 6$).

The treatment declined *de novo* synthesized milk FA proportion in average (Table 7.6) and over week of feeding (Figure 7.3) though decrease in milk proportions of C4:0 to *cis*-9 C15:1 (all $P < 0.01$; Table 7.5). The lower proportions in milk fat of mixed FA ($P < 0.01$; Table 7.6) and C18:0 ($P < 0.05$; Table 7.5) were also observed in the treatment diet. On the contrary, the treatment diet improved ($P < 0.001$) milk preformed FA proportion in average (Table 7.6) and over week of feeding (Figure 7.3) because milk proportions of almost C18 UFA (except *cis*-9 C18:1 and C18:3n-6) were numerously enhanced (all $P < 0.001$; Table 7.5). Even though milk fat concentration and yield were strongly depressed, similar to the results for milk FA on a concentration basis, the yields of milk *de novo* synthesized and mixed FA were decreased ($P < 0.001$) by the treatment diet, but preformed FA did not (Table 7.6).

Table 7.6 Milk composition (g/100 g FA) and yield (g/d) of FA groups and indices (n = 6)

Item	FA composition		SEM	*P*-value	FA yield		SEM	*P*-value
	Control[1]	Treatment[1]			Control	Treatment		
FA groups								
De novo[2]	30.37	20.40	2.43	<0.001	143.80	69.93	15.01	<0.001
Mixed[3]	40.28	34.08	2.68	0.001	190.33	116.52	14.57	<0.001
Preformed[4]	29.35	45.53	3.59	<0.001	139.16	156.97	25.15	0.228
C18 UFA	20.30	38.31	2.46	<0.001	96.23	131.82	18.51	0.006
SFA (S)	75.77	56.70	2.55	<0.001	358.48	194.51	27.79	<0.001
UFA	24.23	43.30	2.55	<0.001	114.81	148.91	20.21	0.012
MUFA (M)	22.71	38.46	2.58	<0.001	107.64	132.22	18.59	0.037
PUFA (P)	1.52	4.84	0.78	<0.001	7.18	16.68	3.53	<0.001
n-6 PUFA	0.95	1.93	0.42	<0.001	4.49	6.69	1.86	0.009
n-3 PUFA	0.12	0.58	0.07	<0.001	0.57	1.97	0.33	<0.001
Total CLA	0.42	2.20	0.59	<0.001	2.00	7.58	2.29	<0.001
Indices								
M/S	0.30	0.68	0.06	<0.001				
P/S	0.02	0.09	0.01	<0.001				
n-6/n-3	8.10	3.44	2.08	<0.001				
AI	4.09	1.70	0.44	<0.001				
TI	4.92	2.11	0.47	<0.001				
Desaturation ratios								
c-9 C14:1/C14:0	0.09	0.12	0.03	0.020				
c-9 C16:1/C16:0	0.05	0.08	0.01	<0.001				
c-9 C18:1/C18:0	2.08	3.05	0.04	<0.001				

[1] Control : basal diet at 27.16% RUP in dietary CP; Treatment : 3.24% roasted SBM + 5.82% CDDGS replaced for concentrate to meet 35.00% RUP in dietary CP then supplemented with 3% oil mixture.

[2] *De novo* FA originate from mammary *de novo* synthesis (<16 carbons).

[3] Mixed FA originate from both mammary *de novo* synthesis and extraction from plasma (C16:0 and *cis*-9 C16:1).

[4] Preformed FA originate from extraction from plasma (>16 carbons).

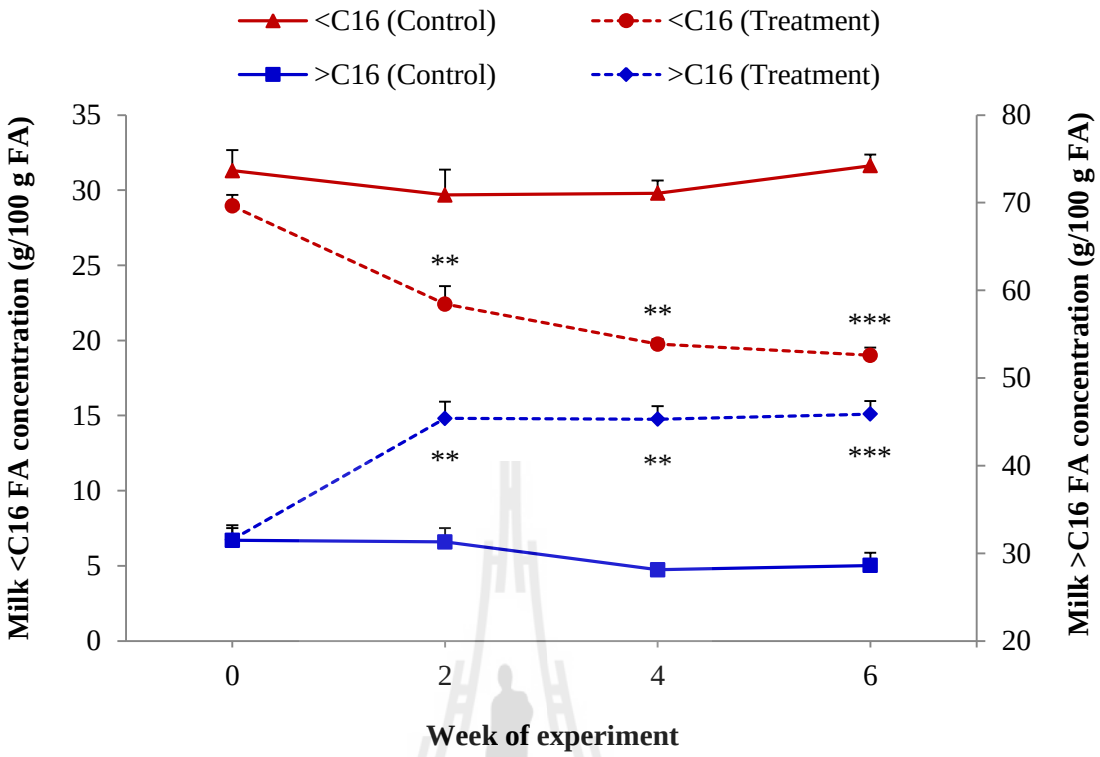

Figure 7.3 Milk <C16 FA and >C16 FA changes during the experiment. The standard error of the mean is indicated by the error bars over each point. ** : $P < 0.01$ and *** : $P < 0.001$ ($n = 6$).

Further calculation of FA groups showed that treatment diet decreased SFA and increased UFA consisting of MUFA and PUFA proportions ($P < 0.001$) in milk fat (Table 7.6). The average daily yields of n-3 PUFA and total CLA were respectively increased ($P < 0.001$) from 0.57 and 2.00 g/d in the control group to 1.97 and 7.58 g/d in the treatment group. The treatment diet increased the ratios of MUFA to SFA (P/S) and of PUFA to SFA (M/S) but decreased n-6 to n-3 ratio ($P < 0.001$). The increased n-3 PUFA ($P < 0.001$) and declined n-6/n-3 ($P < 0.05$) were detected after two-week feeding (Figure 7.4).

