FACTORS AFFECTING CONJUGATED LINOLEIC ACID CONTENT OF COW'S MILK

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ปัจจัยที่มีผลต่อปริมาณของ CONJUGATED LINOLEIC ACID ในน้ำนมโค

นายพิพัฒน์ เหลืองลาวัณย์

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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วิทยานิพนธ์นี้ได้ศึกษาถึงปัจจัยที่มีผลต่อการเปลี่ยนแปลงปริมาณของ Conjugated linoleic acid (CLA) ในน้ำนมโคและการเพิ่มปริมาณ CLA ในน้ำนมโคโดยการเสริมน้ำมันพืช และ lactic acid bacteria ในอาหารโคนม โดยแบ่งออกเป็น 1 การศึกษา และ 2 การทดลอง ดังนี้

การศึกษาที่ 1 การศึกษาปัจจัยที่มีผลต่อการเปลี่ยนแปลงปริมาณของ Conjugated linoleic acid (CLA) ในน้ำนม โดยทำการสุ่มเก็บตัวอย่างน้ำนมโคจากฟาร์มมหาวิทยาลัยทุกเดือนๆ ละ 1 ครั้งๆ ละ 24 ตัว ในรอบ 1 ปี เพื่อบันทึกปริมาณผลผลิตนม องค์ประกอบทางเคมีของน้ำนม และ CLA ในน้ำนม และข้อมูลจำนวนวันให้นม (days in milk; DIM) อุณหภูมิ ความชื้นสัมพัทธ์ และปริมาณน้ำฝน การได้รับโภชนะต่างๆ ในอาหาร พบว่าปริมาณ CLA ในน้ำนมจะอยู่ในช่วง 4.45 – 6.13 mg/g. milk fat ปัจจัยด้านสัตว์ทดลอง ปัจจัยด้านการให้ผลผลิต ปัจจัยด้านสิ่งแวดล้อมและปัจจัยด้านอาหารสัตว์ที่นำมาศึกษามีความสัมพันธ์ค่อนข้างต่ำต่อปริมาณ CLA ในน้ำนม ยกเว้นการได้รับ linoleic acid และ linolenic acid มีความสัมพันธ์ต่อการผลิต CLA ในน้ำนมสูง (R = 0.59, 0.52 และ R^2 = 0.34, 0.27 ตามลำดับ) และจากการศึกษาความสัมพันธ์ของตัวแปรต่างๆ ต่อการผลิต CLA ในน้ำนม โดยวิธีการวิเคราะห์ความถดถอยเชิงซ้อน (Multiple regression) ซึ่งได้สมการดังนี้ CLA = 2.5993 -0.004583AGE + 0.00605DIM - 0.35067MP + 0.02549LA. เมื่อ; CLA = ปริมาณ CLA (มิลลิกรัม/กรัม ไขมันนม), AGE = อายุ (เดือน), DIM = จำนวนวันให้นม (วัน), MP = โปรตีนนม (เปอร์เซ็นต์) และ LA = การได้รับ Linoleic acid (กรัม/วัน). โดยสมการดังกล่าวมีค่า R^2 = 0.458

การทดลองที่ 1 การศึกษาผลของการเสริมน้ำมันพืชในอาหารต่อการให้ผลผลิต และการเพิ่มปริมาณ CLA ในน้ำนมของโครีดนมลูกผสม Holstein Friesian โดยจัดกลุ่มโคนม 24 ตัว มีน้ำหนักเฉลี่ย 451 $\pm$ 45 ก.ก. จำนวนวันของการให้นม 97 $\pm$ 41 วัน ปริมาณน้ำนมเฉลี่ย 22.9 $\pm$ 4.6 ก.ก. ออกเป็น 3 กลุ่มๆ ละ 8 ตัว ได้แก่ กลุ่มที่ 1โคนมกลุ่มควบคุม กลุ่มที่ 2 โคนมที่ได้รับอาหารที่เสริมน้ำมันทานตะวันที่ระดับ 200 กรัม และ กลุ่มที่ 3 โคนมที่ได้รับอาหารที่เสริมน้ำมันถั่วเหลืองที่ระดับ 200 กรัมโดยวางแผนการทดลองแบบ Randomized Complete Block Design (RCBD) ผลการทดลองพบว่า การกินได้วัตถุแห้ง การกินได้ของโปรตีน ปริมาณน้ำนม องค์ประกอบของน้ำนมและน้ำหนักตัวที่เปลี่ยนแปลง ไม่มีความแตกต่างอย่างมีนัยสำคัญทางสถิติ ($p>0.05$) ในส่วนปริมาณกรดไขมันในน้ำนมพบว่า กรดไขมัน $C_{6:0}$, $C_{8:0}$ และ $C_{16:0}$ มีปริมาณลดลงเมื่อเปรียบเทียบกับโคนมที่ได้รับอาหารควบคุม ($p<0.05$) อย่างไรก็ตาม กรดไขมัน $C_{18:0}$, $C_{18:1n9t}$, $C_{18:1n9c}$, $C_{18:2n6t}$ มีปริมาณสูงขึ้น

เมื่อเปรียบเทียบกับโคนมกลุ่มควบคุม ($p<0.05$) และในส่วนของ CLA (*cis*-9, *trans*-11 octadecadienoic) พบว่า มีปริมาณสูงขึ้นเมื่อเปรียบเทียบกับโคนมกลุ่มควบคุม อย่างมีนัยสำคัญทางสถิติ ($p<0.05$)

การทดลองที่ 2 การศึกษาผลของการเสริมจุลินทรีย์กลุ่ม Lactic acid bacteria ในอาหารต่อการให้ผลผลิต และปริมาณ CLA ในน้ำนมของโคนมลูกผสม Holstein Friesian โดยจัดกลุ่มโคนมลูกผสมโฮลสไตน์ฟรีเชี่ยน 24 ตัว มีน้ำหนักเฉลี่ย 457 $\pm$ 54 ก.ก. จำนวนวันของการให้นม 96 $\pm$ 55 วัน ปริมาณน้ำนมเฉลี่ย 22.6 $\pm$ 5.7 ก.ก. ออกเป็น 3 กลุ่มๆ ละ 8 ตัว กลุ่มที่ 1. โคนมกลุ่มควบคุม กลุ่มที่ 2. โครีดนมที่ได้รับอาหารร่วมกับ *Lactobacillus. plantarum* ที่ระดับ 1 x 10^9 cfu/cow/day และกลุ่มที่ 3. โครีดนมที่ได้รับอาหารร่วมกับ *Lactobacillus acidophilus* ที่ระดับ 1 x 10^9 cfu/cow/day โดยที่อาหารทั้ง 3 กลุ่มเสริมน้ำมันถั่วเหลืองที่ระดับ 200 กรัม/ตัว/วัน โดยวางแผนการทดลองแบบ RCBD จากการทดลองพบว่า การกินได้วัตถุแห้ง การกินได้ของโปรตีน การกินได้พลังงานสุทธิ ปริมาณน้ำนม และองค์ประกอบของน้ำนม และปริมาณ CLA ไม่มีความแตกต่างอย่างมีนัยสำคัญทางสถิติ ($p>0.05$) ในขณะเดียวกันใช้โคเจาะกระเพาะจำนวน 6 ตัว จัดการทดลองแบบ 3x3 Replicated Latin squares โดยกลุ่มที่ 1. กลุ่มควบคุม กลุ่มที่ 2. โคเจาะกระเพาะที่ได้รับ *L. plantarum* ที่ระดับ 1 x 10^9 cfu/cow/day และกลุ่มที่ 3. โคเจาะกระเพาะที่ได้รับ *L. acidophilus* ที่ระดับ 1 x 10^9 cfu/cow/day โดยที่อาหารทั้ง 3 กลุ่มเสริมน้ำมันถั่วเหลืองที่ระดับ 200 กรัม/ตัว/วัน พบว่า ระดับของ pH ภายในกระเพาะหมัก ปริมาณกรดไขมันระเหยง่าย (Acetate, Propionate, butyrate และ A/P) และการเสริม lactic acid bacteria ไม่มีผลต่อจำนวนของแบคทีเรียกลุ่มต่างๆ ในกระเพาะหมัก ($p>0.05$)

สาขาวิชาเทคโนโลยีการผลิตสัตว์
ปีการศึกษา 2548

ลายมือชื่อนักศึกษา.....
ลายมือชื่ออาจารย์ที่ปรึกษา.....
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DAIRY CATTLE/MILK PRODUCTION/MILK COMPOSITIONS/FATTY ACIDS/ CONJUGATED LINOLEIC ACID

The objectives of this study were to determine factors affecting change in milk CLA, effect of supplementation of plant oil in dairy cattle diet on production and CLA in milk in dairy cattle and effect of supplementation of Lactic acid bacteria on milk production and CLA in milk in dairy cattle. The present research divided into 1 study and 2 experiments.

The first study was carried out to determine the studies of factors affecting change in milk CLA. Milk samples were collected from University farm every month and a time per month. The records of milk yield, milk compositions, CLA content, day in milk, temperature, humidity, rain and feed intake during March 2004 – February 2005. CLA change all year round by CLA content has between 4.45 – 6.13 mg/g. milk fat. The factors of animal, production, environment and feed intake has low correlation on milk CLA. However, linoleic acid and linolenic acid intake has high correlation on milk CLA (R = 0.59, 0.52 and R^2 = 0.34, 0.27 respectively). All variables were submitted to the multiple regressions with stepwise backward elimination for a variable to remain in the predictions equation. CLA = 2.5993 -

0.004583AGE + 0.00605DIM - 0.35067MP + 0.02549LA. Where; CLA = CLA Yield (mg/g milk fat), AGE = Age (month), DIM = Day in milk (day), MP = Milk protein (%) and LA = Linoleic acid intake (g/day). (R^2 = 0.458).

The first experiment was carried out to determine the effect of supplementation of plant oil in dairy cattle diet on production and CLA in milk in dairy cattle. Twenty-four Crossbred Holstein-Friesian (>87.5 Holstein-Friesian), with averaging 22.9 $\pm$ 4.6 kg milk yield, 97 $\pm$ 41 days in milk, 451 $\pm$ 45 kg body weight, were assigned into 3 treatment groups (8 cows in each group). The first group was the unsupplemented group (control), the second group was supplemented with 200 g/cow/day sunflower oil and the third group was supplemented with 200 g/cow/day soybean oil. The experimental design was a Randomized Complete Block Design (RCBD). Dry matter intake, milk yield, milk compositions and body weight change were unaffected (P>0.05) by supplementation of soybean and sunflower oils. Concentrations of $C_{6:0}$, $C_{8:0}$ and $C_{16:0}$ in milk were significantly decreased (P<0.05) while concentrations of $C_{18:0}$, $C_{18:1n9t}$, $C_{18:1n9c}$ and $C_{18:2n6t}$ in milk were significantly increased (P<0.05) when compared to the control group. Supplementation of the 2 plant oils resulted in increased CLA in milk when compared to the unsupplemented control group. However, there was no significant different (P>0.05) in CLA in milk between the supplementation of the 2 oils.

The second experiment was conducted to investigate the effect of supplementation of Lactic acid bacteria on production and CLA in milk in dairy cattle. Twenty-four Crossbred Holstein-Friesian (>87.5 Holstein-Friesian), with averaging 22.6 $\pm$ 5.7 kg milk yield, 96 $\pm$ 55 days in milk, 457 $\pm$ 54 kg body weight, were assigned into 3 treatment groups (8 cows in each group). The first group was

unsupplemented group (control), the second group was supplemented with 1 x 10^9 cfu of *Lactobacillus plantarum* and the third group was supplemented with 1 x 10^9 cfu of *Lactobacillus acidophilus*. All groups supplementation of soybean oil in dairy cattle diet the experimental design was a RCBD. There were no significant differences between treatment (P>0.05) in milk production, milk composition body weight change and CLA content by supplementation of lactic acid bacteria. Six fistulated non-lactating dairy cows were used to determine population of rumen microorganisms. The experimental design was 3x3 Replicated Latin square designs. The first group was the unsupplemented control group, the second group was supplemented with 1 x 10^9 cfu of *L. plantarum* and the third group was supplemented with 1 x 10^9 cfu of *L. acidophilus*. Rumen pH, acetate, propionate, butyrate, A/P ratio and population of bacteria in rumen were no significant different (P>0.05)

School of Animal Production Techology

Academic Year 2005

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LIST OF ABBREVIATIOS

ADF = acid detergent fiber

ADICP = acid detergent insoluble crude protein

ADIN = acid detergent insoluble N

ADL = acid detergent lignin

CLA = Conjugated linoleic acid

C 4:0 = Butyric acid

C 6:0 = Caproic acid

C 8:0 = Caprylic acid

C 10:0 = Capric acid

C 11:0 = cis-10-Pentadecenoic acid

C 12:0 = Lauric acid

C 14:0 = Myristic acid

C 16:0 = Palmitic acid

C 16:1 = Palmitoleic acid

C 17: 0 = Heptadecanoic acid

C 18:0 = Stearic acid

C 18:1n9t = Elaidic acid

C 18:1n9c = Oleic acid

C 18:2n6t = Linolelaidic acid

C 18:2n6c = Linoleic acid

C 18:3n3 = Linoleic acid

C 20:0 = Arachidic acid

C 22:0 = Behenic acid

C 22:1n9 = Erucic acid

FCM = Fat corrected milk

NDF = neutral detergent fiber

NDICP = neutral detergent insoluble crude protein

NDIN = neutral detergent insoluble N

NE = net energy

NFC = non-fiber carbohydrate

NPN = non protein nitrogen

NRC = national research council

RDP = rumen degradable protein

RDP_{req} = rumen degradable protein requirement

LIST OF ABBREVIATIOS (cont.)

RDP_{sup} = rumen degradable protein supplement

RUP = rumen undegradable protein

RUP_{req} = rumen undegradable protein requirement

RUP_{sup} = rumen undegradable protein supplement

tdCP = truly digestibility crude protein

CHAPTER I

Introduction

1.1. Rationale of the Study

Advance development of human consumption of fat begins after the finding of close relationship between saturated fatty acid consumption and abnormal problem in the body. There are campaigns to promote consumption of unsaturated fatty acids. Furthermore, medical researches support the role of unsaturated fatty acids on reduction in the risk of many diseases. Researchers found fat from seafood containing a high n-3 unsaturated fatty acids particularly Eicosapentaenoic acid (EPA) and Docosahexaenoic acid (DHA). These two fatty acids play a major role in improving human's health status (Baer et al. 2001). Besides these fatty acids, there is another group of unsaturated fatty acid which has anticarcinogenic properties, conjugated linoleic acid (CLA) which can be found in ruminant products (Chouinard et al., 2001).

Conjugated linoleic acids are isomers of fatty acids found in small amount milk and meat of ruminants. Conjugated linoleic acid has been known to inhibit development of tumors in mice (Pariza and Hargrances, 1985). In addition, many publications reported that CLA could inhibit the development of tumor in fore stomach, mammary gland, lung and intestines of rats (Ha et al., 1990; Ip et al., 1991). There are many factors affecting CLA content in dairy cow's milk including physiological factors and environmental factors. These factors cause variation in milk

yield and composition throughout lactation. Lock and Garnsworthy (2003) studied the effect of season on changes in CLA over a year and found that CLA content in dairy cow's milk was in a range of 0.8 – 1.9 g / 100 g of fatty acid. In addition, CLA content in dairy cow's milk can be increased by supplementation of high linoleic acid raw materials. Donovan et al. (2000) who found an increase in CLA content in milk when supplemented with fish oil. Similarly, Dhiman et al. (1999) also found an increase in CLA content when supplemented with oil seeds or plant oils.

Studies of factors affecting CLA content in milk and increasing CLA content in milk, would, increase consumer's opportunity to receive CLA from milk and thus help to improve health.

1.2. Research objectives

1. To study factors affecting changes in CLA concentration of dairy cow's milk.
2. To study the increase of CLA content of dairy cow's milk by supplementation of high linoleic acid plant oil in the diet.
3. To study the increase of CLA content of dairy cow's milk by supplementation of lactic acid bacteria in the diet.

1.3. Research hypothesis

1. Many factors can affect changes in CLA concentration of dairy cow's milk.
2. Supplementation of high linoleic acid plant oil in diets can increase CLA content of dairy cow's milk.

3. Supplementation of lactic acid bacteria in diets can increase CLA content of dairy cow's milk.

1.4. Scope and limitation of the study

1. Crossbred Holstein Friesian cows from Suranaree University's dairy farm were used in the studies of factors affecting CLA content of dairy cow's milk during the 1-year period from March 2004 to February 2005.
2. Crossbred Holstein Friesian cows from Suranaree University's dairy farm were used in the studies of increasing CLA content of milk by supplementation of plant oil and lactic acid bacteria.

1.5. Expected results

1. To know factors affecting CLA content of dairy cow's milk.
2. To increase CLA content of dairy cow's milk through supplementation of plant oils in the dairy cattle diet.
3. To increase CLA content of dairy cow's milk through supplementation of lactic acid bacteria.

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CHAPTER II

Review of the Literature

2.1. Conjugated linoleic acid

Conjugated linoleic acid (CLA) is a collective term for geometrical and positional conjugated dienoic isomers of linoleic acid. The primary CLA isomer in dairy products, *cis*-9, *trans*-11 CLA, is a potent anticarcinogen in animal models (Ip et al., 1999), and this has created a world-wide interest in the biology of CLA in dairy cows.

Conjugated linoleic acid is positional conjugated dienoic isomers of linoleic acid with two conjugated unsaturated double bonds at various carbon positions. It can be found in dairy products and other animal fats (Lobb and Chow, 2000).

Conjugated linoleic acid is isomers of linoleic acid (cis-9, cis-12 octadecadienoic acid) (Figure 1) which chemical structures are cis-9, trans-11 and trans-10, cis-12 octadecadienoic acid. However, the isomer often found is cis-9, trans-11 octadecadienoic acid. CLA can naturally be synthesized by rumen microorganisms (Baer et al., 2001). In ruminants, dietary polyunsaturated fatty acids undergo biohydrogenation in the rumen. cis-9, trans-11 CLA is an intermediate in rumen biohydrogenation of linoleic acid, and it was originally assumed that this was the source of cis-9, trans-11 CLA in milk fat (Harfoot and Hazlewood, 1988; Griinari and Bauman, 1999). In ruminants, CLA is formed in gastrointestinal tract by

hydrogenation process of linoleic acid by gram positive bacteria such as Butyrivibrio fribrisovens, Ruminococcus albus and Eubacterium sp. (Kepler et al. 1967)

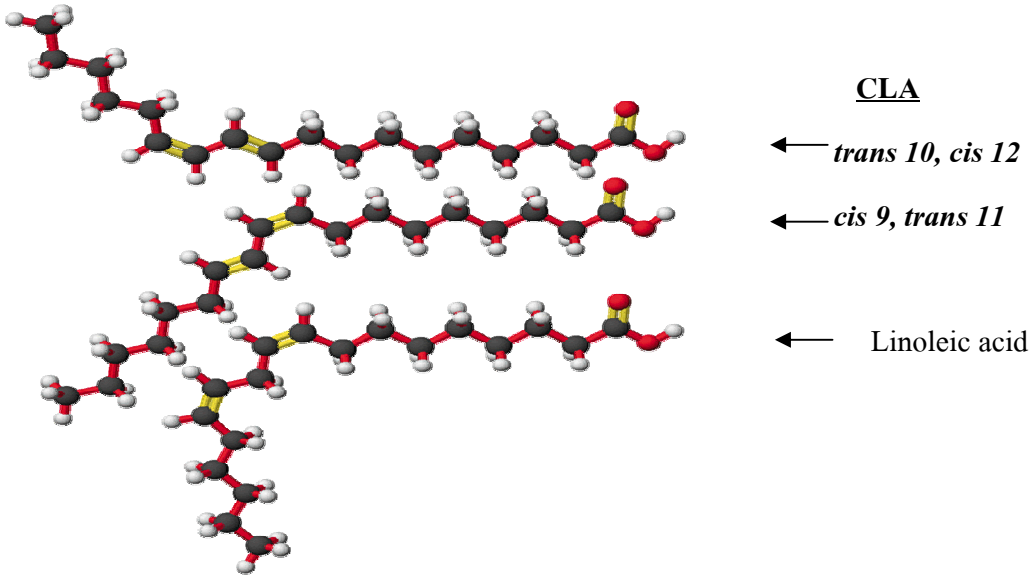

Figure 2.1 Structures of linoleic acid and conjugated linoleic acid (Steinhart, 1996)

Pariza and Hargraves (1985) first reported chemoprotective property of CLA. They found that in grilled beef has CLA which can inhibit growth of cancer in rats induced by 7, 12-diamethylbenz (a) anthracene (DMBA).