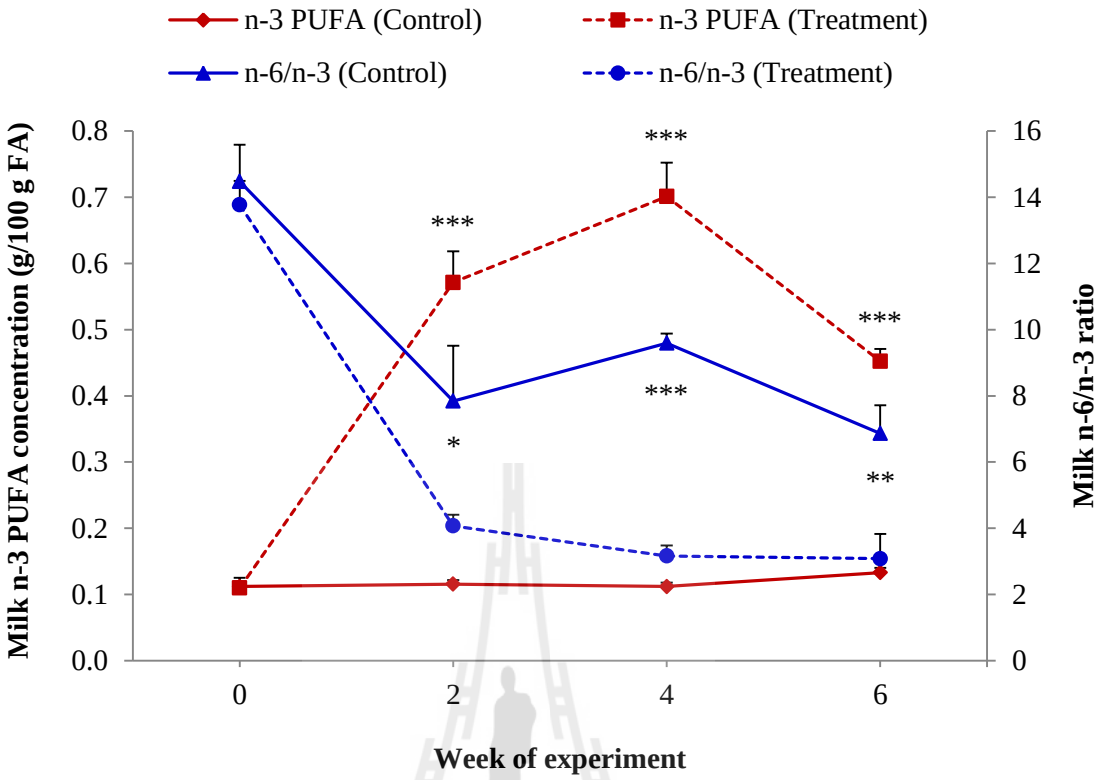

Figure 7.4 Changes of milk n-3 PUFA and n-6/n-3 ratio during the experiment. The standard error of the mean is indicated by the error bars over each point. * : $P < 0.05$, ** : $P < 0.01$, and *** : $P < 0.001$ ($n = 6$).

As the results of decreased proportions of SFA (C12:0-C18:0; Table 7.5) and increased proportions of MUFA and PUFA in the treatment diet, AI and TI in milk fat respectively reduced by 58.44 and 57.11% compared to the control diet ($P < 0.001$; Table 7.6). All desaturation ratios were dramatically improved by the treatment, with 33.33, 60.00, and 46.63% increases for the ratios of *cis*-9 C14:1/C14:0, *cis*-9 C16:1/C16:0, and *cis*-9 C18:1/C18:0, respectively. The transfer of *cis*-9 C18:1, C18:3n-3, and total PUFA from feed into milk fat was lower ($P < 0.01$) for the treatment diet (Table 7.7). However, *cis*-9,*trans*-11 CLA transfer showed a greater tendency ($P = 0.086$) by feeding the treatment.

Table 7.7 Apparent transfer (%) of ingested fatty acids into milk fat (n = 6)

Item	Diet[1]		SEM	*P*-value
	Control	Treatment		
c-9 C18:1	99.44	38.06	14.80	<0.001
C18:2n-6[2]	3.40	2.69	1.21	0.086
c-9,*t*-11 CLA[3]	1.45	2.11	0.79	0.082
C18:3n-3	10.53	1.22	1.06	<0.001
C18:0	312.81	64.20	38.30	<0.001
Total C18 FA	64.49	30.76	6.73	<0.001
C20:5n-3	-	3.24	-	-
C22:6n-3	-	1.15	-	-
UFA	58.74	28.12	6.26	<0.001
PUFA	6.35	4.49	1.40	0.005
n-6 PUFA	4.17	2.96	1.20	0.009
n-3 PUFA	10.53	1.37	1.08	<0.001

[1] Control : basal diet at 27.16% RUP in dietary CP; Treatment : 3.24% roasted SBM + 5.82% CDDGS replaced for concentrate to meet 35.00% RUP in dietary CP then supplemented with 3% oil mixture.

[2] Calculated as 100 × (milk *trans*-9,*trans*-12 C18:2 + milk *cis*-9,*cis*-12 C18:2) / intake of *cis*-9,*cis*-12 C18:2.

[3] Calculated as 100 × milk *cis*-9,*trans*-11 CLA / (intakes of *cis*-9,*cis*-12 C18:2 + intake of C18:3n-3).

7.5 Discussion

7.5.1 Intakes, milk yield, milk composition, and feed efficiency

Decreased total DMI with oil supplementation agrees with the results of Chilliard et al. (2009). Similar results were also found in recent researches (Boerman

and Lock, 2014; Lima et al., 2014). However, Benchaar et al. (2014) observed no effect of oil inclusion on total DMI in dairy cows. Lowered DMI in the current study might relate to the increased concentration of PUFA in the small intestine provided from the rumen bypass of oil. Because the energy content of FA from oil mixture is greater than those of other ingredients in the experimental diets; therefore, intake of DM in the oil-supplemented diet will then decrease in order to maintain a constant of energy ingestion (Lima et al., 2014). Diet supplemented with oil sources rich in unprotected PUFA often causes a decrease in DMI, and the mechanisms of this effect are attributed to the effect on ruminal fermentation, palatability of added fat, and oxidation of fat in the liver (Allen, 2000). Moreover, postruminal delivery of PUFA by feeding oil has been presented to increase plasma concentration of some gut hormones such as cholecystokinin, pancreatic polypeptide (Choi and Palmquist, 1996) and glucagon-like peptide-1 (Relling and Reynolds, 2007) which are responsible for gut motility reduction and postprandial satiety signals (Litherland et al., 2005).

Feeding high RUP diet in this study did not improve milk production; meanwhile, Kurokawa et al. (2013) reported an increase in milk yield as cows were fed 10% CDDGS. The replacement rate of RUP feeds for concentrate in this study might be still low to get the reduce the negative effect on milk performance by supplementing oil mixture at 3% DMI. Depressed milk fat from cows fed the treatment diet in this study was consistent with some previous studies (Angulo et al., 2012; Benchaar et al., 2014). Similar result was also formerly found in the research of Alizadeh et al. (2012), where cows were fed roasted safflower seed and fish oil. This detrimental effect has been known as MFD which often happens in dairy cows supplemented with lipid sources high in PUFA (Huang et al., 2008; Shingfield et al.,

2006). Recently, Ramirez Ramirez et al. (2015) indicated that fat is additive risk factor for MFD in dairy diets containing CDDGS. The MFD is related to an alteration of ruminal BH resulting from the production of different ruminal intermediates that have a negative effect on the gene expression of lipogenic enzymes (Bauman and Griinari, 2001). In general, dietary oil inclusion with a high degree of unsaturation are able to disturb ruminal fermentation and fiber digestibility, leading to lower acetate production and therefore milk fat synthesis (Coppock and Wilks, 1991). Moreover, feeding milk fat-depressing diets to dairy cows showed to inhibit gene expression of mammary lipogenic enzymes, which was associated with increased milk *trans*-10,*cis*-12 CLA (Angulo et al., 2012; Harvatine and Bauman, 2006). In this study, therefore the increased *trans*-10,*cis*-12 CLA proportion in milk fat of the treatment cows caused the strong depression of milk fat concentration and yield. The molecular mechanism for the inhibitory effect of CLA isomers, mainly *trans*-10,*cis*-12 CLA, on MFD in dairy cows is not well understood until now. Nevertheless, Harvatine and Bauman (2006) demonstrated that the sterol response element binding protein transcription factor system, by binding to response elements located in lipogenic enzyme genes, may be a central signaling pathway by which CLA regulates FA synthesis in the mammary gland. Thyroid hormone responsive spot 14, which is down regulated during diet-induced MFD, may also be involved in the molecular mechanism of MFD, possibly as a secondary cellular signal for sterol response element binding protein 1.