Further research found that CLA can inhibit cancer growth in stomach, udder, lung and intestine of rats (Ha et al., 1990; Ip et al., 1991; Liwe et al. 1995). In addition, Ha et al. (1990) and Ip et al. (1990) found that CLA has an antioxidant property. Other research found that CLA can lower body fat (Brodie et al. 1999; Yamazaki et al. 1999; Park et al. 1999).

CLA is fatty acid naturally found in milk and meat products from ruminants. Dhiman et al. (1999a) studied the relationships between different diets and CLA in

cow's milk. They found that cows fed rye grass or natural pasture produced milk containing 50%CLA higher than cows fed conserved forage such as alfalfa, corn silage and grain. Furthermore, researchers found that increase CLA content of milk can be done by supplementation of roasted soybean to cows fed basal diet of alfalfa and corn silage (Dhiman et al., 1999a). Cows supplemented with soybean oil or linseed oil in 2-4% concentrate gave higher CLA content of milk than cows on pasture. Dhiman et al. (1999b) found 2 folds increase in CLA content when cows were supplemented with full-fat extrude soybean and whole cotton seeds.

Milk fat naturally synthesizes from dietary fat and fat from mobilization of body reserve in adipose tissue. However, if cows received an adequate dietary fat, mobilization of fat from adipose tissue would be negligible and directly from the dietary fat. (Holmes and Wilson, 1984)

Generally, animal feeds contain various and different amount of free fatty acids. Chow (1992) reported types and amounts of free fatty acids in plant oils shown in Table 1. Different plant oils contain different free fatty acids. For instance, oil from safflower, sunflower, corn, cottonseed, sesame, rice bran, peanut and palm contain linoleic acid in descending order. If oil seeds or plant oils in Table 1 were added to concentrate for dairy cow, they may increase linoleic acid in the diet and hence increase CLA content of dairy cow's milk.

Table 2.1. Percentage of free fatty acid in plant seed oil and feed stuff

Item	Linoleic acid (18:2)	Linolenic acid (18:3)	Oleic acid (18:1)	Stearic acid (18:0)
Alfalfa silage	14.9	36.2	1.5	2.9
Alfalfa hay	15.3	21.5	3.4	3.3
Corn silage	54.9	2.7	16.8	1.9
Safflower oil	77.5	0.3	12.9	2.2
Sunflower oil	68.2	0.5	18.6	4.7
Corn oil	57.0	0.9	27.5	2.2
Soybean oil	53.3	7.8	23.4	4.0
Cottonseed oil	53.2	0.3	17.6	2.3
Sesame oil	43.3	0.2	41.2	5.2
Rice bran oil	34.0	1.1	43.8	2.1
Peanut oil	30.9	Nr	51.0	2.3
Barley	58.8	2.0	15.4	1.4
Steam-rolled corn	49.2	0	31.2	1.8
Soybean meal	41.9	7.5	8.5	3.5
Extruded soybeans	53.2	9.1	19.5	3.8
Extruded cottonseed	57.4	0	16.5	2.2
Blood meal	17.0	0	35.8	20.2

Adapted from Chow (2000) and Dhiman et al. (1999)

Nr = not report

2.2 Synthesis of CLA in the rumen

When animals receive dietary fat into the rumen, there are three processes that occur in the rumen. Firstly, hydrolysis, lipid will be digested into fatty acids and glycerol by extracellular enzymes produced by bacteria. Fatty acids (linoleic acid, *cis*-9 *cis*-12) are then subjected to isomerization to change a cis form into trans form at position *cis*-12 to *trans*-11 or CLA (*cis*-9 *trans*-11) (Figure 2). Some fatty acids are subjected to hydrogenation at position *cis*-9 into single bond in the form of *trans*-11 (vaccenic acid) and further hydrogenation into stearic acid. Every forms of fatty acids passed to small intestines will be synthesized again in tissues into CLA by Δ^9 – desaturase enzyme through addition of double bonds at position 9 into *cis*-9 *trans*-11 form.

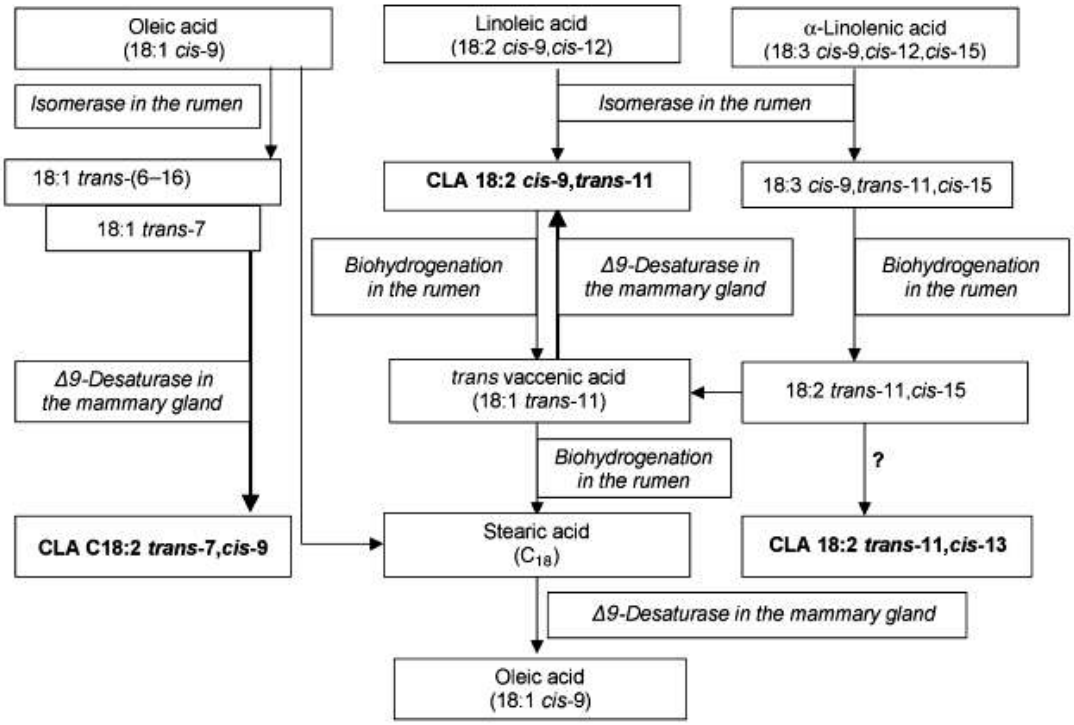

Figure 2.2 Synthesis of CLA in the rumen. (Collomb et al., 2004)

2.3 CLA production from bacteria

In ruminants, ruminal bacteria, *B. fibrisovens*, can synthesize CLA from dietary fat by biohydrogenation (Kepler et al, 1967). Recently, Kamlage et al. (2000) and Alonso et al. (2003) found that bacteria from human small intestine can also produce CLA. Thus, a bacterium is one method to produce CLA. In recent year, commercial CLA can be produced by 5 methods; 1) Alkali isomerization 2) Dehydration of hydroxyl fatty acid 3) Reduction of acetylenic bonds 4) Multiple step syntheses and 5) Biochemical synthesis (Yang et al., 2000 and Mattila-Sandholm and Saarela, 2003).

Jiang et al. (1998) found that only three strains of bacteria from produced CLA including *Propionibactrium freudenreichii* subsp. *freudenreichii* ATCC6207, *P. freudenreichii* subsp. *freudenreichii* Propioni-6 and *P. freudenreichii* subsp. *shermanii* 9093. This was caused by fatty acids in media lowered ability to produce CLA (Boyaval et al., 1995), but the three strains of bacteria were more tolerate to the inhibition of fatty acids than other strains.

However, Alonso et al. (2003), Lin (2000) and Ogawa et al. (2001) found that *L. acidophilus* could also produce CLA from linoleic acid. In contrast to the study of Jiang et al. (1998) and Kishino et al. (2002b) who found that *L. plantarum* could produce CLA from linoleic acid. Sieber et al. (2004) reviewed that bacteria can produce CLA from many experiments showed in Table 2.2. It is interesting to note that these bacteria may play a major role in producing CLA particularly in dairy products such as yoghurt and cheese.

Table 2.2. Possible CLA formation in a specific growth medium by different microorganism.

Strains
Lactobacillus acidophilus CCRC14079, AKU 1137, IAM 10074,AKU1122
Lactobacillus acidophilus 96
Lactobacillus acidophilus 56, ATCC43121
Lactobacillus acidophilus L1, 016
Lactobacillus brevis IAM 1082
Lactobacillus casei E5, E 10
Lactobacillus delbrueckii ssp. *bulgaricus* CCRC14009
Lactobacillus delbrueckii ssp. *lactis* CCRC14078
Lactobacillus fermentum
Lactobacillus paracasei ssp. *paracasei* IFO12004, JCM 1109, AKU1142, IFO3533
Lactobacillus pentosus AKU1142, IFO12011
Lactobacillus plantarum 4191
Lactobacillus plantarum AKU1009, 1124, JCM8341, 1551
Lactobacillus rhamnosus AKU1124
Lactobacillus reuteri PYR8 (ATCC55739)
Lactococus casei Y2, 210, IO-1
Lactococus lactis M23, 400
Lactococus lactis subsp. *lactis* CCRC10791
Lactococus lactis subsp. *Cremoris* CCRC12586
Streptococus themophilus CCRC12257
Propionibactrium shermanii AKU1254
Propionibactrium freudenreichii subsp. *freudenreichii* ATCC6207
Propionibactrium freudenreichii subsp. *freudenreichii* Propioni-6
Propionibactrium freudenreichii subsp. *shermanii* 9093
Propionibactrium freudenreichii subsp. *freudenreichii* NCIB8896, 5959
Propionibactrium freudenreichii subsp. *shermanii* NCIB10585, 5964, 8099

Source: Sieber et al. (2004)

CLA production from lactic acid bacteria in various media.

Alonso et al. (2003) investigated the effects of producing CLA from 4 lactic acid bacteria using MRS (Man - Rogosa - Sharpe) and skim milk media with an addition of 0.02% linoleic acid. They found that *L. acidophilus L1* had the higher ability to produce CLA in both media when compare with other group (*L. acidophilus O16, L. casei* E5 and *L. casei* E10). The cis-9, trans-11-octadecanoic acid form of CLA was mostly observed.

Table 2.3. CLA production from lactic acid bacteria media containing 0.02% linoleic acid incubated at 37°C for 24 h.

Media	lactic acid bacteria	CLA (µg/ml)			
		c9t11	t10c12	t9t11	Total CLA
MRS broth + 200 µg/ml linoleic acid	L. acidophilus L1	115.1±3.36	13.23±2.20	7.3±0.56	131.63±5.82[a]
	L. acidophilus O16	54.77±0.04	5.7±0.56	0.39±0.06	60.86±0.30[d]
	L. casei E5	93.9±2.25	14.14±0.65	3.14±0.76	111.18±2.36[b]
	L. casei E10	70.66±3.36	7.03±0.45	2.45±0.35	80.14±2.31[c]
Skim milk + 200 µg/ml linoleic acid	L. acidophilus L1	100.33±3.22	9.97±0.49	6.23±0.56	116.53±3.98[a]
	L. acidophilus O16	45.3±4.20	7.83±0.42	1.02±0.40	54.31±4.13[d]
	L. casei E5	85.03±4.72	11.97±0.15	2.90±0.17	99.63±4.48[b]
	L. casei E10	61.0±3.06	8.46±0.57	1.90±0.50	71.36±2.75[c]

Source: Alonso et al. (2003)

[a,b,c,d] significant different at $p<0.05$

Table 2.4. CLA production from lactic acid bacteria in skim milk media containing various level of linoleic acid incubated for 24 h.

Media	Lactic acid bacteria[1]	Total CLA (μg/ml)
Skim milk	L. acidophilus [2]	18.5 [a]
	L. delbrueckii subsp. bulgaricus[2]	18.0 [a]
	L. delbrueckii subsp. lactis[2]	17.5 [a]
	Lc. Lactis subsp. cremoris[3]	15.5 [a]
	Lc. Lactis subsp. lactis[2]	18.0 [a]
Skim milk + linoleic acid 1000 μg/ml	L. acidophilus[2]	105.5 [a]
	L. delbrueckii subsp. bulgaricus[2]	86.5 [b]
	L. delbrueckii subsp. lactis[2]	77.5 [bc]
	Lc. Lactis subsp. cremoris[3]	63.0 [c]
	Lc. Lactis subsp. lactis[2]	77.5 [bc]
Skim milk + linoleic acid 5000 μg/ml	L. acidophilus[2]	91.5 [a]
	L. delbrueckii subsp. bulgaricus[2]	86.0 [ab]
	L. delbrueckii subsp. lactis[2]	52.0 [b]
	Lc. Lactis subsp. cremoris[3]	70.0 [b]
	Lc. Lactis subsp. lactis[2]	76.5 [ab]

Source: Lin et al. (1999)

[1] Log_{10} cfu/ml is in the range 7.5 – 9.1 Log_{10} cfu/ml

[2] incubated at 37 °C

[3] incubated at 26 °C

[a,b] significant different at $p<0.05$

Lin et al, (1999) carried out an experiment by supplementation 3 levels of linoleic acid (0, 1000 and 5000 μg/ml) in addition of 5 type of lactic acid bacteria. They found that media containing non linoleic acid showed low CLA and the efficiency of 5 lactic acid bacteria was similar. However, at 1000 μg/ml linoleic acid supplementation, *L. acidophilus* produced the highest CLA. This was consistent with the work of Alonso et al., (2003). However, at 5000 μg/ml linoleic acid, CLA production was not increased when compared to 1000 μg/ml linoleic acid supplementation. Similarly, Kim et al., (2000) reported an increase in CLA production at 350 μM linoleic acid addition in media while further increases in linoleic acid addition showed no effect on CLA production (Figure 3). This can be attributed to inhibitory effect of fatty acid on activity of bacteria. Short chain fatty acids caused inhibition of metabolism of bacteria and then lysis while long chain fatty acids inhibited growth of bacteria (Boyaval et al., 1995). Boyaval et al. (1995) found that linoleic acid showed negative effect on bacterial growth.

2.4 Roles of CLA on consumer

2.4.1 Role of CLA as an anticarcinogen

Conjugated linoleic acid (CLA) is a mixture of positional and geometric insomers of linoleic acid with two conjugated unsaturated double bonds at various carbon positions. In ruminants, CLA was produced in digestive system by hydrogenation of linoleic acid by Gram positive bacteria such as *B. fribrisovens, Ruminococcus albus* and *Eubacterium sp.* (Kepler and Tove., 1967). Pariza and Hargraves (1985) was first reported chemoprotective property of CLA and suggested

that grilled beef containing CLA could inhibit tumor in mice induced by 7, 12-diamethylbenz(a) anthracene (DMBA).

Table 2.5 Effects of CLA on tumor in mouse's stomach.

Exp.	Additive	Tumor (%)	Tumor in stomach per mouse	Body weight g per mouse	Feed intake Kcal per wk per mouse
1	Olive oil	90.9 [a]	3.6±0.5[a]	31.5±0.7	87.1±3.00
	CLA	70.9[b]	1.4±0.5[b]	33.2±0.9	90.7±3.25
	Linoleic acid	78.9 [a]	3.5±1.3[a]	32.7±0.8	95.7±3.39
2	Olive oil	95.8	5.8±0.8[a]	30.8±0.8	96.3±2.19
	CLA	95.8	3.1±0.6 [b]	29.3±0.6	89.3±1.86
	Linoleic acid	100.0	6.3±1.3[a]	30.6±0.7	95.0±2.30
3	Olive oil	100.0 [a]	5.0±0.6[a]	33.1±0.9	86.9±1.44
	CLA	70.8[b]	1.7±0.4 [b]	30.0±0.6	90.5±1.32
	Linoleic acid	90.0 [a]	3.7±0.7[a]	31.8±0.8	79.2±1.53

Source: Ha et al. (1990)

[a,b] significant different at $p<0.05$

Later research found inhibitory property of CLA on tumor in stomach mammary gland lung and intestines of rats (Table 2.6) (Ha et al., 1990; Ip et al., 1991). Ha et al. (1990) found that CLA significantly reduced tumor occurring percentage in rats induced by Benzo(a)pyrene. Similarly, Ip et al. (1991) found the same result with mammary gland when various levels of CLA were supplemented to rats induced by 7, 12 dimethylbenz(a)anthracen. In addition, They also found antioxidant property of CLA.

Table 2.6 Effects of CLA on tumor in mouse's breast.

CLA level (%)	DMBA	Incidence of tumors (%)	Tumors per rat	Body weight (g)
0	+	80.0[a]	2.7±0.3[a]	148.5
0.5	+	66.7[a]	1.8±0.2[b]	114.3
1.0	+	46.7[b]	1.2±0.2[b]	77.5
1.5	+	40.0[b]	1.1±0.1[b]	68.9
1.5	-	0.0	0.0	0

Source: Ip et al. (1991)

[a,b] significant different at $p<0.05$

2.4.2 Role of CLA on body compositions

Many researchers found lowered body fat by CLA supplementation (Brodie et al. 1999; Yamazaki et al. 1999; Park et al. 1999). They found that 0.5% CLA resulted in reduction in body fat of male and female rats by 57 and 60% respectively and increased body mass by 5 and 14% respectively. Dugan et al (1997) reported increases in lean meat percentage in swine by CLA supplementation. This is in consistent to the reports of Cook and Pariza (1998) and Thiel et al (1998). In addition, swine received CLA showed thinner back fat than the unsupplemented swine. Similarly, Eggert et al (1999) and Wiegand et al (2000) found thinner 10^{th} rib back fat of CLA supplemented swine. Recent researchers found an increase in growth rate of swine receiving CLA (O'Quinn et al, 1999a), increase in carcass percentage (O'Quinn et al, 1999b; 2000a,b) and increase in pork quality (Waylan et al, 1999). Research in human receiving 3 g/d CLA during 3 mo experiment found that CLA reduced body fat and increased body

mass without any effect on body weight change (Hunter, 2000). This is in consistent to reports of Berven et al. (2000), Blankson et al.(2000) and Zambell et al. (2000) who found that human receiving 3 - 4 g/d CLA, not more than 7g/d, through 100 d of the experiment showed reduction in both body weight and body fat.

2.5 Increase CLA content in dairy products

Development of human consumption in recent year recognize an important role of health, therefore, many attempts have been made to bring various compounds that encourage health of consumers to supplement to animal feeds. These compounds include Omega- 3 fatty acid which improve blood circulation, reduce risk of cardiovascular disease and others. Medicinal research found anticarcinogenic property of CLA. Thus, increasing CLA in animal products is beneficial to consumers.

Dairy products consumed in recent day come from ruminants since ruminants can synthesize CLA in the rumen through ruminal microorganisms, *B. fibrisolvens* and other species. Mechanism of CLA synthesis in the rumen used dietary fat for synthesizing CLA. Thus, most researcher try to supplement oil or raw material containing high linoleic acid and transvaccenic acid which are precursors for synthesizing CLA. An experiment reported 50% of CLA synthesized from transvaccenic acid (Santora et al. 2000).

Supplementation of fish oil increased CLA content in dairy cow's milk. Donovan et al. (2000) supplemented dairy cow's diet with fish oil at 0, 1, 2 3 % and found that cows on 2% addition of fish oil in the diet had higher CLA content in milk (2.2 g/100g total fatty acid) than cows on the control group (0.60 g/100g total fatty acid). Similarly, Baer et al. (2001) supplemented 2% fish oil in dairy cattle diet

compared to the control group. They found that cows on supplemented group and unsupplemented control group had 2.43 and 0.66 g/100g fat CLA content in milk respectively. However, high level of fish oil addition caused reduction in feed intake. Besides fish oil, oil seeds or plant oil can also add to the diet for increasing CLA content in milk. Dhiman et al. (2000), in Experiment 1, supplemented dairy cattle diet with 3.6% ground soy bean, roasted soy bean and soybean oil, and 2.2 and 4.4% cotton seed oil. They found that CLA content in milk was highest in soybean oil group. In Experiment 2, Dhiman et al. (2000) supplemented 1, 2, 3 and 4% soybean oil and 1% cotton seed oil in dairy cattle diet compared to the unsupplemented control and found that cows on 4% soybean oil had highest CLA content in milk with no effect on feed intake and milk yield.

Dhiman et al. (1999) using extruded cotton seed (ECS) and extruded soybean (ESB) as ingredients in dairy cattle diets compared to the unsupplemented control found that cows on ESB diet had higher CLA content in milk than cows on control and ECS, and had higher feed intake and milk yield.

Dairy products such as cream, butter and butter cream from raw milk which obtained from cows supplemented with fish oil had higher CLA content compared to milk from cows on the control group. Raw milk contained 2.51 and 0.68 g/100 g fat from supplemented cows and control cows respectively when produced to dairy products they contained 2.75 and 0.61 g/100 g fat of cream, 2.78 and 0.70 g/100 g fat of butter and 2.72 and 0.67 g/100 g fat butter cream respectively. This suggested that processing had little effect on CLA content in the products (Baer et al., 2001).

Table 2.7 Effect of fatty acid composition in the diet on CLA content in dairy cow's milk.

References	Type of supplement	Linoleic acid level (g/d)	Supplement Level	Feed intake KgDM/day	Milk yield (Kg/d)	Milk fat (%)	Milk protein (%)	CLA yield (g/d)
Griinari et al. 1998	Saturated and Unsaturated	8.3	SFA	23.0	29.3	3.6	3.0	3.7 [b]
		418.8	UFA	23.8	31.7	3.4	3.1	21.1 [a]
			SEM	0.6	1.6	0.16	0.12	-
Dhiman et al. 2000. Exp. 1	Type of plant oil	236.9	Control	20.6	27.4	3.4	3.5	4.8 [b]
		308.1	1%SO	21.7	27.9	3.6	3.5	7.1 [a]
		284.2	1%LO	21.7	28.4	3.7	3.5	7.5 [a]
			SEM	0.7	1.0	0.2	0.1	1.1
Exp. 2		274.3	Control	21.6	29.6	3.4 [a]	2.1	4.0 [c]
		618.1	3.6%SO	20.2	29.0	2.8 [b]	1.9	16.9 [a]
		362.0	4%LO	20.0	30.3	2.5 [b]	1.9	12.5 [b]
			SEM	0.7	0.9	0.18	0.03	1.3
Dhiman et al. 2000.	Level of plant oil	234.8	Control	20.6	27.4	3.4 [a]	3.5	4.8 [d]
		308.1	1% SO	21.7	27.9	3.6 [a]	3.5	7.1 [c]
		348.1	2% SO	20.6	28.3	3.6 [a]	3.4	8.5 [c]
		441.3	3% SO	19.7	28.3	2.8 [b]	3.5	13.8 [b]
		692.1	4% SO	21.1	28.5	2.9 [b]	3.6	18.1 [a]
			SEM	0.7	1.0	0.2	0.1	1.1
Donovan et al. 2000.		332.9	Control	28.7 [a]	31.7 [b]	2.9 [a]	3.2	6.7 [c]
		342.2	1% FO	29.0 [a]	34.2 [a]	2.8 [a]	3.2	16.3 [b]
		324.3	2% FO	23.5 [b]	32.3 [b]	2.4 [b]	3.2	19.4 [a]
		308.0	3% FO	20.4 [b]	27.4 [b]	2.3 [b]	3.2	13.4 [ab]
			SEM	1.6	2.9	0.15	0.07	-

Table 2.7 Effect of fatty acid composition in the diet on CLA content in dairy cow's milk. (cont.)

References	Type of supplement	Linoleic acid level (g/d)	Supplement Level	Feed intake KgDM/d	Milk yield (Kg/d)	Milk fat (%)	Milk protein (%)	CLA yield (g/d)
Whitlock et al. 2002	Level of fish oil	235.1	Control	24.3 [a]	32.1	3.51 [a]	3.38	6.76 [b]
		170.6	2% FO	21.6 [b]	29.1	2.79 [b]	3.38	16.8 [a]
			SEM	1.1	2.2	0.18	0.10	-
Chouinard et al. 1998	Ca salts of plant oil	NS	Control	-	-	-	-	3.5 [c]
			canola oil	-	-	-	-	13.0 [b]
			soybean oil	-	-	-	-	22.0 [a]
			linseed oil	-	-	-	-	19.0 [a]
Dhiman et al. 2000.	Raw seeds	274.3	Control	21.6	29.6	3.41	2.13	4.0
		633.6	RAWSB	22.0	29.8	3.53	2.17	3.8
			SEM	0.7	0.9	0.18	0.03	1.3
Dhiman et al. 1999.	Processed seeds	273.8	Control	23.4 [b]	30.9 [b]	3.61	3.25	3.6 [b]
		603.7	ESB	25.8 [a]	39.2 [a]	3.18	2.98	8.6 [a]
		585.6	ECS	25.8 [a]	36.6 [a]	3.31	3.00	7.2 [a]
			SEM	1.9	2.8	0.06	0.03	0.2

Table 2.7 Effect of fatty acid composition in the diet on CLA content in dairy cow's milk. (cont.)

References	Type of supplement	Linoleic acid level (g/d)	Supplement Level	Feed intake KgDM/d	Milk yield (Kg/d)	Milk fat (%)	Milk protein (%)	CLA yield (g/d)
Abu-Ghazaleh et al. 2002.	Processed seeds	442.9	Control	29.4	33.3 [b]	3.74[a]	3.27	4.98 [c]
		1194.8	ESB	29.0	36.9 [a]	3.19 [b]	3.07	10.71 [b]
		1131.8	FM+ESB	28.8	38.0 [a]	3.07 [b]	3.10	18.54 [a]
			SEM	0.84	1.54	0.14	0.12	-
Whitlock et al. 2002		235.7	Control	24.3	32.1	3.51 [a]	3.38	6.76 [b]
		284.2	ESB	24.5	34.6	3.27 [b]	3.30	13.35 [b]
		297.0	FO+ESB	22.5	31.1	3.14 [b]	3.28	18.16 [a]
			SEM	1.1	2.2	0.18	0.10	-
Lawless et al. 1998.		NS	Control	-	20.1	3.81	3.47	13.33
			FFS	-	20.5	3.66	3.37	16.73
			FFR	-	20.3	3.58	3.58	18.09
			SEM	-	0.31	0.12	0.15	-

[a,b,c] significant different at $p<0.05$

Note: SFA = Saturated fatty acid, UFA = Unsaturated fatty acid, FO = fish oil, SO = soybean oil, LO = linseed oil, RAWSB = raw cracked soybeans, RSB = roasted cracked soybeans, ESB = extruded soybeans, ECS = extruded cotton seed, FM = fish meal, FFS = full fat soybean, FFR = full fat rape seed, and NS = not report

Table 2.8 Factors affecting changes in rumen environment and effect on CLA content in milk.

References	Factor	Supplement Level	Feed intake Kg/d	Milk yield (Kg/d)	Milk fat (%)	Milk protein (%)	CLA yield (g/d)
Griinari et al. 1998	Forage : Conc. ratio	50 : 50	23.6 [a]	31.7 [a]	3.36 [a]	3.07	21.09 [a]
		20 : 80	19.5 [b]	26.3 [b]	2.49 [b]	3.24	7.20 [b]
		SEM	0.6	1.6	0.16	0.12	-
Chouinard et al. 1998		100	-	-	-	-	8.8
		81 : 19	-	-	-	-	8.6
		62 : 38	-	-	-	-	6.8
Solomon et al. 2000	NSC level	HS	20.9	35.5	3.33	3.0	5.2 [b]
		HS + FFS	22.0	38.3	3.33	2.87	12.12 [a]
		HP	20.3	34.6	3.38	2.93	5.26 [b]
		HP+ ESB	20.8	38.2	3.30	2.82	12.98 [a]
		SEM	0.3	0.6	0.05	0.02	-
Chouinard et al. 2001	Monansin	Control	-	-	-	-	3.9
		20 mg	-	-	-	-	4.2
		SEM	-	-	-	-	-
Dhiman et al. 1999b	Monansin	Control	24.3	35.1	3.19	3.07	5.3
		250 mg	23.7	35.1	3.00	3.10	6.8
		SEM	0.6	1.6	0.15	0.09	0.5

[a,b,c] significant different at $p<0.05$

Note: HS = high starch, HP = high pectin, ESB = extruded soybeans, FFS = full fat soybean, FFR = full fat rape seed, NSC = Nonstructural carbohydrate

2.6. Factors affecting yield and composition of milk

There are many factors affecting milk yield and milk composition including physiological factors and environmental factors.

2.6.1. Physiological factors

There are many factors related to milk yield and composition such as breeds, varieties, age, number of milking and gestation period.

Breeds. Holstein Friesian cows produce 40-60% higher milk yield than Jersey cows. However, the latter cows give lower milk fat and milk protein than the latter cows. Jersey cow's milk has yellow color of milk fat. (Ensminger, 1992)

Age and body size. Milk yield of dairy cows increased with increasing age up to 6-8 years of age. Heifer given first calf (2 years of age, dairy cows at 3, 4, 5, years of age will approximately produce milk at 75, 85, 92 and 98 % of adult cows. This is because young heifer has less body weight, development and growth of mammary gland than adult cows. After being adult cows, milk yield will gradually reduce with advancing ages. Milk fat and solid not fat will decrease by 0.2 and 0.5% when compared first calf heifer to fifth calf cows.

Larger cows generally produce higher milk yield than smaller cows. However, milk yield will not increase at similar ratio of increase in body weight. Increases in milk yield depend on surface area of cow ($BW^{0.70}$). It can be estimated that when body weight of cow increase 2 folds, milk yield will increase only 70% of the normal previous milk production. (Harding, 1995)

Estrus cycle and pregnancy: During heat period, cows will produce reduced milk yield due to change in hormonal status and reduced feed intake. Milk yield will then increase to normal level. Milk yield will decrease when cow get pregnant,

particularly during the last 5 months of pregnancy. Eight months pregnant cow will produce 20% less milk than non pregnant lactating dairy cow. This is probably due to the fact that some nutrients are taken to support fetal growth. During this period, growth of fetus needs nutrients that equivalent to those needed for 3.5 liters of milk yield. Change in hormonal level in portal blood also causes a reduction in milk yield. During this period, level of progesterone is still high, while level of estrogen increases which causes a reduction in milk yield. Milk fat and milk protein contents increase but glucose and potassium levels in portal blood decrease. However, cows generally given a calf every year will produce higher life time production. (Larson, 1985)

2.6.2 Environmental factors: Climatic, feeding, rearing and milking factors all affect milk yield and milk composition.

Temperature and humidity: suitable temperature for dairy cows is in the range of 4.4°C to 23.8°C. This range of temperature has no effect on milk yield but it causes higher nutrient requirement. Temperature lower than -15.0°C results in decreased milk yield but results in increased fat, SNF and total solid content. Heavier cow is more tolerate to low temperature than lighter cow.

Temperature higher than 23.8°C results in a marked decrease in milk yield but results in little effect on milk fat, SNF and total solid content. Feed intake is reduced while water intake, body temperature and rate of respiration are increased. Lighter cow is more tolerate to high temperature than heavier cow.

The effect of temperature on dairy performances also depends on relative humidity, wind speed and heat from sunshine.

Season: Cow calved in rainy season or early winter gives higher milk yield than cow calved in other period. During these periods, cow receives high quality feeds

and cooler temperature promotes higher milk yield. Milk fat content increases in winter but reduces in summer. (McDowell, 1981)

Dry period: Dry period closely relates to body condition of cow at calving. Cow having suitable long dry period will have good body condition at calving. Cow calving at good body condition will produce higher milk yield than cow calving at low body condition. Cow will mobilize fat accumulated in adipose tissues to produce milk. Approximately 880 kg milk is produced from reserved fat 100 kg. In addition, new cells in mammary gland are produced to replace the old one. Cow should have not more than 60 day dry period since longer period will cause a reduction in milk yield. (Smith and Dodd, 1966)

Milking and milk handling: Number of milking per day and interval of milking affect milk yield and milk composition. Incomplete milking or frighten during milking will cause reduction in milk yield and fat content since milk retained in the udder has higher fat content (8 – 15%) when compares to early milk of milking. Increase number of milking per day (more than 2 times) will lead to higher milk yield particularly in high producing dairy cow

Feeds and feeding: Type of feeds and method of feeding have direct or indirect effects on milk yield and milk composition. Milk yield reflects nutrient intake. If cow receives lower nutrient than normal requirement, milk yield and lactose yield will inevitably be decreased. If cow receives higher nutrient than normal requirement, milk yield will slightly increase. Feeds generally contain 3-4% fat. Change in fat supply has little effect on milk fat content, excepted that cow received high unsaturated fatty acids will produce less milk fat content but show no effect on milk yield. (Holmes and Wilson, 1984)

Increases in milk yield through higher concentrate supplementation and low roughage on offer will cause lower milk fat content. If cow receiving less than30% roughage in the ration, cow will produce milk containing only 2% fat content. Cow should receive roughage at least 1.5% of body weight or the ration should contain at least 1.5% fiber to prevent drop in milk fat content. In addition, particle size of roughage (less than 1/8 inch) (Dhiman et al. 1995) heat treated feeds, pelleted concentrate containing high corn or high quality young grasses will cause a reduction in milk fat content. (MacLeod and Wood, 1972)

Many factors affect changes in milk yield and milk composition. However, there is no evidence whether these factors affect CLA content of milk. It is, therefore, interested to study factors affecting changes in CLA content in milk. The emphasis is placed on feed and environmental factors.

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CHAPTER III

Study of factors affecting CLA concentration in dairy cow's milk

3.1 Abstract

The objective of the present study was to determine factors affecting change in milk CLA content of dairy cows. Milk samples were collected from University's Farm every month and once a month throughout the experimental period. Milk yield, milk compositions, CLA content, day in milk, ambient temperature, relative humidity, rainfall and feed intake were recorded during March 2004 – February 2005. CLA contents changes all year round between 4.45 – 6.13 mg/g. milk fat. The factors of animal, production, environment and feed intake has low correlation on milk CLA. However, linoleic acid and linolenic acid intake has high correlation on milk CLA (R = 0.59, 0.52 and R^2 = 0.34, 0.27 respectively). All variables were submitted to the multiple regressions with stepwise backward elimination for a variable to remain in the predictions equation. CLA = 2.5993 - 0.004583AGE + 0.00605DIM - 0.35067MP + 0.02549LA. Where; CLA = CLA Yield (mg/g milk fat), AGE = Age (month), DIM = Day in milk (day), MP = Milk protein (%) and LA = Linoleic acid intake (g/day). (R^2 = 0.458).

3.2 Introduction

Conjugated linoleic acid (CLA) is a collective term used to describe one or more positional and geometric isomers of linoleic acid with conjugated double bonds. CLA have been reported to have a wide range of beneficial effects, including; anticarcinogenic, antiatherogenic, antidiabetic and immune stimulatory. They have also been shown to alter partitioning and lipid metabolism, and reduce body fat in a number of different animal species (McGuire and McGuire, 2000). The concentration of CLA in milk has been reported to vary considerably (Lin et al., 1995). Banni et al. (1996) found that there were marked seasonal, and consequently dietary, variations in the CLA content of milk. This report found that the CLA content of milk was higher when cows received fresh pasture. This is in accordance with the findings of Kelly et al. (1998) and Stanton et al. (1997), where elevated milk CLA contents were reported with pasture feeding. Little work has been conducted to assess seasonal changes in the CLA concentration of milk. The current study was carried out to evaluate the level of CLA in cow's milk, and how this is influenced by seasonal and certain production parameters.

3.3 Objectives

The objective of this experiment was to study factors affecting CLA concentration in dairy cow's milk.

3.4 Materials and Methods

Cows from the Suranaree University of Technology dairy farm (Crossbred Holstein Friesian), calving all year round, were used and all followed the same dietary regime throughout. Cows were milked twice a day at 04.00 and 15.00 h and milk yields were recorded daily (evening + morning). In any month 24 cows were selected at random at the evening milking and milk taken from them. These same cows were then sampled at the following morning milking. Sampling took place between March 2004 and February 2005. The milk samples were frozen and compositions were analyzed

The following parameters were used in the model:

1. Animal factors: obtained from pedigrees of Suranaree University of Technology dairy farm.

1.1 Blood level (%)

1.2 Age of cow (month)

1.3 Day in milk (day)

2. Production factors

Milk yields and milk samples were taken once a month from 24 cows at random throughout the year. Data recorded included:

2.1 Milk Yield (kg/day)

2.2 Milk Fat (%)

2.3 Milk Protein (%)

2.4 Lactose (%)

2.5 Solid not Fat (%)

2.6 Total Solid (%)

3. Environmental factors

Meteorology data were obtained from the 3rd agricultural and irrigational experimental station (Huay Ban Yang).

3.1 Ambient temperature (°C)

3.2 Rainfall (mm.)

3.3 Relative humidity (%)

4. Feed factors

Feed intakes were recorded on two consecutive days each month. Feed offered and left uneaten samples were taken and analyzed for moisture content, crude protein, ether extract, ash (AOAC, 1990), neutral detergent fiber (NDF), acid detergent fiber (ADF) and acid detergent lignin (ADL) (Georing and VanSoest, 1970). Free fatty acids and linoleic acid contents in feeds were analyzed by gas chromatography (GC). Obtained analyzed data were then calculated for nutrient intakes as follow:

4.1 CP intake (g/day)

4.2 NDF intake (g/day)

4.3 ADF intake (g/day)

4.4 ASH intake (g/day)

4.5 NFC intake (g/day)

4.6 Linoleic acid intake (g/day)

4.7 Linolenic acid intake (g/day)

Analysis of fatty acids by Gas chromatography (GC)

Oil extraction of milk (Kelly et al., 1998)

For fatty acid composition, milk samples were thawed and then centrifuged. Fat cakes were recovered, placed in sample vials, flushed with N_2, capped, and then placed in a –20°C freezer. Subsequently, milk fat samples were combined for each period to create a pool for each cow. Lipid extraction was according to the procedures of Hara and Radin (1978) using a volume of 18 ml of hexane and isopropanol (3:2, vol/vol)/g of fat cake. After vortexing, a sodium sulfate solution (6.7% $NaSO_4$ in distilled H_2O) was added at a volume of 12 ml/g of fat cake. The hexane layer was transferred to a tube containing 1 g of $NaSO_4$, and, after 30 min, the hexane layer was removed and stored under N_2 gas at –20°C until methylation.