Even though total DMI tended to be lower by oil inclusion in the diet, feed efficiency was improved by feeding high RUP diet. This was in agreement with Akbarian et al. (2014) for dairy cows fed roasted SBM. Despite the lower supply of RDP in the treatment, feeding roasted SBM and CDDGS with lower ruminal

degradation resulted in a higher supply of RUP and may be AA digested in the host small intestine (Nasri et al., 2008), accompanied by increased efficiency of milk synthesis. Therefore, increased feed efficiency of the cows fed roasted SBM and CDDGS in this experiment could be attributed to increase consumption of RUP. Moreover, the improved feed efficiency use for milk production is also possibly due to the glucose-sparing effects of FA (Boerman and Lock, 2014). Besides using as precursors for preformed FA in milk fat, FA from dietary oil inclusion can be also used as an energy source for mammary tissues. Bauman and Davis (1975) explained that the decreased *de novo* synthesis of milk FA would decline NADPH requirement from the pentose phosphate pathway, which is produced by glucose oxidation. Thereby, reduced *de novo* synthesis in the mammary gland could be potential to decrease the glucose demand for fat synthesis. Indirectly, as *de novo* synthesis was inhibited or decreased, spared glucose could be used for the osmotic regulator of milk, resulting in improved feed efficiency use for milk production.

7.5.2 Milk fatty acids

Increased C18:1 isomers in milk fat of dairy cows fed oil inclusion rich in PUFA was in agreement with some previous studies (AbuGhazaleh and Holmes, 2007; Benchaar et al., 2014). The lower proportion of C18:0 in milk fat from cows fed LO, SO, and FO could be ascribed to an incomplete BH process in the rumen of either C18:3n-3 or C18:1 to C18:0, resulting in increased milk C18:3n-3, C18:1 isomers, and CLA isomers, particularly *cis*-9,*trans*-11 CLA. The combination of LO and SO with FO resulted in improved milk proportions of *cis*-9,*trans*-11 CLA and C18:3n-3, indicating that a numerous quantity of *trans*-11 C18:1 and *cis*-9,*trans*-11 CLA was able to escape from BH in the rumen, and ruminal BH of C18:3n-3 was diminished.

The proportions of individual FA in milk fat for both control and treatment presented a clear shift in BH pathways associated with oil supplementation. This was demonstrated by the higher concentration of preformed FA (>16 carbons, particularly C18 UFA) compared with *de novo* FA (<16 carbons) and FA originating from mixed sources (16-carbon FA), which is the characteristic response occurred when milk fat synthesis is depressed in the mammary gland of dairy cattle (Harvatine and Bauman, 2011). That the replaced influence of preformed FA for *de novo* synthesized FA in the milk fat was also published in some previous studies (AbuGhazaleh and Holmes, 2007; Boerman and Lock, 2014; He et al., 2012), who showed that the reduction in yield of *de novo* synthesized milk FA was often compensated by an increase in the yield of preformed milk FA when protected oil supplements were fed. In dairy cows, the decrease in *de novo* mammary lipogenesis has been shown to reflect a milk *trans*-10,*cis*-12 CLA coordinated decreases of mRNA abundance for key enzymes involved in the synthesis of milk FA or of activity of lipogenic enzymes in the mammary gland (Baumgard et al., 2002; Gervais et al., 2009).

In the present study, dietary oil mixture inclusion improved milk MUFA and PUFA. This may explain partially by higher consumption and therefore greater supply of MUFA and PUFA in the small intestine of dairy cows. These results were in agreement with previous studies (Lima et al., 2014; Neveu et al., 2014). The dietary inclusion of oil mixture high in PUFA in this study resulted in reduced the AI, TI, and n-6 to n-3 ratio that can counteract the detrimental effect of high SFA and n-6 FA in the milk. Decreased milk n-6 to *n*-3 ratio, atherogenecity and thrombogenicity indices in the present study were supported in previous reports (Caroprese et al., 2010; Huang et al., 2008; Lima et al., 2014). The increased Δ^9-desaturation ratios of milk FA including

cis-9 C14:1 to C14:0, *cis*-9 C16:1 to C16:0, and *cis*-9 C18:1 to C18:0 in the cows fed oil mixture inclusion were similar to the results of Chilliard et al. (2009), indicating that Δ^9-desaturase activity was elevated. This might reflect an adjustment mechanism of the mammary gland to compensate for the simultaneous decrease in short- and medium chain SFA and increase long chain UFA concentrations (Chilliard et al., 2009). That the greater apparent transfer efficiency of selected FA including *cis*-9 C18:1, C18:2n-6, C18:3n-3, n-3 PUFA, and n-6 PUFA from ingested feeds into milk fat for cows fed the control diet agrees with Dewhurst et al. (2003) and Côrtes et al. (2010), where the lower transfer was reported for the diets containing higher UFA concentration.

7.6 Conclusions

Although the treatment diet caused detrimental effects on total DMI and milk fat concentration and yield, dairy cows fed oil mixture rich in PUFA along with RUP had no effect on milk yield and greater feed efficiency use for milk production. Moreover, inclusion of oil mixture high in PUFA into dairy cows' diet effectively enhanced proportions and yields of milk healthy FA including *cis*-9,*trans*-11 CLA, C18:3n-3, C20:5n-3, and C22:6n-3 but decreased milk unhealthy n-6 to n-3 ratio and atherogenecity and thrombogenicity indices suggesting that its consumption benefits for human health.

7.7 References

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CHAPTER VIII

EFFECTS OF OIL MIXTURE ALONG WITH RUMEN UNDEGRADABLE PROTEIN ON *IN VITRO* RUMEN FERMENTATION AND GAS PRODUCTION

8.1 Abstract

This study was designed to investigate the effects of oil addition rich in PUFA along with RUP-high diet on *in vitro* gas and CH_4 production, ruminal fermentation patterns, and digestibility. The study was carried out as a completely randomized design using rumen fluid obtained from three non-lactating Holstein Friesian dairy cows. The diets included : 1) basal ration at 27.13% RUP without addition of oils (Control) and 2) 3.47% medium roasted SBM plus 5.81% CDDGS substituted for concentrate to achieve 35.00% RUP then supplemented with 3% oil mixture (1 : 1 : 1, w/w) from linseed, sunflower, and fish oils (Treatment). Cumulative gas production was recorded at 2, 4, 6, 8, 10, 12, 18, 24, 36, and 48 h incubation. *In vitro* digestibilities were determined after 48 h incubation. Ruminal pH, NH_3-N, VFA, and CH_4 values were measured at 0, 2, 4, 6, and 24 h post incubation. The treatment remarkably reduced ($P < 0.001$) gas production over time points of the incubation. Methane production was not affected by the treatment during the early time points of the incubation (0-6 h), whereas it was strongly reduced ($P < 0.05$) in the treatment at 24 h incubation. Addition of oil and RUP had lower total VFA concentration ($P <$

0.001) and *in vitro* digestibilities of true DM and NDF ($P < 0.001$), whereas no effect ($P > 0.05$) was observed for the molar proportions of individual VFA. Ruminal NH_3-N concentration remained unchanged during the first 6 h of the incubation, but the higher amount ($P < 0.01$) was observed in the treatment at 24 h incubation. In conclusion, addition of linseed, sunflower, and fish oils can mitigate CH_4 production, but it also reduces total VFA concentration and digestibility. The replacement of roasted SBM and CDDGS for concentrate may increase the escape of protein by rumen microbial degradation, but it can be digestible in the intestine.

8.2 Introduction

Consumption of animal products rich in CLA and n-3 PUFA has health benefits in humans, particularly in relation to metabolic syndromes, such as anti-carcinogenic, anti-obesity, anti-diabetic, anti-hypertensive (Koba and Yanagita, 2014), anti-cardiovascular, and anti-inflammatory (Ellulu et al., 2015). Supplementation of oil mixture comprising of linseed, sunflower, and fish oils to dairy cow diet has been reported to improve milk healthy FA, such as CLA, ALA, EPA, and DHA (Thanh and Suksombat, 2015b). Regard to environmental issue, decreasing the potential of global warming by reducing emissions of greenhouse gases (GHG) is a social priority. Among the GHG, CH_4 which produced by specialized bacteria in the rumen has a high capability to cause global warming. A recent review of Hristov et al. (2013) reported that CH_4 yielded from 16 to 26 g per each kg of DMI. Dietary oil supplement is well known as an extensively studied CH_4 mitigation strategy (Hristov et al., 2013; Knapp et al., 2014). However, the limit of using oils to abate CH_4 emission in dairy cattle is that it could cause

negative effects on ruminal fermentation and digestibility (Patra, 2014), reflecting in NEB and therefore milk production (Knapp et al., 2014). Feeding high concentrate diet in dairy cattle could increase total DMI and energy intake, which resulted in increased milk production and reduced NEB (Lawrence et al., 2015). However, the use of high-concentrate diets to maintain high productivity in dairy cows usually causes negative effects on the rumen ecosystem, rumen acidosis, and the economic cost; therefore, feeding rumen undegradable prtotein (RUP)-high diet may represent a proper option in ruminant nutrition in case of low productivity by oil supplementation. Substitution of roasted SBM and CDDGS has been reported to improve milk production in dairy cows (Thanh and Suksombat, 2015a). The addition of dietary oil along with RUP could influence rumen fermentation and digestibility toward the improvement of animal performance and could also mitigate gas and CH_4 production. The objective of this study was to measure the effects of feeding oil mixture (LO, SO, and FO as sources of PUFA) along with roasted SBM and CDDGS mixture (as sources of RUP) on *in vitro* ruminal fermentation pattern, nutrient digestibility, and gas production.

8.3 Materials and methods

8.3.1 Experimental design and diets

This experiment was carried out *in vitro* using a syringe gas production technique at various incubation time intervals. The study was conducted as a completely randomized design with the diets included : 1) basal ration based corn silage and 21% CP concentrate (60 : 40, w/w) at 27.13% RUP without supplementation of oils (Control) and 2) 3.47% medium roasted SBM plus 5.81%

CDDGS replaced for concentrate to obtain 35.00% RUP then supplemented with 3% oil mixture (1 : 1 : 1, w/w) from linseed oil, sunflower oil, and fish oil (Treatment).

8.3.2 Substrates, added oils, and rumen inoculum

Concentrate was produced by SUT feed mill, corn silage and CDDGS were bought from commercial sources in Thailand, whereas roasted SBM was achieved from a roasting method of raw SBM at 100°C for 180 min using a temperature-controlled mixer at SUT farm. Corn silage, 21% CP concentrate, roasted SBM, and CDDGS were ground in a Retsch mill (SR200 model, Retsch, Haan, Germany) to pass a 1-mm mesh prior to analyze for chemical compositions and *in vitro* gas production measurements. The incubation substrates were mixed at above ratios (w/w, DM basis) and stored until incubation. Added lipid including linseed, sunflower, and fish oils were obtained from commercial sources in Thailand. Oils were prepared and added into incubation syringes as an oil-ethanol solution (185 : 15, v/w). Chemical characteristics of feeds and oils used in this study are presented in the Table 8.1.

Table 8.1 Ingredients and chemical composition of feeds used in the experiment

Item	Concentrate	Corn silage	R-SBM	CDDGS	Oils[1]
Ingredient (% DM)					
Cassava distillers dried meal	32.00	-	-	-	-
Soybean meal	20.00	-	-	-	-
CDDGS	17.50	-	-	-	-
Rice bran	10.00	-	-	-	-
Wheat bran	10.00	-	-	-	-
Molasses	8.00	-	-	-	-
Mineral and vitamin mix	2.50	-	-	-	-

Table 8.1 Ingredients and chemical composition of feeds used in the experiment (conc.)

Item	Concentrate	Corn silage	R-SBM	CDDGS	Oils[1]
Chemical composition (% DM)					
DM (%)	90.52	24.04	91.49	88.09	-
OM	90.47	91.68	92.78	95.68	-
CP	21.24	9.33	46.88	27.58	-
RDP[2]	77.40	66.00	36.70	40.00	-
RUP[2]	22.60	34.00	63.30	60.00	-
EE	4.06	1.52	1.43	8.98	100
Ash	9.53	8.32	7.22	4.32	-
NFC[3]	23.05	16.90	24.19	19.56	-
NDF	42.12	63.92	20.28	39.55	-
ADF	25.89	45.88	11.27	20.43	-
Lignin	3.71	5.16	1.31	4.43	-

[1] Included linseed, sunflower, and fish oils.

[2] Obtained from *in situ* bag technique using three fistulated cows (similar to Table 5.3).

[3] Calculated as 100 − (CP + NDF + EE + ash).

Rumen contents were obtained before the morning feeding from three fistulated non-lactating Holstein Friesian dairy cows (approximately 500 kg) fed at maintenance diet based on corn silage and 21% CP concentrate (R : C 70 : 30, w/w on DM basis). The animals were fed twice daily at 08:30 and 17:00 for 1-week period before taking the rumen contents. The 1,000 ml rumen liquor obtaining from donor cows were transported in three thermos flasks to the *in vitro* laboratory within 10 min. The rumen fluid was filtered through 2 layers of cheesecloth into pre-warmed thermos flasks to retain small particles.

8.3.3 Medium preparation

Medium preparation was similar to which in the Chapter 4 (heading 4.3.3).

8.3.4 *In vitro* fermentation

Substrates were weighed to 500 mg of DM into 100-ml glass syringes then supplemented with 200 µl of oil-ethanol solutions which providing 15 mg of added oil/syringe. Three blank syringes for gas production were added 200 µl of absolute ethanol (99.99%) without oil supplementation and substrate. An overview of substrates and oils added to the incubation syringes is given in Table 8.2. Under continuous CO_2 flushing, the filtrated rumen fluid was mixed (1 : 4, v/v) with pre-warmed (39°C) medium and then introduced (50 ml of rumen fluid and medium mixture) into gastight glass syringes. The lower end of syringes was closed afterward, and the syringes were incubated in a water bath at 39°C for 48 h. Gas volume produced was recorded at 2, 4, 6, 8, 10, 12, 18, 24, 36, and 48 h incubation.

Table 8.2 Amount (mg) of substrate and oil added to each incubation syringe

Item	Substrate	LO	SO	FO	Ethanol (µl)
Control	500	-	-	-	200
Treatment	500	5.0	5.0	5.0	185

8.3.5 Sampling, measurements, and chemical analysis

Sampling, measurements, and chemical analysis procedures were similar to Chapter 4 (heading 4.3.5).