Preparation of fatty acid methyl ester (FAME)

Approximately 30 mg of the extracted oil was placed into a 15-mL reaction tube fitted with a teflon-lined screw cap. One and a half mL of 0.5 M sodium hydroxide in methanol was added. The tubes were flushed with nitrogen, capped, heated at 100°C for 5 min with occasional shaking and then cooled to room temperature. One mL of C17 internal standard (2.00 mg/mL in hexane) and 2 mL of boron trifluoride in methanol were added and heated at 100°C for 5 min with occasional shaking. After methylation was completed, 10 mL of deionized water was added. The solution was transferred to a 40-mL centrifuged tube and 6 mL of hexane was added for FAME extraction. The solution was centrifuged at 2000 x g, at 10°C for 20 min and then the hexane layer was dried over sodium sulfate and analyzed by gas chromatography (GC)

FAME analysis by GC

Fatty acid methyl esters were analyzed by GC (Hewlett Packard GC system HP6890 A; Hewlett Packard, Avondale, PA) equipped with a 100 m x 0.25 mm fused silica capillary column (SP2560, Supelco Inc, Bellefonte, PA, USA). Injector and detector temperatures were 240°C. The column temperature was kept at 70°C for 4 min, then increased at 13°C /min to 175°C and held at 175°C for 27 min, then increased at 4°C /min to 215°C and held at 215°C for 31 min.

3.5 Data analysis

All data obtained were subjected to Simple and Multiple Linear Regression and Nonlinear analysis by SAS package (SAS, Procedure Stepwise; backward elimination, 1988)

3.6 Experimental site

The experiment was conducted at Suranaree University of Technology's dairy farm, the center for Scientific and Technological Equipments building 1 and 3, Suranaree University of Technology.

3.7 Duration

March 2004 – August 2005.

3.8 Results

Figure 3.1 showed variation in concentration of CLA over a period of one year, commencing March 2004 until February 2005. Concentrations of CLA in milk were in the range of 4.45 – 6.13 mg/g. milk fat, being highest in August (6.13 mg/g. milk fat) and lowest in March (4.45 mg/g. milk fat).

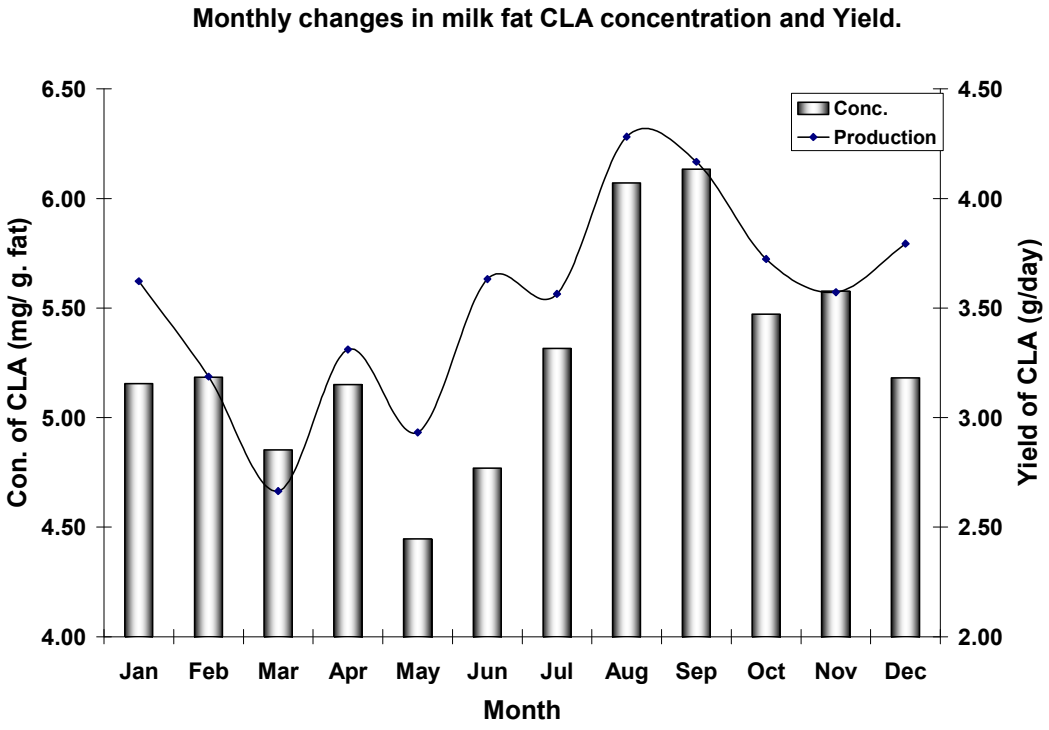


Figure 3.1 Monthly changes in milk fat CLA concentration and yield. (Mar. 2004 – Feb. 2005)

Figure 3.2 showed monthly rainfall (mm), temperature (°C) and relative humidity (%) from March 2004 to February 2005. Monthly average rainfall was in the range of 0 – 214.9 mm. with 0 mm. in March and December 2004, and January and February 2005. The highest rainfall was in September 2004 (214.9 mm). Average monthly temperature was in the range of 22.14 – 33.12 °C with lowest average

monthly temperature in December 2004 (22.14°C) and highest in May 2004 (33.10°C).

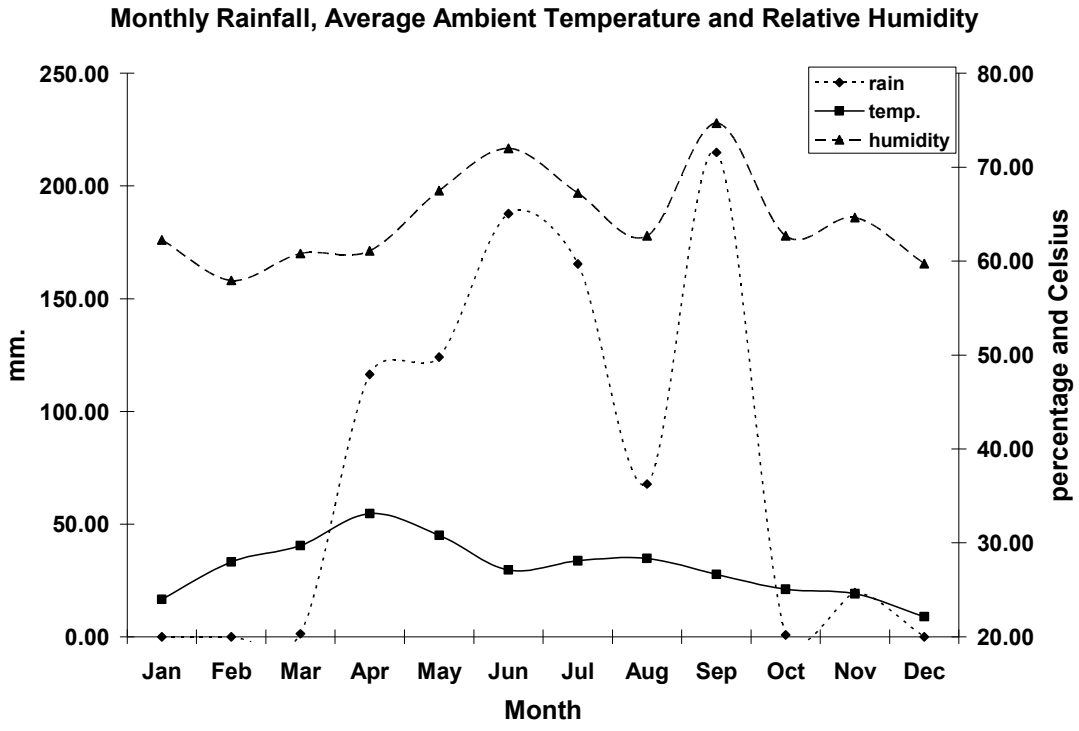


Figure 3.2 Monthly changes in Rainfall, Average Ambient Temperature and Relative Humidity. (Mar. 2004 – Feb. 2005)

Crossbred Holstein Friesian (75.0 – 98.83% Holstein) cows were between 24 and 149 mo old; and between 3 and 422 days in milk.

Milk yield and milk composition are showed in Table 3.1. Average milk yield was 19.82 kg/d with the highest at 39.60 kg/d and the lowest at 7.10 kg/d. Average milk fat, protein, lactose, solid-not-fat and total solid contents were 3.59, 2.91, 4.45, 8.26 and 11.84% respectively.

Nutrient consumptions were 2775, 9715, 4465, 1787, 4089, 122.9 and 11.04 g/day for CP, NDF, ADF, Ash, NFC, linoleic acid and linolenic acid respectively.

Table 3.1 Means, standard deviations and range of various variable. (12 months average)

Variable	Mean	SD	Minimum	Maximum
1. CLA (mg/g. milk fat)	5.34	1.46	2.15	10.07
2. Blood level (%)	92.72	4.34	75.00	98.83
3. Age (month)	65.58	28.04	24.00	149.0
4. Day in milk (day)	143.80	89.63	3.00	422.0
5. Milk yield (kg/day)	19.82	6.14	7.10	39.60
6. Milk fat (%)	3.59	0.80	0.99	6.32
7. Milk protein (%)	2.91	0.39	1.17	4.18
8. Lactose (%)	4.45	0.37	1.82	5.16
9. Solid not fat (%)	8.26	0.67	3.44	9.71
10. Total solid (%)	11.84	1.25	4.43	14.53
11. Temperature (°C)	27.31	2.96	22.10	33.10
12. Rainfall (mm.)	75.05	79.64	0.00	214.9
13. Relative humidity (%)	66.36	6.64	61.10	74.70
14. CP intake (g/day)	2775	247.7	2307	3327
15. NDF intake (g/day)	9715	1143.8	7762	12055
16. ADF intake (g/day)	4465	798.4	3110	6239
17. Ash intake (g/day)	1787	745.3	923	3743
18. NFC intake (g/day)	4089	941.6	2484	6023
19. Linoleic acid intake (g/day)	122.90	32.19	48.00	176.20
20. Linolenic acid intake (g/day)	11.04	4.95	3.30	22.30

Correlation coefficients (R) between CLA content of milk and other factors such as animal factors, performance factors, environmental factors and feed factors, are given in Table 3.3. Breeds had no effect ($p>0.05$) on CLA concentration of milk while days in milk showed high and positive correlation coefficient ($R = 0.42$; $R^2 = 0.17$; Table 3.2).

Milk yield, percent fat, percent lactose, percent SNF and percent total solid had no effect ($p>0.05$) on CLA content of milk, however, percent protein showed high and positive correlation coefficient ($R = 0.21$; $R^2 = 0.043$ respectively).

Table 3.2 Simple linear regression analysis of various variables on milk CLA.

Variables	R^2
1. Linoleic acid intake (g/day)	0.3440*
2. Linolenic acid intake (g/day)	0.2735*
3. Day in milk (day)	0.1743*
4. Milk protein (%)	0.0428*
5. NDF intake (g/day)	0.0280*
6. ADF intake (g/day)	0.0142*
7. Age (month)	0.0138*
8. Solid not fat (%)	0.0088
9. Ash intake (g/day)	0.0082
10. Total solid (%)	0.0068
11. CP intake (g/day)	0.0059
12. Blood level (%)	0.0048
13. Lactose (%)	0.0048
14. Milk yield (kg/day)	0.0041
15. Ambient temperature (°C)	0.0039
16. Milk fat (%)	0.0025
17. Rainfall (mm.)	0.0020
18. Relative humidity (%)	0.0020
19. NFC intake (g/day)	0.0001

* Significant different at $p<0.05$

Correlation coefficients between CLA content of milk and environmental factors such as temperature, rainfall and relative humidity are given in Table 3.3 and showed no effect of environmental factors ($p>0.05$) on CLA concentration of milk.

Correlation coefficients between CLA content of milk and animal feed factors such as protein, NDF, ADF, ash, NFC, linoleic acid and linolenic acid consumptions were low in some factors and high in linoleic acid and linolenic acid consumptions. NDF and ADF consumptions had negative relation to CLA concentration of milk while linoleic acid and linolenic acid consumptions had positive relation to CLA content of milk. Correlation coefficients and regression coefficients between CLA and linoleic acid and linoleic acid consumptions were 0.59 and 0.52; and 0.34 and 0.27 respectively.

Table 3.3 Matrix of correlation coefficients between milk CLA and various variable (n = 286).

Variable	Variable Number																			
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
1. CLA (mg/g milk fat)	1.00	ns	-0.12	0.42	ns	ns	0.21	ns	ns	ns	ns	ns	ns	ns	-0.17	-0.12	ns	ns	0.59	0.52
2. Blood level (%)	(2)	1.00	-0.45	ns	ns	-0.15	-0.18	ns	-0.16	-0.18	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
3. Age (month)		(3)	1.00	ns	ns	0.12	ns	ns	ns	ns	0.19	ns	ns	ns	0.13	0.15	0.19	ns	ns	ns
4. Day in milk (day)			(4)	1.00	-0.65	0.33	0.50	-0.13	0.22	0.33	ns	ns	ns	-0.22	ns	ns	ns	ns	0.18	0.12
5. Milk yield (kg/day)				(5)	1.00	ns	ns	ns	ns	ns	ns	ns	ns	-0.31	ns	ns	ns	ns	ns	ns
6. Milk fat (%)					(6)	1.00	0.50	0.23	0.43	0.88	ns	0.16	ns	-0.25	-0.14	ns	-0.12	0.11	0.25	0.29
7. Milk protein (%)						(7)	1.00	0.37	0.81	0.76	ns	ns	ns	-0.18	ns	ns	ns	0.17	0.22	0.19
8. Lactose (%)							(8)	1.00	0.79	0.57	ns	ns	ns	ns	ns	0.13	ns	ns	0.12	0.12
9. Solid not fat (%)								(9)	1.00	0.82	ns	ns	ns	ns	ns	ns	ns	0.15	0.22	0.20
10. Total solid (%)									(10)	1.00	ns	ns	ns	-0.19	ns	ns	ns	0.15	0.28	0.29
11. Temperature (°C)										(11)	1.00	0.39	-0.11	0.20	0.23	0.45	0.69	0.24	-0.36	-0.29
12. Rainfall (mm.)											(12)	1.00	0.54	ns	0.24	0.24	0.26	0.17	ns	ns
13. Relative humidity (%)												(13)	1.00	-0.34	-0.22	-0.38	-0.50	0.49	0.17	0.20
14. CP intake (g/day)													(14)	1.00	0.67	0.42	0.42	ns	-0.21	-0.25
15. NDF intake (g/day)														(15)	1.00	0.83	0.56	ns	-0.23	-0.25
16. ADF intake (g/day)															(16)	1.00	0.78	-0.22	-0.23	-0.22
17. Ash intake (g/day)																(17)	1.00	ns	-0.33	-0.30
18. NFC intake (g/day)																	(18)	1.00	ns	0.17
19. Linoleic acid intake (g/day)																		(19)	1.00	0.88
20. Linolenic acid intake (g/day)																			(20)	1.00

Note: ns = not significant p>0.05

Table 3.4 Regression equations for predicting milk CLA (mg/g milk fat).

Variable	Regression coefficients for predicting Conjugated linoleic acid (g/d)			
	1	2	3	4
1. Intercept	2.805	2.6374	2.8358	2.5993
2. Age (month)	-0.0046	-0.00487	-0.00469	-0.004583
3. Day in milk (day)	0.00621	0.00619	0.00616	0.00605
4. Milk protein (%)	-0.3750	-0.3675	-0.36251	-0.35067
5. NDF intake (g/day)	-0.000024	.....	.....	.....
6. ADF intake (g/day)	0.000063	0.000043	.....	.....
7. Linoleic acid intake (g/day)	0.02094	0.02081	0.02058	0.02549
8. Linolenic acid intake (g/day)	0.034177	0.03564	0.03544	.....
R^2	0.462	0.462	0.461	0.458
Observation, no.	286	286	286	286

A linear model was fitted with CLA as the dependent variable and other 7 variables as independent variables. The statistical procedure used in the present study was stepwise regression with backward elimination which those variables having no effect on CLA concentration of milk were gradually eliminated from the equation. Eleven variables were eliminated and 4 appropriate equations were obtained (Table 3.4). Those 4 equations had R^2 in the range of 0.462 – 0.458 from 286 data, however, the most appropriate equation was as follow:

CLA = 2.5993 -0.004583AGE + 0.00605DIM -0.35067MP + 0.02549LA (R^2 = 0.458)

Where;

CLA = CLA Yield (mg/g milk fat)

AGE = Age (month)

DIM = Day in milk (day)

MP = Milk protein (%)

LA = Linoleic acid intake (g/day)

This equation was chosen because it used least variables and gave higher R^2 (0.458) which closed to the equation having highest R^2 (0.462).

However, when various nonlinear regression equations were performed between CLA content of milk (mg/g milk fat) and linoleic acid consumption (g/d), the three most appropriate nonlinear regression equations were obtained (Figure 3.3)

1. Exponential

$y = 2.4691e^{0.0059x}$ ($R^2 = 0.415$)

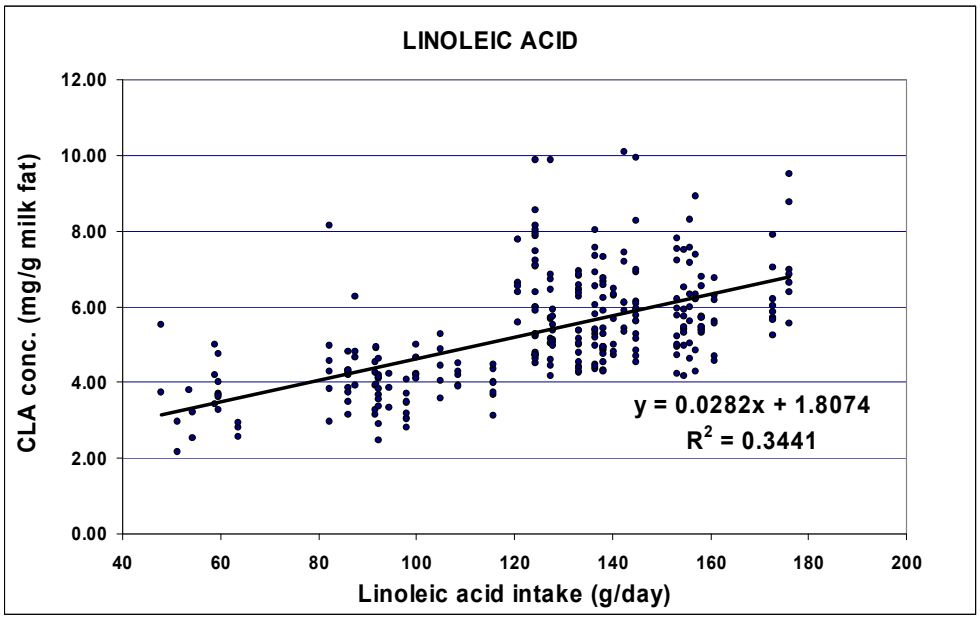
2. Polynomial

$y = 6.6862 – 0.1226 + 0.0014x^2 – 4E\text{-}06x^3$ ($R^2 = 0.357$)

3. Logarithm

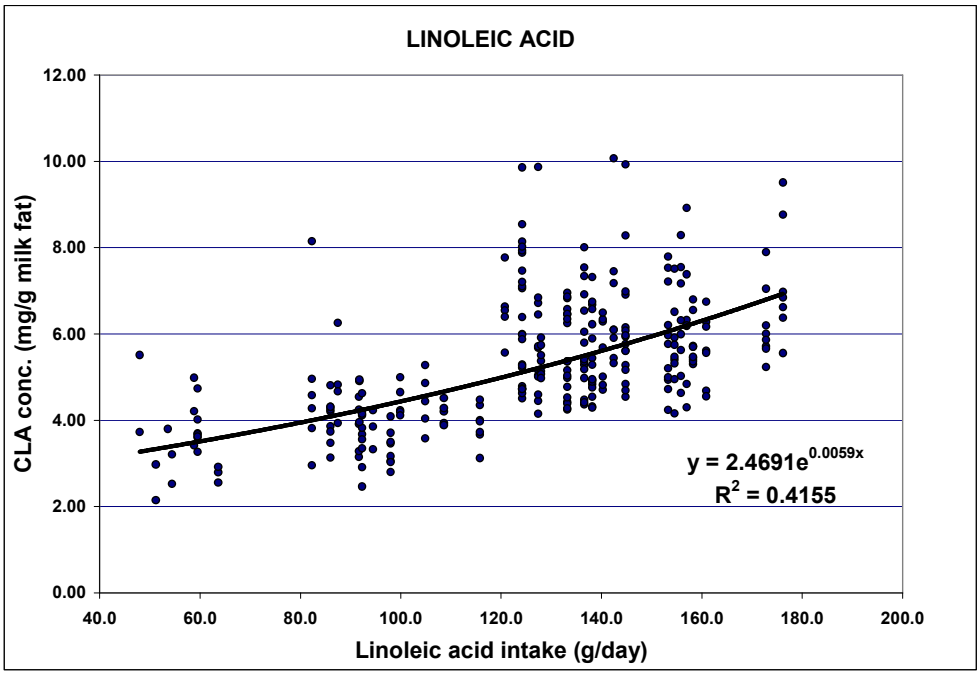
$y = -8.8359 + 2.9566Ln(x)$ ($R^2 = 0.333$)

The equation obtained highest R^2 (0.42) was the exponential equation.

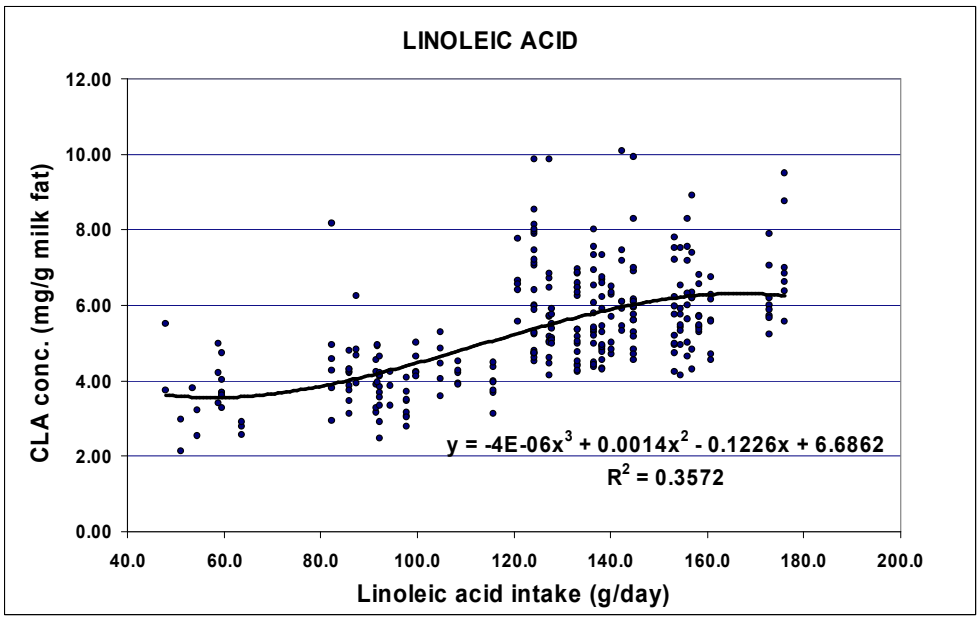


Simple linear regression

Figure 3.3 Relationship between linoleic acid intake and conjugated linoleic acids in milk fat.

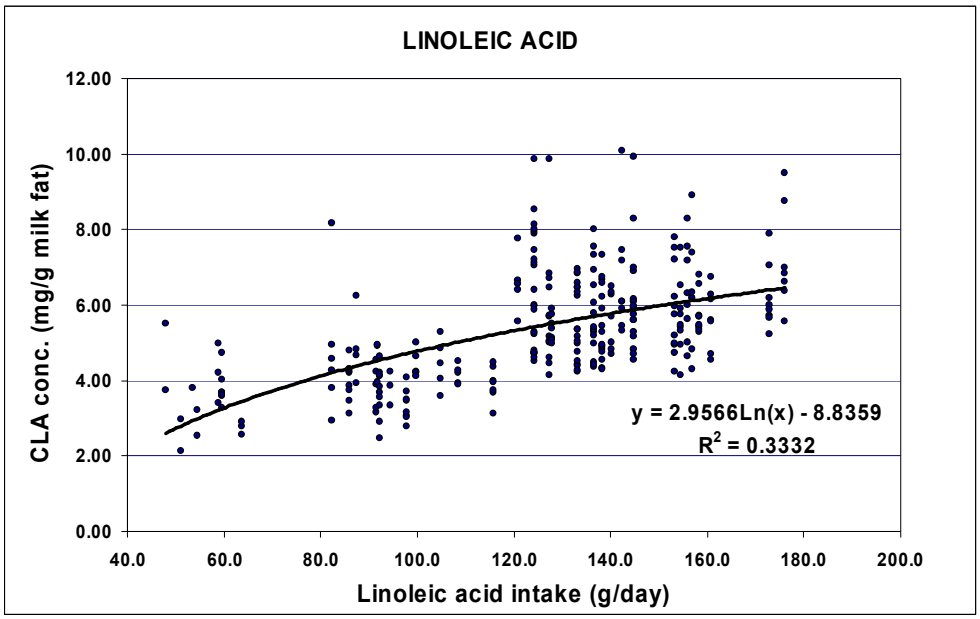


Exponential



Polynomial

Figure 3.3 Relationship between linoleic acid intake and conjugated linoleic acids in milk fat. (cont.)



Logarithm

Figure 3.3 Relationship between linoleic acid intake and conjugated linoleic acids in milk fat. (cont.)

3.9 Discussion

The present study found that concentrations of CLA of milk were in the range of 4.45 – 6.13 mg/g. milk fat, being highest in August (6.13 mg/g. milk fat) and lowest in March (4.45 mg/g. milk fat). Lock and Garnsworthy. (2003) found CLA concentration of milk were in the range of 6 – 17 mg/g. milk fat, being highest in May and June which were in the mid of spring where grasses were young and leafy (Dhiman et al., 1999; Kelly et al., 1998; Stanton et al., 1997). August is in the mid of rainy season in Thailand where fresh grasses or fresh cut corn are in adequate supply. CLA concentration, therefore, was highest in August.

Lawless et al. (1999) reported that cows from different breeds gave different CLA content of milk. CLA content of milk was higher in Holstein > Brown Swiss > Normandes > Jersey respectively. It is possible that Δ^9 –desaturase in Holstein cows

had more efficiency to produce CLA than Brown Swiss cows (Griinari et al., 2000). The present study used Crossbred Holstein Friesian cows which were not different in breed; therefore, breeds did not affect CLA concentration of milk.

Milk yield of first calved heifer increases with increasing age up to 6-8 years whereas 2, 3, 4 and 5 years cows produce 75, 85, 92 and 98% of adult cows. This is due to body weight development and growths of udder are lower in younger cows. Beyond the adult age milk, fat and SNF yield gradually reduce as age increases. Milk fat and milk SNF will decrease 0.2 and 0.5% respectively when compared first calved heifer and fifth calved cows (Nickerson, 1995). There were no relationships between age and CLA content of milk.

Kelsey et al. (2003) found that little effect of days in milk on CLA content of milk (R^2 = 0.07), however, regression analysis of this study showed lower R^2 (R^2 = 0.013). Relationships between milk yield and CLA content of milk were low. Regression coefficients between milk yield and percent fat, and CLA content were 0.17 and 0.122 respectively which were higher than those of Kelsey et al. (2003), being 0.01 and 0.08 respectively.

Relative humidity had direct relation to rainfall. During period of high rainfall, relative humidity is also high (R = 0.54). Effect of temperature on milk yield depends on other environmental factors such as relative humidity and heat from sunshine. In addition, season has effect on milk yield and milk composition (Stanton et al., 1997). Fat yield increases during winter while milk yield remains high then decreases in summer (Riel, 1963). Riel (1963) recorded 327 data and found that average CLA content was 11.3 mg/g. milk fat (2.4 to 20.1 mg/g milk fat). CLA content during summer was higher than during winter (14.6 and 7.8 mg/g milk fat respectively). In

contrast, Banni et al. (1996) found lowest CLA content of milk in summer and highest CLA content of milk in winter when cows received fresh grasses. This suggested that season had direct effect on feed supply rather than direct effect on the cows. (Banni et al., 1996; Chouinard et al., 1998; Dhiman et al., 1999; Jahreis et al., 1997; Kelly et al., 1998; Riel, 1963; Stanton et al., 1997). Contrast to the present finding, Lock and Garnsworthy. (2003) who studied relationships between CLA content and average temperature and relative humidity found regression coefficients being 0.90 and 0.99 respectively.

Crude protein, NDF, ADF, ash and NFC intakes showed a low relation to CLA content of milk. Composition of feed had direct and indirect effects on milk yield and milk compositions. Milk yield and milk lactose are reduced if cows receive inadequate supply of nutrients. The major finding of the present study is high relationships between CLA content of milk and linoleic and linolenic acid intakes (0.59 and 0.52; and 0.34 and 0.27 respectively). The reason for elevation of CLA content of milk when cows receive increased linoleic and linolenic acids is that when cows receive dietary fat into the rumen. Fatty acids (linoleic acid, *cis*-9 *cis*-12) were then isomerized from cis form to be trans form at cis-12 position to be trans-11 or CLA (*cis*-9 *trans*-11) by Δ^{12} *cis*, Δ^{11} *trans* isomarase (Chouinard et al., 1999). Some fatty acids were hydrogenated at cis-9 position to be single bond in the form of *trans*-11 (vaccenic acid) and further hydrogenated to be stearic acid. All the form of fatty acids can transfer to small intestine and absorbed to portal blood. These fatty acids were then synthesized again at tissues to be CLA by Δ^9 –desaturase by adding double bond at 9^{th} position to be in the form of *cis*-9 *trans*-11. (Abu-Ghazaleh et al., 2001; 2002; Griinari et al., 1999; Baer et al., 2000 and Whitlock et al., 2002).

Besides the predicting equation for CLA content of milk in the present study, many researchers suggested equations for predicting CLA content of milk by using dependent variables as fatty acid yield in cheeses, fermented products and fluid milk and found R^2= 0.47, 0.70 and 0.96 respectively with highest predictability in fluid milk. In addition, Bargo et al. (2005); Solomon et at. (2000); Jiang et al. (1996) and Lawless et al. (1998) found 0.66, 0.77, 0.61 and 0.69 R^2 of relationships between CLA content of milk and *trans*-11 (vaccenic acid) content of milk.

With nonlinear equation study, Loor et al., (2002) fed soybean oil to dairy cows and found that *trans*11-18:1 concentration in plasma increased to the greatest extent. O'Kelly and Spiers (1993) observed increased proportions of *trans*11-18:1 in phospholipids (PL) and triglycerides (TG) when steers were fed safflower oil. An exponential relationship (R^2 = 0.69) between 18:2n-6 intake and concentrations of *trans*11-18:1 in free fatty acids, TG, and PL fractions provided the best fit for their data. Fatty acids *trans*11-18:1 in plasma will be synthesized to CLA through the Δ^9 – desaturase enzymatic system (Bauman et al., 2000). Thus an increase in *trans*11-18:1 in plasma free fatty acids would be resulted in an increase in CLA content of milk.

3.10 Conclusion

The present study found that CLA content of milk range from 4.45 – 6.13 mg/g milk fat over the year. Animal, performance, environmental and feed factors had low relation to CLA content of milk except for linoleic acid and linolenic acid consumptions that had high relation to CLA content of milk (0.59 and 0.52; and 0.34 and 0.27 respectively). Study of relationships between various variables and CLA

content of milk using multiple regression procedure showed that the most appropriate equation was CLA = 2.5993 - 0.004583AGE + 0.00605DIM - 0.35067MP + 0.02549LA. Where; CLA = CLA Yield (mg/g milk fat), AGE = Age (month), DIM = Day in milk (day), MP = Milk protein (%) and LA = Linoleic acid intake (g/day). (R^2 = 0.458). When relationship between linoleic acid consumption and CLA content of milk was fitted with various nonlinear model, the exponential equation was most appropriate since it gave highest R^2 (0.415).

Increases in CLA content of milk can be done by increased supplementation of linoleic acid and linolenic acid in the diet.

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CHAPTER IV

The study of plant oil supplementation on performance and CLA accumulation in milk of Crossbred Holstein Friesian dairy cows

4.1 Abstract

The objective of the research is to study the increasing of CLA in dairy cow's milk and performance of them through supplementation of high linoleic acid plant oils in dairy cattle feeds. Twenty four Crossbred Holstein Friesian lactating dairy cows, averaging 22.9 $\pm$ 4.6 kg milk/d, 97 $\pm$ 41 days in milk and 451 $\pm$ 45 kg body weight, were block into 3 groups of 8 cows. The first group was fed with the control diet, the second and the third groups were fed with the control diet together with 200 g of soybean and sunflower oils per day respectively. The experimental design was a Randomized Complete Block Design (RCBD). Dry matter and protein intakes, milk yield, milk composition and body weight change were similar ($p>0.05$) in all treatment groups, however, net energy intakes of both supplemented groups were higher than the control group. The $C_{6:0}$, $C_{8:0}$ and $C_{16:0}$ fatty acids in milk of cows supplemented with plant oils were reduced ($p<0.05$) when compared to the unsupplemented control cows. However, the $C_{18:0}$, $C_{18:1n9t}$, $C_{18:1n9c}$ and $C_{18:2n6t}$ fatty acids were significantly increased ($p<0.05$) compared to the control cows. Plant oils significantly increased CLA (*cis*-9, *trans*-11 octadecadienoic) when compared to the control group, however,

there was no significant difference between sunflower oil and soy bean oil on CLA content.

4.2. Introduction

When dairy cows received diets containing fat into the rumen, there will be three processes occur in the rumen. Firstly, lipid was hydrolyzed to be fatty acids and glycerol by extracellular enzymes produced by ruminal bacteria. Fatty acids (linoleic acid, *cis*-9 *cis*-12) were then isomerized from cis form to be tran form at cis-12 position to be tran-11 or CLA (*cis*-9 *trans*-11). Some fatty acids were hydrogenated at cis-9 position to be single bond in the form of *trans*-11 (vaccenic acid) and further hydrogenated to be stearic acid. All the form of fatty acids can transfer to small intestine and absorbed to portal blood. These fatty acids were then synthesized again at tissues to be CLA by Δ^9 –desaturase by adding double bond at 9^{th} position to be in the form of *cis*-9 *trans*-11. Supplementation of high linoleic acid plant oil in the diet can increase CLA content in dairy cow's milk.

4.3. Objective

The objective of this experiment was to determine the effect of high linoleic acid plant oil supplementation in the diet on CLA content of dairy cow's milk.

4.4. Materials and methods

Dairy cattle and feeding managements

Soybean and sunflower oils were randomly sampled from the markets. They were then analyzed for free fatty acids, especially linoleic acid. These plant oils were used in the experiment. Twenty four Crossbred Holstein Friesian lactating dairy cows, averaging 22.9 $\pm$ 4.6 kg milk/d, 97 $\pm$ 41 days in milk and 451 $\pm$ 45 kg body weight, were blocked into 3 groups of 8 cows each. The first group was fed with control diet, the second and the third groups were fed with control diet together with 200 g of soybean and sunflower oils per day respectively. The experimental design was a Randomized Complete Block Design (RCBD).

Group 1. Eight cows received the control diet.

Group 2. Eight cows received the control diet plus sunflower oil 200 g/d

Group 3. Eight cows received the control diet plus soybean oil 200 g/d

The experiment lasted for 40 days including 10 days for adjustment period followed by six five-day periods for measurements.

Feed intake and milk production.

Feed offered and left after eating were weighed on two consecutive days of each period. Feed samples were then taken for proximate analysis (AOAC, 1990), detergent analysis (Georing and Van Soest, 1970), free fatty acids and linoleic acid. All cows were weighed at the start and at the end of the experiment. Milk yield was recorded daily while milk samples (evening + morning) were taken on two consecutive days in each period. Milk samples were then analyzed for milk

compositions (Milko Scan S50, Tecator, Denmark). On day 0, 10, 20 and 30 of the experiment, milk samples were taken for free fatty acids and CLA analyses (Gas chromatography; Hewlett Packard GC system HP 6890).

Fatty acids analysis by Gas chromatography (GC)

Fatty acids analysis by GC was done as reported in chapter 3.

4.5 Data analysis

Data were subjected to analysis of variance in RCBD. The differences between means were subjected to orthogonal comparison using Statistical Analysis System (SAS, 1988).

4.6 Experimental location

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

4.7 Experimental period

June 2004 – February 2005

4.8 Results

Chemical composition of the control diet, the diet supplemented with 200 g/d sunflower oil, the diet supplemented with 200 g/d soybean oil and corn silage are presented in Table 4.1. Mean values for chemical composition of the control diet, the diet supplemented with 200 g/d sunflower oil, the diet supplemented with 200 g/d soybean oil and corn silage were as follows: DM = 93.23, 92.11, 92.46 and 27.61%; CP = crude protein were 21.52, 20.61, 20.21 and 7.57%; CF = 10.36, 10.12, 9.98 and 32.37%; NDF = 47.87, 44.68, 43.87 and 62.13%; ADF = 18.31 and 5.16, 17.99 and 4.60%; and ADL = 17.78 and 4.59, and 38.24% and 5.29% respectively. The evaluation of energy concentration in the concentrate, concentrate plus 200 g/d sunflower oil, concentrate plus 200 g/d soybean oil and grass silage were as follows: TDN_{1x} = 64.82, 69.48, 69.96 and 47.26%; DE_p = 3.18, 3.49, 3.51 and 2.25 Mcal/kgDM; ME_p = 2.76, 3.10, 3.11 and 1.82 Mcal/kgDM; and NEL_p = 1.75, 1.98, 1.99 and 1.09 Mcal/kgDM respectively.

Fatty acid compositions of sunflower and soybean oil are given in Table 4.2. Both plant oils contain high linoleic acid which will be changed to CLA in the rumen. Thus, the two oils were used in this study.

Table 4.1 Chemical composition of feeds.

Item	Concentrates			Corn silage
	Control	Sunflower oil	Soybean oil	
Chemical composition	---------- % of DM ----------			
Dry matter	93.23	92.11	92.46	27.61
Crude protein	21.52	20.61	20.21	7.57
Ether extract	3.80	5.79	5.81	1.37
Ash	7.51	7.36	7.21	15.37
Crude fiber	10.36	10.12	9.98	32.37
Neutral detergent fiber	47.87	44.68	43.87	62.13
Acid detergent fiber	18.31	17.99	17.78	38.24
Acid detergent lignin	5.16	4.60	4.59	5.29
Neutral detergent insoluble N	1.28	1.24	1.28	0.56
Acid detergent insoluble N	0.85	0.74	0.74	0.47
TDN_{1X} (%)	64.82	69.48	69.96	47.26
DE_P (Mcal/kgDM)	3.18	3.49	3.51	2.25
ME_P (Mcal/kgDM)	2.76	3.10	3.11	1.82
NE_{LP} (Mcal/kgDM)	1.75	1.98	1.99	1.09

[1] TDN_{1X} (%) = tdNFC + tdCP + (tdFA x 2.25) + tdNDF – 7

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

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

DE_P (Mcal/kgDM) = DE_{1X} x Discount

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

[4] $NE_{Lp} = ([0.703 \times ME_p (Mcal/kg)] – 0.19) + ([(0.097 \times ME_p + 0.19)/97] \times [EE – 3])$

Table 4.2 Fatty acid compositions of feeds and plant oil.