8.3.6 Calculations

The equations used to calculate experimental data were similar to Chapter 4 (heading 4.3.6).

8.3.7 Statistical analysis

Data on mean values of CH_4 production, protozoa population, pH, NH_3-N, and VFA were analyzed according to a completely randomized design with the repeated measures (hours) using PROC MIXED procedure of SAS (2002) with the statistical model $Y_{ijk} = \mu + T_i + H_j + (T \times H)_{ij} + \varepsilon_{ijk}$, where Y_{ijk} = the dependent variable, μ = the overall mean, T_i = the fixed effect of treatment, H_j = the fixed effect of incubation time (hour), $(T \times H)_{ij}$ = the fixed effect of interaction between treatment and time, and ε_{ijk} = the random residual error. The replicate within treatment was considered as a random effect.

Data on gas production, MCP, and digestibility were analyzed by ANOVA procedure of SAS (2002) for a completely randomized design with the statistical model $Y_{ij} = \mu + T_i + \varepsilon_{ij}$, where Y_{ij} = the dependent variable, μ = the overall mean, T_i = the treatment effect, and ε_{ij} = the random residual error. Overall differences between treatment means were considered to be significant as $P < 0.05$. Significant differences among treatment means were assessed by Tukey's studentized range test after a significant F-test. Data are expressed as mean ± SEM, which represents the pooled SEM for the model.

8.4 Results

8.4.1 Gas production, methane production, and protozoa

The supplementing oil mixture rich in PUFA along with RUP-high diet had remarkably influenced on ruminal gas production, CH_4 production as well as protozoa population (Table 8.3). As expected, the gas production expressed as ml/g DM at 48 h incubation was lower ($P < 0.001$) for diet supplemented with oils and RUP than for

the control, accounting for 3.40% decrease. The treatment could decrease gas production since the early time points (2 h) until the end (48 h) of the incubation. It ranged from 45.14 to 298.46 ml/g DM in the treatment was different ($P < 0.001$) from the control, which ranged from 40.16 to 288.08 ml/g DM (Figure 8.1). Methane production which calculated from net molars of individual VFA showed that oil supplement along with RUP resulted in depressed ($P < 0.001$) CH_4 emission expressed as mmol/g DM, accounting for 10.83% decrease relative to the control. Further analysis across the time of incubation (Figure 8.2) showed that CH_4 production was not affected by the treatment during the early time points of the incubation (0-6 h), whereas it was strongly reduced ($P < 0.05$) in the treatment at 24 h incubation. The oil supplement had lower ($P < 0.05$) protozoa population than that in the control (8.87 and 11.93 × 10^5 cfu/ml, respectively).

Table 8.3 Gas production, CH_4 production, and protozoa

Item	**Diet**		**SEM**	***P*-value**
	Control	**Treatment**		
Gas (48 h)				
ml/g DM	298.46	288.30	6.02	<0.001
mmol	6.67	6.43	0.14	<0.001
mmol/g DM	13.32	12.87	0.27	<0.001
Methane				
mmol	0.60	0.53	0.03	<0.001
mmol/g DM	1.20	1.07	0.07	<0.001
Protozoa (×10^5 cfu/ml)	11.93	8.87	3.09	0.013

n = 12 for gas production and *n* = 3 for methane production and protozoa

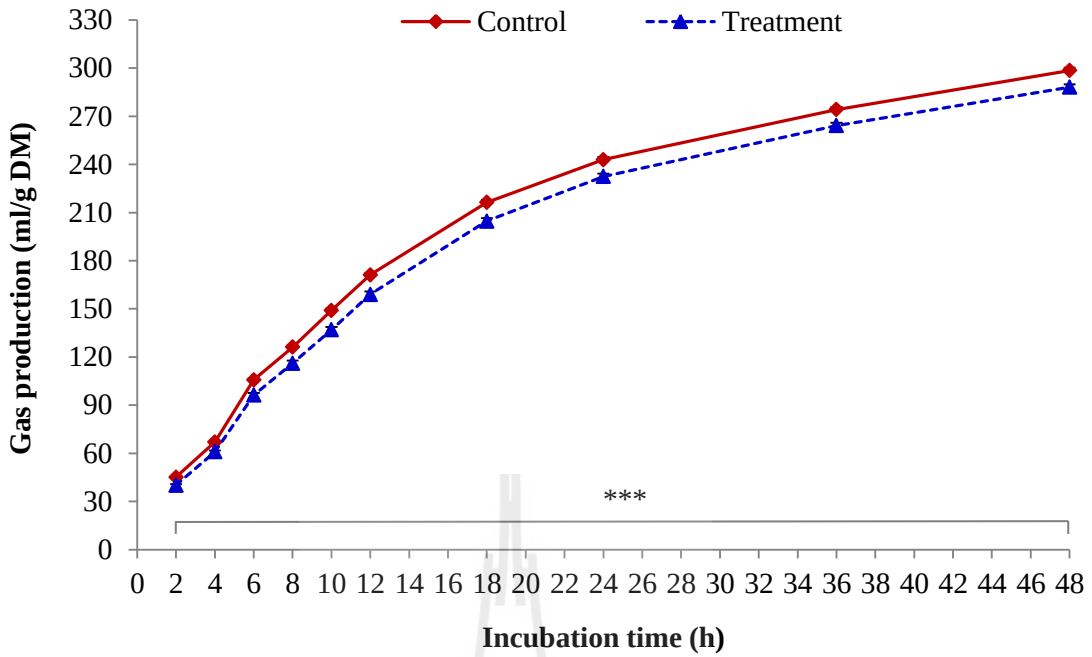

Figure 8.1 Cumulative gas production changes during the incubation. The standard error of the mean is indicated by the error bars over each point. *** : $P < 0.001$ ($n = 12$).

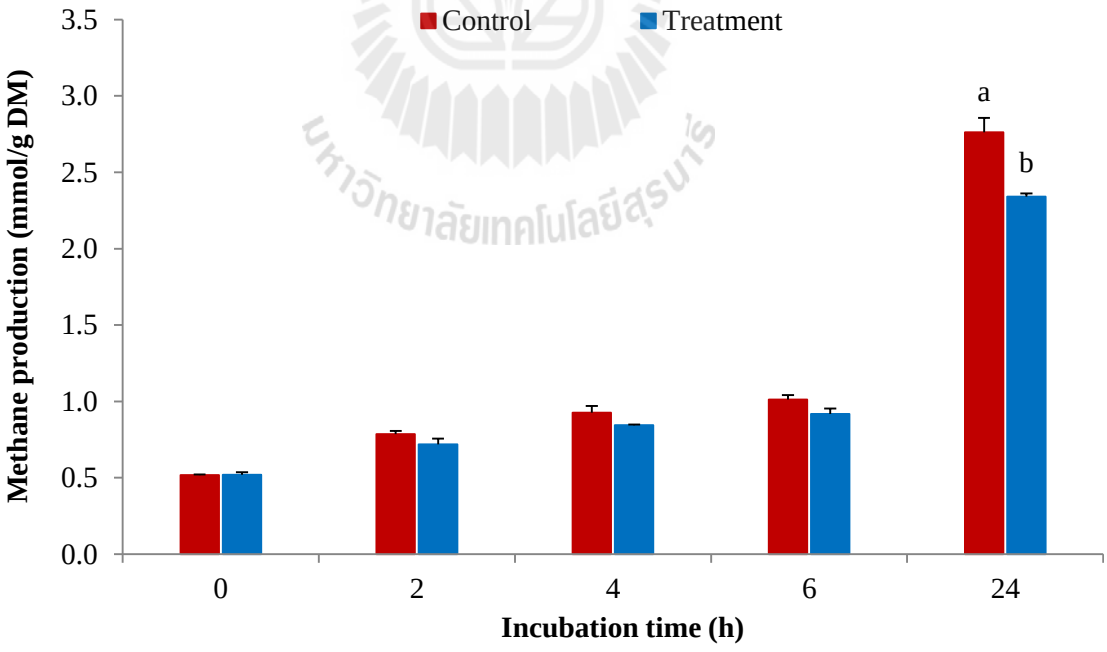

Figure 8.2 Methane production changes during the incubation. The standard error of the mean is indicated by the error bars over each point. The statistical significance is indicated by different letters over the columns ($P < 0.05$, $n = 3$).