Item	Concentrate	Corn silage	Sun flower oil	Soybean oil
	---------- % of total fatty acid ----------			
$C_{14:0}$	7.74	1.36	0.06	0.06
$C_{16:0}$	1.57	36.85	10.88	8.23
$C_{18:0}$	2.81	6.69	4.19	3.75
$C_{18:1n9c}$	24.99	11.83	21.28	30.01
$C_{18:2n6c}$	20.16	24.39	55.45	53.03
$C_{20:0}$	0.40	1.68	0.33	0.29
$C_{18:3n6}$	0.05	0	0.62	0.33
$C_{20:1}$	0.23	0	6.74	3.60
$C_{22:0}$	0.23	2.14	0.36	0.53
$C_{24:0}$	-	0.43	0.09	0.16
Others	41.82	14.63	-	-

Dry matter, crude protein and net energy for lactation intakes are presented in Table 4.3. There were no significant differences in concentrate, grass silage and total dry matter and crude protein intakes of the experimental cows. Total DM intakes of the control, sunflower oil and soybean oil cows were 15.04, 14.19 and 14.48 kg/d respectively. Grass silage DM consumption of the control, sunflower oil and soybean oil cows were 5.55, 4.74 and 5.29 kg/d respectively. However, cows fed with plant oils

consumed significantly more ($p<0.05$) net energy than cows fed with the control group.

Table 4. 3 Dry matter intake of cows fed plant oil.

Item	Control (1)	Sunflower oil (2)	Soybean oil (3)	SEM	%CV	Contrast	
						1 vs 2 & 3	2 vs 3
DM intake (KgDM)							
Concentrate	9.46	9.46	9.46	-	-	-	-
Roughage	5.55	4.74	5.29	0.81	28.75	0.4155	0.4698
Total	15.04	14.19	14.48	0.74	10.09	0.2857	0.7025
CP intake (g/d)							
Concentrate	2036	2036	2036	-	-	-	-
Roughage	518	460	471	43.51	18.00	0.3700	0.2956
Total	2554	2496	2508	43.49	3.45	0.3697	0.2956
NE_{LP} intake (Mcal/d)							
Concentrate	16.60	18.80	18.80	-	-	-	-
Roughage	6.03	5.16	5.74	0.80	28.90	0.4250	0.4887
Total	22.63	23.99	24.57	0.87	6.92	0.0305	0.4827

Milk yield and milk composition of the 3 groups of cows are given in Table 4.5 and 4.6. Milk yield, 3.5% fat corrected milk yield, and fat, protein, lactose, solid not fat and total solid concentration from the 3 group of cows were similar ($P>0.05$). Similarly, fat, protein, lactose, solid not fat and total solid yields were also similar ($P>0.05$).

Table 4.7 shows body weight at the start and at the end of the experiment and live weight change. There were no significant differences in those 3 parameters (P>0.05).

Table 4.4 Intake of individual fatty acid.

Item	Control (1)	Sunflower oil (2)	Soybean oil (3)
	---------- g/day ----------		
$C_{12:0}$	55.31	55.29	55.30
$C_{14:0}$	21.12	21.18	21.19
$C_{15:0}$	0.12	0.12	0.12
$C_{16:0}$	39.78	52.52	50.14
$C_{16:1}$	0.44	0.41	0.43
$C_{18:0}$	7.34	12.34	12.10
$C_{18:1n9c}$	61.41	87.00	99.69
$C_{18:2n6c}$	50.72	117.52	118.36
$C_{20:0}$	1.10	1.48	1.46
$C_{18:3n6}$	0.11	0.86	0.53
$C_{20:3n6}$	0.16	0.16	0.16
$C_{22:0}$	0.55	8.71	5.15

Table 4.5 Effect of plant oil supplement on milk yield and milk composition (%).

Item	Control (1)	Sunflower oil (2)	Soybean oil (3)	SEM	%CV	Contrast	
						1 vs 2 & 3	2 vs 3
Milk yield (kg/d)	17.8	18.5	18.6	0.96	14.95	0.5907	0.9858
3.5%FCM	18.7	17.7	18.3	1.09	18.19	0.5959	0.7532
Fat (%)	3.79	3.29	3.48	0.22	17.92	0.1620	0.5694
Protein (%)	2.68	2.82	2.99	0.09	9.38	0.0617	0.2330
Lactose (%)	4.52	4.60	4.66	0.08	5.04	0.3416	0.7045
SNF (%)	8.14	8.34	8.61	0.14	4.88	0.0582	0.3443
Total solid (%)	11.92	11.68	12.10	0.31	7.27	0.9189	0.3880

Table 4.6 Effect of plant oil supplement on milk composition yield (g/d).

Item	Control (1)	Sunflower oil (2)	Soybean oil (3)	SEM	%CV	Contrast	
						1 vs 2 & 3	2 vs 3
Fat yield (g/d)	682	599	636	54.36	24.06	0.3381	0.6817
Protein (g/d)	478	514	548	23.52	12.99	0.0875	0.3669
Lactose (g/d)	807	848	860	46.04	15.54	0.4503	0.9389
SNF (g/d)	1451	1540	1586	76.05	14.12	0.2681	0.7500
Total solid (g/d)	2132	2139	2222	119.3	15.60	0.7828	0.6965

Table 4.7 Effect of plant oil supplement on body weight change.

Item	Control (1)	Sunflower oil (2)	Soybean oil (3)	SEM	%CV	Contrast	
						1 vs 2 & 3	2 vs 3
BW (kg)							
Pre – exp.	452	451	446	17.5	11.0	0.7890	0.8566
Post – exp.	450	453	447	16.3	10.3	0.9792	0.8080
BWC (g/d)	-70.84	79.17	19.05	130	42.4	0.4320	0.8348

Note : BW = Body weight

BWC = Body weight change

Fatty acid compositions in milk fat of the 3 groups of cows were shown in Table 4.8. Fatty acids $C_{6:0}$, $C_{8:0}$ and $C_{16:0}$ were significantly reduced ($p<0.05$) by supplementation of the two oils when compared to the control group. However, $C_{18:0}$, $C_{18:1n9t}$, $C_{18:1n9c}$ and $C_{18:2n6t}$ fatty acids were significantly increased ($p<0.05$) by oil supplementation compared to the control group while $C_{18:2n6c}$ fatty acids were similar ($p>0.05$) in all treatment groups. CLA (cis-9, trans-11 octadecadienoic) were significantly increased ($p<0.05$) by oil supplementation being 4.09, 5.50 and 6.12 mg/g milk fat for the control, sunflower oil and soybean oil cows respectively.

There were no significant differences in fatty acid composition between the two oils. Supplementation of both oils significantly reduced short and medium chain fatty acids while they significantly increased long chain fatty acids and unsaturated fatty acids. Saturated fatty acids were not affected by both plant oils supplementation.

Table 4.8 Effect of plant oil supplement on fatty acid profile of milk fat.

Item	Control (1)	Sun flower oil (2)	Soybean oil (3)	SEM	%CV	Contrast	
						1 vs 2 & 3	2 vs 3
	---------- mg/g milk fat ----------						
$C_{4:0}$	17.30	15.71	15.87	0.79	13.72	0.1222	0.9996
$C_{6:0}$	13.09	11.19	10.57	0.66	15.90	0.0130	0.4967
$C_{8:0}$	7.62	6.23	5.53	0.49	21.67	0.0106	0.3394
$C_{10:0}$	15.97	15.65	11.70	1.61	31.31	0.2490	0.0978
$C_{11:0}$	1.93	1.35	1.72	0.24	41.10	0.1981	0.3238
$C_{12:0}$	50.49	45.96	44.81	2.23	13.39	0.0830	0.7466
$C_{13:0}$	1.54	1.44	1.32	0.18	34.49	0.4616	0.6209
$C_{14:0}$	101.13	95.28	91.90	4.86	14.27	0.2348	0.6711
$C_{14:1}$	9.74	8.04	10.08	0.95	29.14	0.5974	0.1427
$C_{15:0}$	6.03	5.18	5.42	0.33	16.98	0.0906	0.6404
$C_{16:0}$	262.35	223.63	236.12	10.10	11.86	0.0193	0.3782
$C_{16:1}$	18.65	14.87	19.13	0.85	13.82	0.1504	0.0024
$C_{18:0}$	76.51	111.48	97.91	5.22	15.51	0.0004	0.0648
$C_{18:1n9t}$	16.39	26.49	30.29	3.05	35.75	0.0051	0.4136
$C_{18:1n9c}$	181.84	228.12	232.64	13.03	17.27	0.0079	0.8612
$C_{18:2n6t}$	0.430	1.06	1.49	0.10	29.63	0.0001	0.0072
$C_{18:2n6c}$	12.13	12.71	14.55	1.82	39.29	0.5299	0.5277
$C_{20:0}$	1.31	1.46	2.73	0.51	79.81	0.2290	0.1071
$C_{18:3n6}$	0.99	1.06	1.58	0.11	25.92	0.0304	0.0045
CLA[1]	4.09	5.50	6.12	0.57	31.02	0.0246	0.4572
$C_{22:0}$	>0.01	0.08	>0.01	0.03	31.72	0.3317	0.0781
$C_{20:3n3}$	0.04	0.16	0.09	0.06	41.53	0.2795	0.5052
$C_{22:1n9}$	0.84	0.44	0.39	0.11	53.84	0.0051	0.7709
Short[2]	107.95	97.55	91.52	5.96	12.00	0.0177	0.3174
Medium[3]	397.90	346.99	362.66	21.07	11.41	0.0334	0.4446
Long[4]	294.56	388.62	387.86	26.59	14.95	0.0008	0.9002
Saturated	553.97	533.11	522.87	27.11	10.09	0.2830	0.7046
Unsaturated	246.46	300.05	319.16	22.29	15.52	0.0047	0.4452

[1] CLA = *cis-9, trans-11 octadecadienoic acid*
[2] Short chains FA: ($C_{4:0} - C_{13:0}$)
[3] Medium chains FA: ($C_{14:0} - C_{17:0}$)
[4] Long chains FA: ($\geq C_{18:0}$)

4.9 Discussion

Analyses of chemical composition of feeds found that both concentrates supplemented with the 2 oils showed lower chemical composition than the unsupplemented control concentrate except for fat and energy that were increased. This is due to the fact that plant oil contains higher energy concentration and has 86%

true digestibility (NRC, 2001). Estimates of TDN_{1X}, DE_P and NE_{LP} in oil supplemented concentrate were relatively high.

Feeds contain various fat content and fatty acid composition. Analyses of type and content of fatty acids in this experiment were close to those reported by Chow (2000) and Dhiman et al. (1999). They reported that plant oils containing linoleic acid contents in descending order were safflower, sunflower, corn, soybean, cottonseed, sesame, rice bran, peanut and palm oil. Supplementation of sunflower and soybean oils to dairy cow ration should increase linoleic acid content in the diet and thus should increase CLA content in milk.

When dairy cows received diets containing fat, there will be three processes occur in the rumen. Firstly, lipid was hydrolyzed to be fatty acids and glycerol by extracellular enzymes produced by ruminal bacteria. Fatty acids (linoleic acid, *cis*-9 *cis*-12) will then isomerized from cis form to be trans form at cis-12 position to be trans-11 or CLA (*cis*-9 *trans*-11) by Δ^{12} *cis*, Δ^{11} *trans* isomarase (Chouinard et al., 1999). Some fatty acids will be hydrogenated at cis-9 position to be single bond in the form of *trans*-11 (vaccenic acid) and further hydrogenated to be stearic acid. All the form of fatty acids can transfer to small intestine and absorbed to portal blood. These fatty acids will then synthesized again at the tissues to be CLA by Δ^9 –desaturase by adding double bond at the 9^{th} position to be in the form of *cis*-9 *trans*-11. (Abu-Ghazaleh et al., 2001;2002; Griinari et al., 1999; Baer et al., 2000 and Whitlock et al. 2002). Corl et al. (2000) reported that 65% of CLA synthesis depened on Δ^9 – desaturase.

There were no significant differences in DM and CP consumption in the present study. Two hundreds gram/cow/day of plant oils did not affect feed intake.

Khorasani and Kennelly (1998) reported that supplemented more than 2% fish oil reduced DM intake. Similarly, Donovan et al. (2000) suggested that DM intake and milk yield were decreased by 2% and 3% fish oil supplementation when compared to 1% supplementation. The reason of reducing feed intake when high oil supplementation was probably due to palatability and feed degradation in the rumen. When oil was supplemented at high level, oil may coat fiber particles in the rumen and thus decreased rate of fiber degradation and reduced feed intake (Murphy et al., 1987; Khorasani and Kennelly. 1998). Mohammed et al. (1988) also reported similar results when 4% of oil was supplemented in the diet. Cant et al. (1997) suggested that milk yield would have been affected by oil when the rate of addition was 500 g/day onwards. Cows received less than 500 g/d of oil showed no effects on milk yield, fat and protein content. However, cows on plant oils had higher energy intake than those cows on control group. Increase in energy intake reflected the energy concentration in plant oils.

Milk yield were not affected by plant oil supplementation. This agreed with research done by Dhiman et al. (2000) which supplement 1, 2, 3 and 4% of soybean oil to dairy cattle diet. Thus found those milk yields were similar in all treatment groups. However, previous work of Dhiman et al. (1999) found increases in milk yield when the diet was supplemented with extruded soybeans or extruded cotton seeds. This may be attributed to the fact that extruded oil seeds contained bypass protein and thus higher amino acids absorbed at intestines (Solomon et al., 2000; Madron et al., 2002).

Although, there were no significant differences in milk composition among treatment groups, fat yield and fat content tended to be reduced in supplemented cows.

This may be due to coated fiber by oil reduced fiber digestion in the rumen and thus lowered milk fat content and fat yield. End products of fiber digestion were acetate and butyrate which were the precursors for milk fat synthesis in mammary glands. Reduced fiber digestion affected reduction of these volatile fatty acids and thus reduced milk fat synthesis (Khorasani and Kennelly, 1998).

Both sunflower and soy bean oils contained higher long chain fatty acids ($C_{18:0}$ - $C_{22:6}$) and fatty acid profile in milk fat reflected fatty acids in feeds, thus long chain fatty acids increased while short ($C_{4:0}$ - $C_{13:0}$) particularly $C_{6:0}$ and $C_{8:0}$ and medium chain ($C_{14:0}$ - $C_{17:0}$) fatty acids particularly $C_{16:0}$ decreased in the present study. Donovan et al. (2000) and Dhiman et al. (1999; 2000) also found similar results. Short and medium chain fatty acids were synthesized de novo in mammary glands which acetate was believed to be precursor for short chain fatty acids in tissues. Oil supplementation reduced fiber digestion and thus acetate, short chain fatty acids therefore decreased (Banks et al., 1984; Grummer, 1991; Palmquist et al., 1993). However, Ney (1991) suggested that reduction in medium chain fatty acids reduced risk of accumulation of cholesterol in blood stream. Long chain fatty acids ($C_{16:0}$, $C_{18:1n9t}$, $C_{18:1n9c}$, $C_{18:2n6c}$) increased with oil supplementation in this study which was similar to the finding of Donovan et al. (2000) and Dhiman et al. (1999; 2000).

Milk fat is generally synthesized from dietary fat and fat from body reserved in the adipose tissues. However, if cows received adequate fat from feeds, mobilization of fat from body reserved is reduced and directly from the dietary fat (Holmes and Wilson, 1984)

Cows on oil supplementation groups showed significantly higher CLA (cis-9, trans-11 octadecadienoic) content in milk than cows on the control diet. Similarly,

Dhiman et al. (2000) found significantly increases in CLA content in milk (237% and 314%) when 3 and 4% of soy bean oil were supplemented respectively. Kelly et al. (1998) also found an increase in 500% CLA in milk when 5.3% of oil was supplemented to dairy cattle diet. However, milk fat content was reduced from 3.38% to 2.25%.

4.10 Conclusion

Supplementation of 200 g/d sunflower oil or soybean oil to dairy cattle diets increased CLA content in milk without affecting other performances such as DM intake, milk yield, milk composition and body weight gain. Short and medium chain fatty acids were reduced while long chain fatty acids were increased when the diet was supplemented with plant oils, compared to the unsupplemented control. However, an increase in CLA content in milk is considered to be relatively low. Therefore, other methods to increase CLA content in milk should be further researched.

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CHAPTER V

The study of soybean oil and lactic acid bacteria supplementation on performance and CLA accumulation in milk of Crossbred Holstein Friesian dairy cow

5.1 Abstract

The objective of this study is to increase CLA content in dairy cow's milk and their performances through addition of lactic acid bacteria and soy bean oil in diets. Twenty four Crossbred Holstein Friesian lactating dairy cows, averaging 22.6 $\pm$ 5.7 kg milk/d, 96 $\pm$ 5.5 days in milk and 457 $\pm$ 54 kg body weight, were blocked according to milk yield and days in milk. They were then randomly assigned into three treatments, being control group, addition of *Lactobacillus plantarum* at 1 x 10^9 cfu/cow/day plus 200 g/d soy bean oil and addition of *Lactobacillus acidophilus* at 1 x 10^9 cfu/cow/day plus 200 g/d soy bean oil. The experiment was a Randomized Complete Block Design (RCBD).

There were no significant differences in DMI, CPI, NELI, milk yield and milk composition ($p>0.05$). There were no significant differences ($p>0.05$) in CLA (cis-9, trans-11 and trans-10, cis-12 octadecadienoic acid) levels among the three groups. Thus lactic acid bacteria addition had no effect on CLA concentration in milk. However, short-medium chain fatty acids and saturated fatty acids were significantly increased ($p<0.05$) by lactic acid bacteria addition. Furthermore, ruminal pH, volatile

fatty acids including acetate, propionate, butyrate and acetate: propionate ratio were unaffected (P>0.05) by lactic acid bacteria addition. Lactic acid bacteria addition had no effect (p>0.05) on number of microorganisms in the rumen.

5.2 Introduction

Conjugated linoleic acid can be synthesized by rumen microbes, particularly *Butyrivibrio fibrisovens*, from dietary fat through biohydrogenation process. Further researches found that some bacteria can also produce CLA by converting linoleic acid added to media (Jiang et al., 1998; Lin et al., 1999; and Alonso et al., 2003). In addition, Ogawa et al. (2001) found that *L. acidophilus* and *L. plantarum* can produce CLA *in vitro*. It is, therefore, interested to study the effect of lactic acid bacteria addition to dairy cattle diet on CLA content in milk.

5.3 Objective

The objective of this experiment was to investigate the effect of lactic acid bacteria supplementation in the diet on CLA content of dairy cow's milk.

5.4 Materials and methods

In this study, there were many steps as follow:

Preliminary study of producing CLA from Lactic acid bacteria

1. Lactic acid bacteria, *L. acidophilus* and *L. plantarum*, were selected from Microbiology Laboratory, the Center for Scientific and Technological Equipments 2. (Gift from Dr. Sureelak Rodtong)

2. Study of CLA production from the 2 Lactobacilli was adapted from the method of Kishino et al. (2003) as follow:

1.1 Microorganism cultivation and preparation of washed cells.

L. acidophilus and *L. plantarum* were aerobically cultivated in MRS (pH 6.5). The strains were inoculated in 15 ml of liquid medium in screw-cap tubes. The liquid medium occupied approximately 80 to 90% of the volume of the tubes. Cultivations were carried out for 24 hour at 28°C with shaking in water bath. The cells were harvested by centrifugation (14,000 × *g*, 30 min), washed twice with 0.85% NaCl, centrifuged again, and then used as the washed cells.

1.2 Reaction conditions.

Reactions were carried out at 28°C with gentle shaking in screw-cap tubes under aerobic conditions with or without replacement of the air in the tubes by pure nitroge. The reaction mixture contained, in 1 ml of 100 mM potassium phosphate buffer (pH 6.5), 5 mg of linoleic acid in a complex with bovine serum albumin (BSA) (0.2 mg of BSA/mg of linoleic acid) and the washed cells from 15 ml of culture broth.