8.4.2 Volatile fatty acids, nitrogen metabolism, and digestibility

Oil mixture and RUP supplementation affected total VFA concentration (Table 8.4), where the treatment had lower ($P < 0.001$) total VFA concentration (3.17 mmol/g DM) compared to 3.54 mmol/g DM in the control. The total VFA concentration linearly increased from 0 to 24 h incubation for both diets; however, difference ($P < 0.01$) was only detected at 24 h incubation (Figure 8.3). Under this study it was observed that oil supplementation along with RUP-high diet didn't modified ($P > 0.05$) molar proportions of individual VFA. Ruminal NH_3-N concentration remained unchanged during the first 6 h of the incubation, but the higher amount ($P < 0.01$) was observed in the treatment at 24 h incubation (Figure 8.4). In this study, addition of oil mixture to RUP-high diet resulted in reduced ($P < 0.05$) microbial protein synthesis in the rumen (12.43 g/kg OM) compared to the control (12.64 g/kg OM). The treatment diet affected *in vitro* digestibilities of true DM, OM, and NDF. In particular, the IVTD was lower (48.62%) in the treatment versus the higher value (56.46%) in the control ($P < 0.001$). The IVNDF was too low ($P < 0.001$) in the treatment (5.39%) compared to 21.12% in the control.

Table 8.4 Volatile fatty acid production, nitrogen metabolism, and digestibility

Item	Diet		SEM	*P*-value
	Control	Treatment		
pH	6.70	6.67	0.04	0.032
Volatile fatty acid				
Total (mmol)	1.77	1.59	0.09	<0.001
Total (mmol/g DM)	3.54	3.17	0.17	<0.001
Acetate, C2 (%)	65.84	65.64	0.52	0.309
Propionate, C3 (%)	21.64	21.79	0.28	0.153

Table 8.4 Volatile fatty acid production, nitrogen metabolism, and digestibility (conc.)

Item	Diet		SEM	*P*-value
	Control	Treatment		
Butyrate (%)	12.52	12.57	0.28	0.685
C2/C3 ratio	3.09	3.05	0.07	0.176
Nitrogen metabolism				
NH_3-N (mg N/dl)	31.57	31.36	2.35	0.810
MCP (g/kg OM)	12.64	12.43	0.21	0.027
Digestibility (%)				
IVTD	56.46	48.62	0.96	<0.001
IVOMD	64.49	64.45	1.08	0.028
IVNDFD	21.12	5.39	1.73	<0.001

n = 12 for MCP and IVOMD and *n* = 3 for other parameters

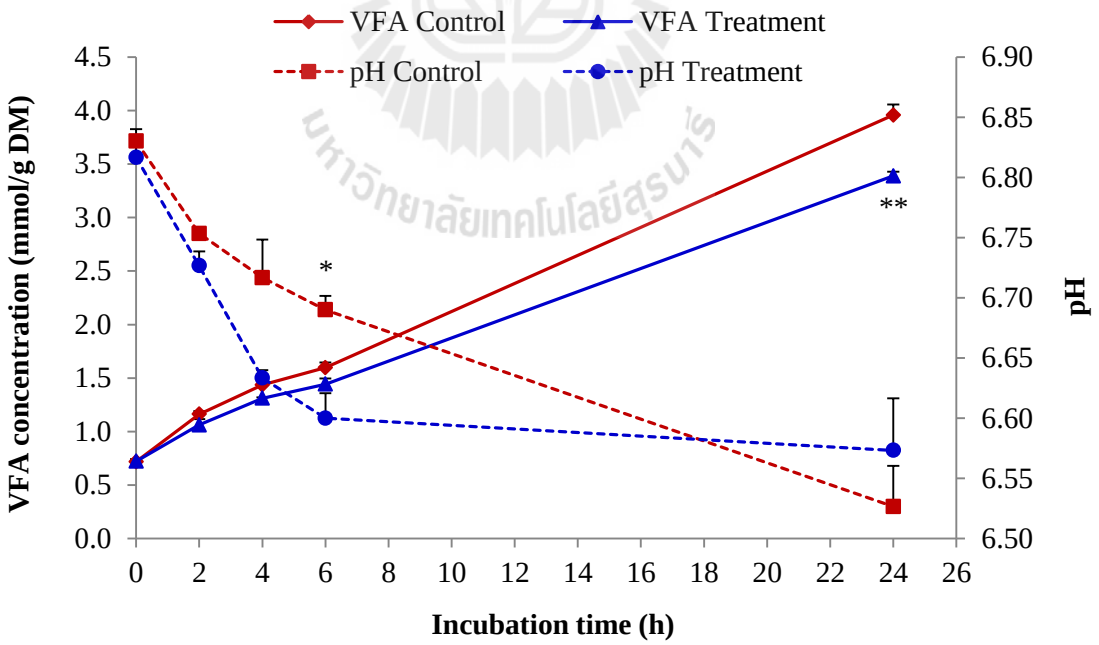

Figure 8.3 Volatile fatty acid concentration and pH changes during the incubation. The standard error of the mean is indicated by the error bars over each point. * : $P < 0.05$ and ** : $P < 0.01$ ($n = 3$).

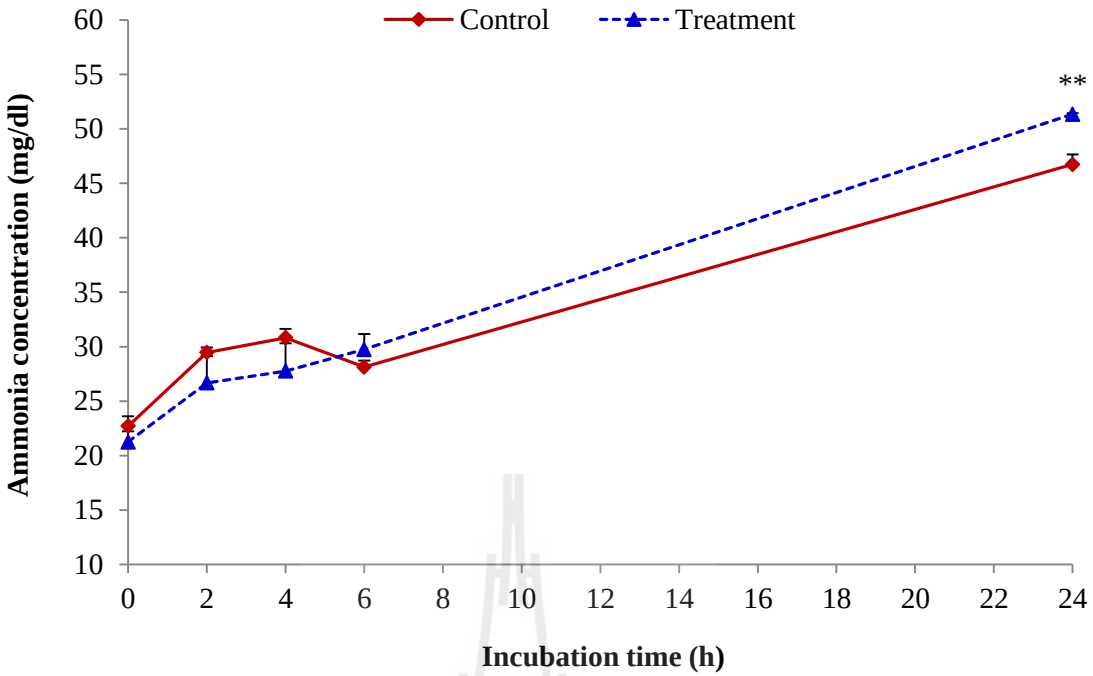

Figure 8.4 Ammonia concentration changes during the incubation. The standard error of the mean is indicated by the error bars over each point. ** : $P < 0.01$ ($n = 3$).

8.5 Discussion

8.5.1 Gas and methane production

The reduction of cumulative gas production during the whole incubation time in this study was in agreement with Wu et al. (2015). Toral et al. (2009) found that sunflower oil and fish oil addition *in vivo* significantly declined gas production *in vitro*. Supplementation of oils rich in PUFA usually reduces CH_4 production through a negative effect on protozoa and methanogens. In a research of linseed supplement in forms of crude, extruded, and oil to dairy cattle diet, Martin et al. (2008) observed that all the forms of linseed could mitigate daily CH_4 emission. Recently, Li et al. (2015) found that inclusion of oils *in vitro* reduced total CH_4 production, and the higher effect on CH_4 depression was detected with addition of

linseed oil and fish oil mixture rather than linseed alone. Eugène et al. (2008) reported a 9% decrease in CH_4 production in dairy cows due to dietary addition of lipid, but this was also accompanied by a 6% reduction in DMI, which resulted in no change in CH_4 production per unit of DMI. In this study, however, oil mixture supplementation showed a depression of CH_4 production expressed as mmol/g DM. This may be the result of synergistic effect of oil combination in the ruminant diet (Soliva et al., 2004). Moreover, the remarkable mitigation of CH_4 production by oil supplementation in this study suggested that the amount of added oil was high enough to disturb the growth or function of rumen microbes, such as protozoa and methanogens, which corresponding to CH_4 synthesis. Patra (2013) found that CH_4 production in the ruminants could be only mitigated as oils were added to meet the dietary lipid content above 5%. In the current study, the negative effects of supplementing linseed, sunflower, and fish oils on protozoa, hence protozoa-associated methanogens and CH_4 emission could relate to predominant UFA in these oils, such as ALA, LA, EPA, and DHA. Supporting this notion, Zhang et al. (2008) showed that ALA and LA reduced the growth of rumen protozoa and methanogens, and the higher effect on these rumen microbes was observed in the ALA treatment. Methane production was linearly decreased with increasing level of LA addition to *in vitro* rumen incubation (Wu et al., 2015). Moreover, Maia et al. (2007) concluded that PUFA inhibit the growth of rumen microbes involving FA hydrogenation, and the toxicity to microbial growth was respectively ranked from EPA, DHA, ALA to LA. In fact, lipid addition can mitigate CH_4 production by decreasing methanogenesis through inhibition of protozoa and cellulolytic bacteria, increasing production of propionic acid, and by BH of UFA (Martin et al., 2010).

Besides, FA can inhibit methanogens directly through binding to the cell membrane and interrupting membrane transport (Dohme et al., 2001).

8.5.2 Ruminal fermentation and digestibility

The shift in ruminal pH as well as total VFA and NH_3-N concentrations occurred in the cultures over times of the incubations (Figures 8.3 and 8.4) indicates that the current batch cultures were an adequate model of rumen activity and the effect of oil and RUP supplement was comparable to what would occur in the rumen. Mean ruminal pH was normal over the first 4 h post suggesting that effect of oil addition in ruminal fermentation pattern could only detected after this incubation times. The decreased ruminal pH in this study was similar to the research of Wu et al. (2015), whereas Li et al. (2015) and Toral et al. (2009) didn't find any different effect of oil supplement on ruminal pH. The inhibition of oil addition on microbial activity in the rumen could lead to a shift in total VFA concentration; however, published results were inconsistent. Toral et al. (2009) observed a reduction tendency of total VFA concentration with sunflower oil and fish oil inclusion, Razzaghi et al. (2015) reported a greater total VFA concentration in dairy goats supplemented with 3.7% DM of sunflower oil, whereas other authors (Li et al., 2015; Pirondini et al., 2015; Wencelová et al., 2015) found no significant effect of oil inclusion on total VFA concentration. The reduction of total VFA seemed beneficial in term of mitigating CH_4 production, but it could also cause a negative effect on animal performance. Oil supplementation rich in PUFA has been reported to result in a reduction in acetate proportion and an increase in propionate proportion (Li et al., 2015; Razzaghi et al., 2015). Similar result was also found in the experiment of Zhang et al. (2008). However, the changes in ruminal molar proportions of acetate and propionate in this

study were too tiny so that they could be significantly detectable. In the present study, ruminal NH_3-N concentration in the treatment remained lower the control during the first 4 h incubation while it was increased from 6 h incubation and significantly increased ($P < 0.01$) at 24 h incubation. This revealed that protein of roasted SBM and CDDGS could be protected from rumen microbial degradation for some hours, but it was then digestible at the lower parts of digestive tract. That decreased *in vitro* nutrient digestibility in this study was in line with the results of some previous studies (Wencelová et al., 2015; Wu et al., 2015). The lower fiber digestibility could be a result of decreasing amounts or function of dominant cellulolytic bacteria, such as *Fibrobacter succinogenes*, *Ruminococcus albus*, and *Ruminococcus flavefaciens*, which are more susceptible to oil addition (Maia et al., 2007; Zhang et al., 2008). The *Fibrobacter succinogenes* population was decreased markedly by increasing degree of unsaturation and inclusion level of unsaturated C18-FA while growth of *Ruminococcus flavefaciens* was inhibited by linoleic and linolenic acids at a high level added (Zhang et al., 2008).

8.6 Conclusions

Based on the current results, it can be concluded that replacement of concentration with roasted SBM and full-fat CDDGS then supplemented with oil mixture rich in PUFA could reduce gas and CH_4 production through reduction of total VFA concentration. Feeding oil mixture and RUP didn't affect molar proportions of individual VFA, but shifts in microbial protein synthesis and digestibility were occurred. Further researches involving longer term study, rumen microbe quantification, and nutrient digestion in the small intestine should be considered.

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CHAPTER IX

OVERALL CONCLUSIONS AND IMPLICATION

9.1 Conclusions

This study aimed to investigate whether supplementation of PUFA-rich oils in the RUP-high diet can enhance healthy fatty acids in milk, but mitigate CH_4 production without affecting milk production. For these purposes, the study was carried out comprising 6 experiments. The first two experiments were conducted to test the effects of supplementing PUFA-rich oils on milk yield, milk composition, milk fatty acid profiles (Chapter 3), CH_4 production, and rumen fermentation patterns (Chapter 4). The next two experiments were done to evaluate the effects of replacing concentrate with medium-roasted SBM and CDDGS alone or in combination on milk production and composition, income over feed costs (Chapter 5), CH_4 production, and rumen fermentation patterns (Chapter 6). The last two experiments, which were designed from the best treatments of the above experiments, were conducted to test the effects of PUFA-rich oil supplementation in RUP-high diet on milk production, milk composition, milk fatty acid profiles (Chapter 7), CH_4 production, and rumen fermentation patterns (Chapter 8). The summary results from this study are presented as below.