1.3 Lipid analyses and methylation.

Lipids were extracted from the reaction mixture with chloroform-methanol (1:2, vol/vol) according to the procedure of Bligh and Dyer (1959) and methylation with boron trifluoride according to the procedure of Ostrowska et al. (2000).

The study of utilization of lactic acid bacteria in crossbred Holstein Friesian diet

Feed and animal management

Dairy cattle and feeding managements

Soybean oil was chosen because it can give appropriate CLA content of milk and cheaper than other linoleic acid contained plant oil (chapter 4).

Twenty four Crossbred Holstein Friesian lactating dairy cows, averaging 22.6 $\pm$ 5.7 kg milk/d, 96 $\pm$ 5.5 days in milk and 457 $\pm$ 54 kg body weight, were blocked according to milk yield and days in milk. They were then randomly assigned into three treatments of 8 cows in each group, being control group, addition of *L. plantarum* at 1 x 10^9 cfu/cow/day plus 200 g/d soybean oil and addition of *L. acidophilus* at 1 x 10^9 cfu/cow/day plus 200 g/d soybean oil. The experiment was a Randomized Complete Block Design (RCBD).

Group 1. Eight cows received concentrate plus 200 g/d soybean oil (control).

Group 2. Eight cows received concentrate plus 200 g/d soybean oil and *L. plantarum* at 1 x 10^9 cfu/cow/day.

Group 3. Eight cows received concentrate plus 200 g/d soybean oil and *L. acidophilus* at 1 x 10^9 cfu/cow/day.

The experiment lasted for 40 days including 10 days for adjustment period followed by six five-day periods for measurements.

Feed intake and dairy cow's performances

Feed offered and left uneaten were sampled on 2 consecutive days in each period of 6 five – day periods. Samples were then dried at 60 °C until dry and were ground through 1 mm sieve. Samples taken were subjected to several analysis as

follow; proximate analysis (CP, DM, EE and ash) (AOAC, 1990); detergent analysis (NDF, ADF and ADL) (Georing and Van Soest, 1970) and fatty acids in the diets by Gas chromatography.

Milk yields were recorded daily while milk samples were taken on 2 consecutive days in each period of 6 five – day periods. They were then analyzed for fat, protein, lactose, SNF and total solid) by MilkoScan S50. On day 10, 20 and 30 of the experimental period, milk samples were taken to analyze for fatty acids and CLA (gas chromatography; Hewlett Packard GCD system HP 6890).

Analysis of fatty acid by Gas chromatography (GC)

Fatty acids analysis by GC was done as reported in chapter 3.

The study of microorganism population in the rumen

Six fistulated non-lactating dairy cows were used to determine population of rumen microorganisms. The experimental design was 3x3 Replicated Latin square designs including:

	Cow No.					
	Square 1			**Square 2**		
	1	**2**	**3**	**4**	**5**	**6**
Period 1	control	L. plantarum	L. acidophilus	control	L. plantarum	L. acidophilus
Period 2	L. plantarum	L. acidophilus	control	L. plantarum	L. acidophilus	control
Period 3	L. acidophilus	control	L. plantarum	L. acidophilus	control	L. plantarum

The control diet was added 200 g/d soybean oil. Cows were allowed 1 week for adjustment period followed by 7-day periods of measurement.

Sampling for determination of rumen microorganisms population

Digesta from the rumen were collected 6 h after morning feeding (Ghorbani et al, 2002). They were then placed in plastic bag and then on to plates and into an anaerobic jar with anaerocult A to obtain oxygen free jar. Samples were plated on 4 different media; PDA (Potato Dextrose Agar), Rogosa, Streptococcus selective agar and E-Medium for anaerobes for plate count.

Collection of rumen fluid for protozoa count.

Rumen fluid was collected from rumen after 6 h. feeding (Ghorbani et al, 2002). Digesta were squeezed through nylon cloth, 5 ml of rumen fluid was then diluted with normal saline (10%, v/v) formaldehyde solution in 0.85% (w/v) NaCl 20 ml. Protozoa were then counted by microscopic count method (Ogimoto and Imai, 1981).

Volatile fatty acid (acetate, butyrate and propionate) by High performance liquid chromatography (HPLC)

Duplicate samples (5 ml) were added to 1 ml of protein precipitant (metaphosphoric acid/Formic acid: 18.75% (w/v)/25% (v/v)). One ml of the internal standard (isocaproic acid: 0.52% v/v) was added to one sample (internal standard sample), and 1 ml of distilled water was added to the other sample (control sample). Both samples were centrifuged at 1895 x g for 15 min and stored at -20°C until analysis. The concentrations of individual VFAs were measured by High performance liquid chromatography (HPLC) (Pecina et al., 1984).

Concentration of 0.1, 0.3 and 0.6 mol acetate, butyrate and lactate standard solutions were prepared for calibration curve and recovery rate (%) of VFAs.

Rumen fluid samples were through filter membrane with pore size of 0.4 μm, they were then injected to HPLC with condition of: Column: Aminex HPX-87H, Guard column, Detector: UV at 210 nm., Flow rate : 0.6 ml/min, sample size 10 μl, column temperature : 41°C, Mobile phase : 0.0025M H_2SO_4

5.5 Data analysis

Data were subjected to analysis of variance in RCBD and 3x3 Replicated Latin square designs. Differences between means were subjected to orthogonal comparison using SAS (SAS, 1988).

5.6 Experimental site

The experiment was conducted at Suranaree University of Technology's dairy farm, the center for Scientific and Technological Equipments building 1 and 3, Suranaree University of Technology.

5.7 Duration

May – August 2005.

5.8 Results

Table 1 shows the preliminary study of lactic acid bacteria's ability to produce CLA. *L. plantarum* and *L. acidophilus* were used in this study. Both lactic acid bacteria have the ability to produce CLA although they produced very low level of CLA (11.00 and 8.48% of linoleic acid in the media respectively). However, both lactic acid bacteria were used in the following experiment.

Table 5.1 Fatty acid produced from linoleic acid by lactic acid bacteria.

Item	*L. plantarum*	*L. acidophilus*
fatty acid profile	**---------- mg/5 mg linoleic acid ----------**	
$C_{14:0}$	0.000	0.000
$C_{16:0}$	0.004	0.005
$C_{18:0}$	0.000	0.000
$C_{18:1n9c}$	0.081	0.087
$C_{18:2n6c}$	0.541	0.497
Total CLA[1]	0.550	0.424
Other	2.210	2.806

[1] CLA1 = *cis-9, trans-11 octadecadienoic acid* and*, trans-9, trans-11 octadecadienoic acid*

Table 5.2 Chemical compositions of feeds.

Item	Concentrate	Grass silage
Chemical composition	---------- % of DM ----------	
Dry matter	94.57	24.86
Crude protein	20.91	9.25
Ether extract	6.31	2.08
Ash	7.87	10.94
Crude fiber	11.46	36.71
Neutral detergent fiber	41.76	68.38
Acid detergent fiber	16.52	46.96
Acid detergent lignin	3.94	7.59
Neutral detergent insoluble N	0.97	0.45
Acid detergent insoluble N	0.79	0.52
TDN_{1x} (%)[1]	70.79	46.56
DE_P (Mcal/kgDM) [2]	3.58	2.34
ME_P (Mcal/kgDM) [3]	3.18	1.92
NE_{LP} (Mcal/kgDM) [4]	2.05	1.15

[1] TDN_{1X} (%) = tdNFC + tdCP + (tdFA x 2.25) + tdNDF – 7

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

Discount = [(TDN_{1X} – [(0.18 x TDN_{1X}) – 10.3]) x Intake)]/TDN_{1X}

DE_P (Mcal/kgDM) = DE_{1X} x Discount

[3] ME_p = [1.01 x (DE_p) – 0.45] + [0.0046 x (EE – 3)]

[4] NE_{Lp} = ([0.703 x ME_p (Mcal/kg)] – 0.19) + ([(0.097 x ME_p + 0.19)/97] x [EE – 3])

Chemical composition of concentrate and grass silage used in the present experiment is given in Table 5.2. Mean values for DM, CP, EE, CF, NDF, ADF and ADL of concentrate and grass silage were 94.57 and 24.86, 20.91 and 9.25, 6.31 and 2.08, 11.46 and 36.71, 41.76 and 68.38, 16.52 and 46.96, and 3.94 and 7.59% respectively.

An evaluation of energy concentration in concentrates and grass silage showed that concentrates and grass silage contained 70.79 and 46.56% TDN1x, 3.58 and 2.34 Mcal/kgDM DEp, 3.18 and 1.92 Mcal/kgDM MEp, and 2.05 and 1.15 Mcal/kgDM NELp respectively.

Fatty acid composition of concentrate, grass silage and soybean oil are showed in Table 5.3. Soybean oil contains high level of linoleic acid and has similar content as in Experiment 1. However, linoleic acid content in concentrate in this experiment was higher in Experiment 1(Chapter 4), while linoleic acid content in grass silage in this experiment was lower than that of corn silage in Experiment 1. (Chapter 4)

Table 5.3 Fatty acid composition of feeds and soybean oil.

Item	Concentrate	Grass silage	Soybean oil
	---------- % of total fatty acid ----------		
$C_{14:0}$	5.24	0.75	0.09
$C_{16:0}$	13.25	20.57	10.68
$C_{18:0}$	3.13	2.78	4.22
$C_{18:1n9c}$	24.57	4.26	22.41
$C_{18:2n6c}$	31.65	13.21	54.74
$C_{20:0}$	0.43	1.59	0.39
$C_{18:3n6}$	0.08	0.00	0.32
$C_{20:1}$	3.38	15.45	6.58
$C_{22:0}$	0.32	1.64	0.42
$C_{24:0}$	-	0.51	0.15
Others	17.94	39.24	-

Table 5.4 showed feed consumption of the experimental cows. Concentrate DM intake of the three groups was similar at 9.6 kg/d, while grass silage DM intakes were 5.3, 5.9 and 5.4 kg/d for cows on control, *L. plantarum* and *L. acidophilus* respectively which were not significant differences among treatments. Similarly, total DM intakes were unaffected by the treatments.

Concentrate, grass silage and total CP and NE_L consumptions were similar in all treatment groups being 1941 g/d; 534, 585 and 614; 2525, 2544 and 2526 for cows on control, *L. plantarum* and *L. acidophilus* respectively.

Table 5.4 Effect of lactic acid bacteria supplement on DM, CP and NE intake.

Item	Control (1)	*L. plantarum* (2)	*L. acidophilus* (3)	SEM	%CV	Contrast	
						1 vs 2 & 3	2 vs 3
DM intake (KgDM)							
Concentrate	9.6	9.6	9.6	-	-	-	-
Roughage	5.3	5.7	5.4	0.20	7.79	0.2835	0.1330
Total	14.9	15.3	14.9	0.22	2.84	0.2799	0.1214
CP intake (g/d)							
Concentrate	1941	1941	1941	-	-	-	-
Roughage	584	614	585	10.52	3.66	0.2980	0.1023
Total	2525	2544	2526	10.86	9.88	0.3098	0.1111
NE_{LP} intake (Mcal/d)							
Concentrate	19.55	19.55	19.55	-	-	-	-
Roughage	6.17	6.59	6.21	0.24	7.74	0.2816	0.1299
Total	25.72	26.15	25.76	0.25	1.89	0.2863	0.1297

Milk yield and milk composition are given in Table 5.5 and 5.6. There were no significant differences (P>0.05) in milk yield and milk composition yield, percent fat, percent protein, percent lactose, percent solid not fat and percent total solid.

Initial and final live weights were similar (P>0.05) in all treatment groups (Table 5.7). However, there were significant differences (P<0.05) in live weight changes. Cows on both strain of lactic acid bacteria addition lost weight while cows on the control feed gained weight.

Table 5.5 Effect of lactic acid bacteria supplement on milk yield and milk compositions (%).

Item	Control (1)	*L. plantarum* (2)	*L. acidophilus* (3)	SEM	%CV	Contrast	
						1 vs 2 & 3	2 vs 3
Milk yield (Kg/d)	18.5	19.2	18.2	1.34	14.40	0.8650	0.4755
3.5%FCM	17.8	18.9	19.0	1.49	16.06	0.3835	0.9288
Fat (%)	3.27	3.44	3.79	0.29	16.75	0.1921	0.2386
Protein (%)	2.69	2.63	2.63	0.09	7.07	0.4544	0.9895
Lactose (%)	4.51	4.50	4.60	0.10	4.47	0.6040	0.3239
SNF (%)	8.10	8.05	8.15	0.16	3.87	0.9818	0.5253
Total solid (%)	11.38	11.49	11.95	0.36	6.17	0.2868	0.2148

Table 5.6 Effect of lactic acid bacteria supplement on milk composition yield (g/d).

Item	Control (1)	*L. plantarum* (2)	*L. acidophilus* (3)	SEM	%CV	Contrast	
						1 vs 2 & 3	2 vs 3
Fat yield (g/d)	603.6	652.1	686.0	66.55	20.56	0.2697	0.6154
Protein (g/d)	488.8	501.4	476.5	30.87	12.62	0.9934	0.4294
Lactose (g/d)	828.5	857.5	837.9	64.23	15.27	0.7333	0.7632
SNF (g/d)	1483.0	1534.5	1482.0	103.5	13.81	0.7850	0.6180
Total solid (g/d)	2087.0	2186.5	2186.0	154.0	14.35	0.5056	0.9056

Table 5.7 Effect of lactic acid bacteria supplement on body weight change.

Item	Control (1)	*L. plantarum* (2)	*L. acidophilus* (3)	SEM	%CV	Contrast	
						1 vs 2 & 3	2 vs 3
BW (Kg)							
Pre – exp.	456	463	452	28.93	12.66	0.9509	0.6984
Post – exp.	456	442	440	25.38	11.36	0.4957	0.9612
BWC (g/d)	17.9	-317	-607	264.7	-175	0.0491	0.2874

Note : BW = Body weight

BWC = Body weight change

Fatty acid compositions in dairy cow's milk of the three groups are presented in Table 5.8. Lactic acid bacteria supplementation significantly increased $C_{6:0}$ ($p<0.01$) compared to the control while *L. plantarum* addition significantly increased $C_{6:0}$ ($p<0.05$) compared to *L. acidophilus*.

$C_{10:0}$ short chain fatty acids were significantly increased ($p<0.05$) by lactic acid bacteria supplementation compared to the control group. Total short chain fatty acids were also significantly increased ($p<0.05$) by lactic acid bacteria addition. However, there were no significant differences ($p>0.05$) in other fatty acids when compared to the control cows.

CLA (cis-9, trans-11 and trans-10, cis-12 octadecadienoic acid) was not significantly different ($p>0.05$) among treatment groups due to lactic acid bacteria addition. However, medium and long chain fatty acids were significantly increased ($p<0.05$) when lactic acid bacteria were included in the diet.

Table 5.8 Effect of lactic acid bacteria supplement on fatty acid profile of milk fat.

Item	Control (1)	*L. plantarum* (2)	*L. acidophilus* (3)	SEM	%CV	Contrast	
						1 vs 2 & 3	2 vs 3
	---------- mg/g milk fat ----------						
$C_{4:0}$	21.74	22.81	23.23	1.25	11.04	0.3879	0.3980
$C_{6:0}$	11.26	12.95	13.85	0.63	9.93	0.0044	0.0143
$C_{8:0}$	7.70	6.77	7.84	1.28	34.52	0.5937	0.4746
$C_{10:0}$	11.55	13.75	20.52	4.29	56.16	0.0467	0.6136
$C_{11:0}$	1.21	1.39	1.51	0.16	23.36	0.1434	0.2634
$C_{12:0}$	37.79	41.64	41.33	2.60	12.92	0.4820	0.1539
$C_{13:0}$	1.11	1.14	1.58	0.33	51.15	0.4697	0.1639
$C_{14:0}$	63.08	78.84	78.89	5.29	14.37	0.0989	0.7400
$C_{14:1}$	10.59	9.54	14.14	3.23	56.64	0.1619	0.7484
$C_{15:0}$	4.93	4.99	5.09	0.31	12.41	0.6340	0.8301
$C_{16:0}$	179.58	188.50	197.62	11.08	11.75	0.1723	0.4301
$C_{16:1}$	20.46	22.36	22.06	2.13	19.75	0.7281	0.3844
$C_{18:0}$	73.58	80.28	88.29	8.89	22.05	0.1559	0.4600
$C_{18:1n9t}$	31.17	23.26	38.231	9.75	63.12	0.2068	0.4267
$C_{18:1n9c}$	293.74	306.86	291.20	16.62	11.19	0.5345	0.4390
$C_{18:2n6t}$	2.81	2.52	2.31	0.41	32.12	0.3261	0.4823
$C_{18:2n6c}$	25.04	25.22	24.19	1.89	15.91	0.2690	0.9258
$C_{20:0}$	2.70	1.24	2.12	1.06	105.36	0.8682	0.1854
$C_{18:3n6}$	1.30	1.32	1.23	0.09	13.82	0.2949	0.8892
CLAa[1]	6.86	6.76	7.01	0.61	17.78	0.7088	0.8701
CLAb[2]	0.13	0.06	0.06	0.05	116.63	0.3982	0.1787
$C_{22:0}$	0.41	0.37	0.45	0.05	24.69	0.1836	0.4082
$C_{20:3n6}$	0.91	0.93	1.07	0.09	19.62	0.2050	0.1969
$C_{22:1n9}$	0.79	0.67	0.78	0.08	22.35	0.4867	0.1425
Short[3]	92.34	100.88	109.41	5.57	11.05	0.0153	0.1413
Medium[4]	278.64	304.23	317.79	15.54	10.35	0.0259	0.3930
Long[5]	449.51	449.70	442.48	12.58	5.64	0.6628	0.4260
Saturated	413.50	453.50	475.44	22.52	10.07	0.0167	0.3465
Unsaturated	406.99	401.32	394.24	21.30	10.66	0.5716	0.6413

[1] CLAa = *cis-9, trans-11 octadecadienoic acid*
[2] CLAb = *trans-10,cis-12 octadecadienoic acid*
[3] Short chains FA: ($C_{4:0} - C_{13:0}$)
[4] Medium chains FA: ($C_{14:0} - C_{17:0}$)
[5] Long chains FA: ($\geq C_{18:0}$)

Levels of rumen pH at various hours after feeding of experimental cows are given in Table 5.9. After feeding, pH in the rumen measured from rumen fluid decrease as the time after feeding increased up to hours 5, then gradually increase. However, there were not statistically different (P>0.05) among treatments.

Table 5.9 showed concentrations of acetate, propionate, butyrate and A:P ratio in the rumen. Concentration of acetate, propionate, butyrate and A:P ratio were similar (P>0.05) in all treatments by lactic acid bacteria addition.

Table 5.9 Effect of lactic acid bacteria supplement on pH level and VFAs of ruminal fermentation.

Item	Control (1)	*L. plantarum* (2)	*L. acidophilus* (3)	SEM	%CV	Contrast.	
						1 vs 2 & 3	2 vs 3
pH level							
Hour 0	6.64	6.54	6.66	0.10	6.19	0.4012	0.374
Hour 1	6.34	6.40	6.54	0.11	6.73	0.1655	0.3462
Hour 2	6.34	6.31	6.47	0.16	2.28	0.3365	0.8489
Hour 3	6.41	6.23	6.48	0.17	4.12	0.2786	0.2986
Hour 4	6.33	6.22	6.40	0.17	3.51	0.3572	0.4835
Hour 5	6.45	6.41	6.57	0.13	5.24	0.2286	0.7394
Hour 6	6.36	6.20	6.35	0.14	2.58	0.4168	0.1364
VFAs mol/100mol							
Acetate	73.17	75.15	75.27	1.41	3.86	0.5153	0.3244
Propionate	15.49	18.39	15.98	1.11	14.91	0.4224	0.0575
Butyrate	5.66	6.33	6.89	1.04	19.19	0.2745	0.4364
Acetate: Propionate	4.87	4.17	4.75	0.34	16.31	0.5423	0.1217

Table 5.10 Effect of lactic acid bacteria supplement on bacteria and protozoa population in the rumen.