Milk yield was not affected by supplementation of all oil mixtures rich in PUFA at 3% DMI. However, the cattle diet supplemented with SOFO depressed milk fat concentration as well as yields of milk fat, protein and total solid, whereas

supplementation of LOFO reduced yield of milk total solid. The LOFO improved proportions of milk *cis*-9,*trans*-11 CLA and C18:3n-3, whereas the MIXO enhanced proportions of preformed FA and n-3 PUFA in milk, but reduced AI and TI (Chapter 3). The supplementation of a high-concentrate diet with combination of either LO or SO or both with FO at 3% DM showed a good strategy to reduce gas and CH_4 production *in vitro*. Both LOFO and SOFO showed disturbances in microbial protein synthesis and nutrient digestibility in the rumen, but only SOFO impaired total VFA concentration. The rumen fermentation patterns and digestibility were maintained in the MIXO diet (Chapter 4). Therefore, to improve health UFA in milk and to reduce CH_4 production without affecting rumen fermentation, digestibility and hence animal productivity, an ideal oil supplementation would be MIXO at 3%.

The partially substitute of full-fat CDDGS alone or in combination with roasted SBM for concentrate in lactating dairy cattle diet improved milk production and net income over feed costs without affecting total DMI. Partially replacing concentration with either roasted SBM or full-fat CDDGS or both had no effect on milk composition of dairy cows. The higher yields of milk and composition in the DDGS and SB-DG resulted in improved feed efficiency in these treatments. Feeding roasted SBM alone replaced for concentrate seemed to show the intermediate values in almost parameters (Chapter 5). Methane production was reduced when only or both roasted SBM and full-fat CDDGS were incorporated in the diet at the expense of concentrate. However, replacement of either roasted SBM or full-fat CDDGS for concentrate disturbed total VFA concentration; moreover, concentrate substituted with full-fat CDDGS alone shower lower molar proportions of acetate and propionate, microbial protein synthesis as well as *in vitro* digestibility of OM. These results

suggested that CH_4 production and ruminal fermentation patterns in response to added RUP sources are dependent on not only RUP content but also fat content of RUP-high feed sources. Therefore, method to improve fat extraction from CDDGS could decrease its capacity to depress CH_4 emissions in cattle feeding (Chapter 6). Both results from *in vivo* and *in vitro* studies revealed that to reduce environmental impact mainly causing by CH_4 emission without affecting rumen fermentation and to improve milk production, replacement of concentrate in dairy cattle diet with a mixture of roasted SBM and full-fat CDDGS should be an ideal approach.

Supplementation of oil mixture including LO, SO, and FO to RUP-high diet had no effect on milk yield and greater feed efficiency use for milk production, but it could cause detrimental effects on total DMI, milk fat concentration as well as milk fat yield. Feeding oil mixture rich in PUFA along with RUP effectively enhanced proportions and yields of milk healthy FA including *cis*-9,*trans*-11 CLA, C18:3n-3, C20:5n-3, and C22:6n-3 but decreased milk unhealthy n-6 to n-3 ratio and atherogenecity and thrombogenicity indices suggesting that its consumption benefits for human health (Chapter 7). Replacement of concentration with roasted SBM and full-fat CDDGS then supplemented with oil mixture rich in PUFA could reduce gas and CH_4 production through reduction of total VFA concentration. Feeding oil mixture and RUP didn't affect molar proportions of individual VFA, but shifts in microbial protein synthesis and digestibility were also occurred (Chapter 8).

Overall, enrichment of milk healthy FA and mitigation of CH_4 production in the dairy cattle can be achieved by feeding oil mixture including LO, SO and FO at 3% along with medium-roasted SBM and full-fat CDDGS partially replaced for concentration.

9.2 Implication

Over the last few decades researches have been done so far to improve milk production, milk fatty acid profiles, and methane mitigation by dietary supplementation. To date these findings have not yet delivered a comprehensive approach to balance animal production, product quality as well as environmental aspects. The researches described in this thesis represent a contribution to these overall objectives. An attractive feature of the feeding approach in these experiments was that it was able to integrate into not only commercial farming system but also local farming system. The novelty of this work is that we explore a feeding method to solve the negative effect of oil supplement on milk production and feed efficiency. Based on the results of this study, it is proposed that :

The dairy cattle diet in early lactation should be supplemented with oil mixture including LO, SO, and FO (1 : 1 : 1, w/w) at 3% DMI. This would allow one to enrich beneficial fatty acids in milk, mitigate CH_4 production with low effects on milk production and composition.

The medium-roasting method of SBM at 100°C for 3 h which applied in this thesis could be considered to apply as a heating technology to obtain the greater RUP content in raw SBM. However, further improving and testing with post-abomasum digestibility of amino acids are considered. The concentrate in the dairy cattle diet should be replaced with 3.58% medium-roasted SBM and 5.75% full-fat CDDGS because this feed substitution in early lactation could result in greater milk productivity and feed efficiency, accompanied by higher income over feed costs. The feeding duration which is able to get the positive income over feed costs is at least 28

d. Moreover, feeding medium-roasted SBM and full-fat CDDGS could be also a potential method to reduce CH_4 production.

Another application might be the implementation of oil mixture comprising of LO, SO, and FO (1 : 1 : 1, w/w) at 3% DMI to dairy cattle diet, where concentrate is already replaced with 3.58% medium-roasted SBM and 5.75% full-fat CDDGS. Feeding this diet could have high efficacy in enhancing both milk healthy fatty acids and feed efficiency use for milk production, and mitigating CH_4 production.

However, feeding unprotected oil mixture at 3% DMI along with RUP-high diet could cause detrimental effect on milk fat synthesis. Therefore, to manipulate feeding approach so that it could enhance milk beneficial fatty acids, improve feed efficiency, and mitigate CH_4 production without or less negatively affecting milk fat synthesis, a proper amount of oils added or protection technology of oils from rumen BH should be further perspectives. Additionally, further researches involving longer term study, molecular-based on experiment in rumen microbes, and nutrient digestion in the small intestine should be advisable.

BIOGRAPHY

Mr Lam Phuoc Thanh was born on 28th February 1984 in Tra Vinh, Viet Nam. In 2007, he graduated a Bachelor degree in Animal Husbandry at Can Tho University, Viet Nam. He has been employed by the Department of Animal Sciences, College of Agriculture and Applied Biology, Can Tho University to work as a researcher for rabbit and ruminant production in the MEKARN project funded by Swedish Government on "Sustainable Livestock Systems in the Tropics". In 2010, he got an award for a MSc program from Chulalongkorn University, Thailand, and he studied in the field of Animal Nutrition at Department of Animal Husbandry, Faculty of Veterinary Science, Chulalongkorn University from June 2010 to May 2012. In 2012, he got an award for a PhD program from Surnanaree University of Technology, Thailand in the program of "SUT-PhD Scholarship Program for ASEAN". He studied in the field of Animal Production Technology at School of Animal Production Technology, Institute of Agricultural Technology, Suranaree University of Technology from June 2012 to May 2015 with the thesis entitled "Optimizing milk quality and methane emission in dairy cows : Feeding oils along with rumen undegradable protein". The results of this project have been published with 2 papers in Asian-Australasian Journal of Animal Sciences. During his PhD program, he also had an opportunity to go abroad to conduct some *in vitro* researches focusing on fatty acid biohydrogenation at Department of Animal Production, Faculty of Bioscience Engineering, Ghent University, Belgium from May 2014 to March 2015 funded by Lotus 3 project - Erasmus Mundus Action 2.