Item	Control (1)	*L. plantarum* (2)	*L. acidophilus* (3)	SEM	%CV	Contrast.. 1 vs 2 & 3	Contrast.. 2 vs 3
Grouping bacteria	-------- x 10^6 cfu / 1 g. digesta -------						
Lactobacilli	1.72	1.60	1.79	0.18	21.96	0.4750	0.5259
Clostridia	1.66	1.78	1.96	0.29	18.55	0.2472	0.5776
Yeast + Mold	1.25	1.03	1.00	0.22	31.44	0.6479	0.2410
Streptococci	1.52	1.55	1.20	0.54	38.99	0.1307	0.8980
Protozoa (x 10^5/ ml)	3.20	2.60	3.21	0.56	30.56	0.4812	0.2366

Table 5.10 showed numbers of microorganisms in the rumen of fistulated cows received control diet and *Lactobacilli sp.* The microorganisms measured were Lactobacilli, Clostridia, Yeast and Mold, Streptococci and protozoa. The results showed that supplementation of lactic acid bacteria had no effects on number of microorganisms in the rumen ($p>0.05$).

5.9 Discussion

Preliminary study found an increase in CLA content when *L. plantarum* and *L. acidophilus* were added to the media. However, such increase is considered to be very low. Only 10.82 and 9.94% of CLA respectively were produced from linoleic acid added to the media by those two bacteria. Lin et al. (1999) also found 6.3 – 10.55% of CLA were produced when 1000 μg/ml linoleic acids were added to the media and 1.04 – 1.85% of CLA were produce when 5000 μg/ml linoleic acids were added.

Alonso et al. (2003) found much higher CLA produced (65.85%) when using *L. acidophilus.* Ogawa et al. (2001); Kishino et al. (2002) and Ando et al. (2003) found 82.5, 76.8 and 65.4% CLA produced respectively from linoleic acids by *L. acidophilus, L. plantarum* and *L. plantarum*.

Many published documents reported inhibition of metabolism and growth of bacteria by short and long chain fatty acids. Boyaval et al. (1995) found that linoleic acid had a negative effect on growth and metabolism of bacteria. Furthermore, inhibition effect can be reduced by protein source such as Tween-80 in media since Tween-80 has preventive characteristic against fatty acids (Dubos, 1947; Ledeoma et al., 1977; Baker et al, 1983). CLA can also produce by other bacteria, for example, Jiang et al. (1998) using 3 strains of *Propionibactrium freudenreichii* and found that 23.2, 35.37 and 22.86% CLA respectively were converted from linoleic acid by those bacteria. However, the present study not only determined the efficiency of producing CLA by *L. acidophilus* and *L. plantarum* but also investigated the effect of these bacteria on milk yield. (Kung et al., 2000)

Chemical composition analysis showed higher fat content and energy in soybean oil supplementation groups and has 86% true digestibility (NRC, 2001). Estimates of TDN_{1X}, DE_P and NE_{LP} in oil supplemented concentrate therefore were relatively high. Moreover, fatty acids content in feed and soybean oil in this experiment fit with the experiment 1. (chapter 4). But, fatty acids content of grass silage found $C_{16:0}$, $C_{18:0}$ and $C_{18.2n6c}$ lower than corn silage in the experiment 1. (chapter 4)

The present study found no significant differences in DM, CP and NE_L consumptions among treatment groups. Milk yields and fat corrected milk yields were

unaffected by bacteria addition, although bacteria addition cows tended to produce 0.9 – 1.2 kg/d higher fat corrected milk than the control cows.

This is consistent with the finding of Jaquette et al., (1988) and Ware et al., (1988) who found that milk yield increased when 1 x 10^9 cfu/cow/day *L. acidophilus* were added to the diet. Jeong et al. (1998) also found an increase in 0.8 kg/d milk yield when *Lactobacillus sp.* was included in the diet. Increases in milk yield reflected higher lactate produce and thus propionate produced when lactic acid bacteria were added to the diet. Propionate has been known to be precursors for glucose synthesis and thus lactose synthesis in mammary glands. Higher lactose was synthesized, higher milk yield was produced.

The $C_{6:0}$ and $C_{10:0}$ fatty acids were increased when lactic acid bacteria were added to the diet in the present experiment. Although, acetate and butyrate contents in the rumen were not statistically different between supplemented and unsupplemented groups, they were numerically higher in lactic acid bacteria supplemented groups. Short and medium chain fatty acids were subjected to de novo synthesis in mammary glands from acetate (Banks et al., 1984; Grummer, 1991; Palmquist et al., 1993). A tendency towards increases in acetate resulted in increases in short ($C_{4:0}$- $C_{13:0}$) and medium ($C_{14:0}$- $C_{17:0}$) chain fatty acids and saturated fatty acids in milk.

The present experiment found that after feeding, pH in the rumen measured from rumen fluid decreased as the hour after feeding increased up to 5 hours, then slightly increased. Levels of pH in the rumen below 5.9 can cause rumen acidosis (Seal and Parker, 1994 and Garrett et al., 1999). The lowest rumen pH in the present experiment was at 5 hours after feeding and was higher than 5.9. Feeding 1 x 10^9 cfu/cow/day lactic acid bacteria had no toxic effect on rumen pH. Kim et al. (2000)

also found unchanged rumen pH when fed *P. acidipropionici* and *L. plantarum* to the cows. In contrast, Nocek et al. (2000) found reduction in rumen pH up to 5.5 when *Enterococcus* and *Lactobacillus* were fed to the cows. This will cause a risk of sub clinical ruminal acidosis.

Concentrations of acetate, propionate, butyrate and A:P ratio in the rumen. Concentration of acetate, propionate, butyrate and A:P ratio were similar (P>0.05) in all treatments by lactic acid bacteria addition in the present experiment. Similarly, Kim et al. (2000) observed increases in propionate when lactate-producing and –utilizing bacteria (*L. plantarum* and *P. acidipropionici*) were fed. Acetate:Propionate lower than 2.2:1 can cause rumen acidosis. The present experiment observed acetate:propionate ratios of all cows were in the range of 2.99 – 3.32 which were higher than the risk level. Garrett et al. (1999) suggested that when ruminal pH reduced to below 5.9, acetate:propionate ratio dropped below 2.2:1.

The results of the present experiment showed that supplementation of lactic acid bacteria had no effects on number of microorganisms in the rumen. Boyaval et al. (1995) found that linoleic acid had a negative effect on growth and metabolism of bacteria. Soybean oil was included in concentrate in the present experiment since it contained high amount of long chain fatty acids. These fatty acids probably inhibit the growth and metabolism of the microorganisms in the rumen. Galbraith and Miller (1973ab) reported that unsaturated fatty acids can inhibit cell respiration and thus cause cell lysis.

5.10 Conclusion

The present study revealed that lactic acid bacteria supplementation had no effect on CLA content of milk, DM, CP, and NE_L consumptions, milk yield and milk compositions. However, lactic acid bacteria inclusion in the diet significantly increased short and medium chain fatty acids, and saturated fatty acids in milk while long chain fatty acids and CLA content in milk, rumen pH, VFAs and microorganism population in the rumen were unaffected by the addition of lactic acid bacteria.

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CHAPTER VI

Overall Discussion and Implication

Studies of factors affecting change in CLA content in dairy cow's milk used recorded data from crossbred Holstein Friesian reared at Suranaree University Dairy Farm during March 2004 and February 2005. Increasing CLA content in dairy cow's milk by supplementation of high linoleic acid plant oils and by supplementation of lactic acid bacteria were also conducted at the same location with similar dairy cows. Results from these studies can be summarized as follows:

The first study found that CLA content in milk varied from 4.45 – 6.13 mg/g. milk fat throughout the year. Most factors studied had low correlation to CLA content except for linoleic acid and linolenic acid intakes which had high correlation to CLA content in milk. Using backward stepwise multiple regression procedure to study relationship between various variables to CLA content in milk obtained the best equation as follows:

CLA = 2.5993 -0.004583AGE + 0.00605DIM - 0.35067MP + 0.02549LA.

Where; CLA = CLA Yield (mg/g milk fat), AGE = Age (month), DIM = Day in milk (day), MP = Milk protein (%) and LA = Linoleic acid intake (g/day). (R^2 = 0.458).

From this equation, it can be concluded that increase in CLA content in milk largely depended on amounts of linoleic acid and linolenic acid content in the diet. If we want more accumulation of CLA in milk, we should supplement the diet with high

linoleic acid and linolenic acid ingredients such as sunflower seeds, soybean seeds, sunflower oil and soybean oil. However, other factors such as cost of ingredients should be taken into account before making the final decision.

The first experiment found that supplementation of 200 g/d of sunflower oil or soybean oil increased CLA content in milk without having any adverse effects on performances of the cows. Short and medium chain fatty acids reduced while long chain fatty acids increased when compared to the unsupplemented control. However, such increase was still low.

The second experiment suggested that lactic acid bacteria had the ability to produce CLA of milk in laboratory study but had only small effect in the field trial. The present study found that adding lactic acid bacteria to dairy cattle diet did not increase CLA content in milk and had no adverse effect to dairy cow's performances. Short and medium chain fatty acids and saturated fatty acids increased when compared to the unsupplement control cows. Long chain fatty acids and CLA were unaffected by lactic acid bacteria addition. Furthermore, addition of lactic acid bacteria to dairy cattle diet had no effect on ruminal pH, VFAs and ruminal microorganism population.

Future research should emphasize on other factors affecting CLA content in milk such as amount of *trans*-11 (vaccenic acid). Since many studies found relatively high relationship (R^2) between *trans*-11 (vaccenic acid) and CLA content of milk.

From the two present experiments, relative low increases in CLA content of milk (Appendix A) of 0.27 g/liter (Experiment 1) and 0.26 g/liter (Experiment 2) would not be sufficient to meet a minimum requirement of 1-3 g/d of adult.

Although supplementation of plant oils resulted in an increase in CLA content in milk, such increase was considered relatively low. Manipulations of feeding method, types of diets and balance of microbes in the rumen can all improve CLA production in dairy cow's milk and thus enhance chances of receiving more CLA by consumers and reduce risk of diseases.

APPENDIX A

Table 1 A. Effect of plant oil supplement on percent of total fatty acid.

(Chapter 4)

Item	Control (1)	Sun flower oil (2)	Soybean oil (3)	SEM	%CV	Contrast	
						1 vs 2 & 3	2 vs 3
	------ % of total fatty acid -----						
$C_{4:0}$	2.17	1.88	1.94	0.08	11.81	0.0193	0.6462
$C_{6:0}$	1.64	1.37	1.30	0.08	16.42	0.0077	0.6018
$C_{8:0}$	0.95	0.77	0.69	0.07	25.64	0.0228	0.4268
$C_{10:0}$	1.99	1.92	1.47	0.21	32.64	0.2517	0.1408
$C_{11:0}$	0.42	0.17	0.19	0.03	40.52	0.1175	0.5296
$C_{12:0}$	6.30	5.56	5.41	0.23	11.19	0.0080	0.6357
$C_{13:0}$	0.19	0.22	0.15	0.05	77.71	0.9770	0.3328
$C_{14:0}$	12.58	11.49	11.17	0.43	10.26	0.0265	0.6084
$C_{14:1}$	1.20	0.99	1.12	0.12	29.54	0.3039	0.4339
$C_{15:0}$	0.75	0.63	0.66	0.03	13.43	0.0110	0.4678
$C_{16:0}$	32.9	26.79	28.9	0.81	7.74	0.0001	0.0819
$C_{16:1}$	2.36	1.78	2.17	0.35	17.19	0.0239	0.0470
$C_{18:0}$	9.52	13.33	12.04	0.52	12.59	0.0001	0.0939
$C_{18:1n9t}$	2.01	3.12	3.49	0.34	33.42	0.0054	0.4574
$C_{18:1n9c}$	22.64	27.28	26.27	1.20	13.36	0.0113	0.5561
$C_{18:2n6t}$	0.06	0.13	0.16	0.02	47.96	0.0015	0.2975
$C_{18:2n6c}$	1.47	1.52	1.68	0.20	36.05	0.6161	0.5630
$C_{20:0}$	0.16	0.17	0.31	0.06	77.01	0.2851	0.1006
$C_{18:3n6}$	0.12	0.12	0.17	0.02	37.86	0.3542	0.1161
CLA[1]	0.49	0.66	0.70	0.07	33.59	0.0474	0.7000
$C_{22:0}$	>0.001	0.009	>0.001	0.004	349.93	0.3340	0.1019
$C_{20:3n3}$	0.004	0.02	0.01	0.008	198.07	0.2663	0.4244
$C_{22:1n9}$	0.11	0.05	0.04	0.01	54.24	0.0006	0.5719
Short[2]	13.50	11.91	10.93	0.77	12.70	0.0061	0.2309
Medium[3]	49.98	41.68	43.22	1.34	5.95	0.0001	0.2064
Long[4]	36.62	46.42	45.78	1.81	8.43	0.0001	0.6350
Saturated	69.35	64.14	62.36	1.62	4.94	0.0004	0.3178
Unsaturated	30.64	35.86	37.58	1.62	9.36	0.0004	0.3387

[1] CLA = *cis-9, trans-11 octadecadienoic acid*
[2] Short chains FA: ($C_{4:0} - C_{13:0}$)
[3] Medium chains FA: ($C_{14:0} - C_{17:0}$)
[4] Long chains FA: ($\geq C_{18:0}$)

Table 2 A. Effect of plant oil supplement on milk Fatty acid yield (g/d).

(Chapter 4)

Item	Control (1)	Sun flower oil (2)	Soybean oil (3)	SEM	%CV	Contrast	
						1 vs 2 & 3	2 vs 3
	---------- g/day ----------						
$C_{4:0}$	11.77	9.41	10.32	1.42	27.15	0.1359	0.6070
$C_{6:0}$	8.99	6.75	6.96	1.23	32.42	0.0591	0.9053
$C_{8:0}$	5.24	3.77	3.57	0.75	35.69	0.0272	0.7758
$C_{10:0}$	11.00	9.44	7.70	1.90	40.20	0.1492	0.3514
$C_{11:0}$	1.31	0.81	1.07	0.23	43.81	0.0844	0.3244
$C_{12:0}$	34.19	27.55	28.22	3.62	24.07	0.0602	0.8713
$C_{13:0}$	1.03	0.88	0.82	0.19	41.99	0.2867	0.7225
$C_{14:0}$	68.49	57.09	58.43	8.00	26.04	0.1414	0.8808
$C_{14:1}$	6.47	4.70	6.35	1.00	34.51	0.3070	0.1212
$C_{15:0}$	4.06	3.09	3.37	0.40	22.97	0.0283	0.5344
$C_{16:0}$	179.06	133.57	150.39	18.75	24.26	0.0362	0.3954
$C_{16:1}$	12.97	8.83	12.09	1.57	27.87	0.0861	0.0566
$C_{18:0}$	52.05	66.31	63.92	7.99	26.38	0.0921	0.6646
$C_{18:1n9t}$	10.87	15.67	18.77	2.76	36.97	0.0176	0.3069
$C_{18:1n9c}$	124.00	135.54	147.30	17.22	25.49	0.2811	0.5664
$C_{18:2n6t}$	0.32	0.63	0.90	0.10	32.18	0.0001	0.0136
$C_{18:2n6c}$	7.47	7.53	9.26	1.21	30.13	0.4172	0.2061
$C_{20:0}$	0.89	0.87	1.67	0.40	70.91	0.2933	0.0710
$C_{18:3n6}$	0.70	0.62	1.01	0.02	36.04	0.3856	0.0146
CLA[1]	2.88	3.36	4.08	0.67	47.77	0.2567	0.4099
$C_{20:3n6}$	0.03	0.09	0.04	0.05	185.84	0.4760	0.4383
$C_{22:1n9}$	0.60	0.25	0.25	0.10	54.74	0.0010	0.9726
Short[2]	73.50	58.61	58.65	8.41	26.37	0.0548	0.9645
Medium[3]	271.04	207.28	230.63	28.36	23.98	0.0499	0.4356
Long[4]	199.83	230.89	247.21	27.11	24.09	0.1281	0.6358
Saturated	377.16	318.67	334.76	41.11	23.91	0.1709	0.7395
Unsaturated	167.21	178.09	201.73	21.68	23.89	0.2622	0.3346

[1] CLA = *cis-9, trans-11 octadecadienoic acid*

[2] Short chains FA: ($C_{4:0} - C_{13:0}$)

[3] Medium chains FA: ($C_{14:0} - C_{17:0}$)

[4] Long chains FA: ($\geq C_{18:0}$)

Table 3 A. Effect of plant oil supplement on fatty acid per liter of milk.

(Chapter 4)

Item	Control (1)	Sun flower oil (2)	Soybean oil (3)	SEM	%CV	Contrast	
						1 vs 2 & 3	2 vs 3
	---------- mg/liter ----------						
$C_{4:0}$	616	643	543	101	28.8	0.8930	0.3747
$C_{6:0}$	509	417	467	88.8	33.1	0.5001	0.6547
$C_{8:0}$	306	247	238	56.6	37.1	0.3215	0.9000
$C_{10:0}$	654	524	522	132.8	40.6	0.3812	0.9893
$C_{11:0}$	83	56	59	15.7	40.9	0.1582	0.8896
$C_{12:0}$	2044	1649	1704	282.7	27.2	0.2554	0.8783
$C_{13:0}$	65	62	47	14.4	42.5	0.5306	0.4193
$C_{14:0}$	4084	3442	3686	564.4	26.2	0.4105	0.7332
$C_{14:1}$	426	372	293	75.3	35.7	0.2752	0.4164
$C_{15:0}$	237	184	201	31.7	26.1	0.2129	0.6770
$C_{16:0}$	9577	7868	9305	1366.7	26.54	0.5135	0.4156
$C_{16:1}$	686	529	725	104.2	27.9	0.6095	0.1632
$C_{18:0}$	2910	3593	4062	529.9	26.1	0.1413	0.4903
$C_{18:1n9t}$	733	792	1020	232.3	47.4	0.5019	0.4461
$C_{18:1n9c}$	7329	7593	9125	1197.1	25.9	0.4406	0.3267
$C_{18:2n6t}$	19	38	48	6.9	34.2	0.0099	0.2473
$C_{18:2n6c}$	382	418	528	87.3	34.1	0.3565	0.3348
$C_{20:0}$	52	48	82	17.7	50.2	0.5045	0.1477
$C_{18:3n6}$	43	32	59	11.2	43.5	0.8379	0.0834
CLA[1]	153	230	265	54.5	43.7	0.1424	0.6170
$C_{20:3n6}$	>0.001	5	>0.001	2.7	47.5	0.1999	0.4109
$C_{22:1n9}$	34	10	18	8.3	69.2	0.00517	0.4249
Short[2]	4279	3699	3501	3019.2	25.9	0.4608	0.4605
Medium[3]	15011	14290	12317	1629.5	25.9	0.2991	0.3414
Long[4]	11657	12761	15209	654.6	25.8	0.6943	0.5345
Saturated	9806	9942	12162	2078.2	26.5	0.4914	0.2983
Unsaturated	21142	18637	21037	1976.8	29.6	0.3566	0.8109

[1] CLA = *cis-9, trans-11 octadecadienoic acid*

[2] Short chains FA: ($C_{4:0} - C_{13:0}$)

[3] Medium chains FA: ($C_{14:0} - C_{17:0}$)

[4] Long chains FA: ($\geq C_{18:0}$)

Table 4 A. Effect of lactic acid bacteria supplement on percent of total fatty acid. (Chapter 5)

Item	Control (1)	*L. plantarum* (2)	*L. acidophilus* (3)	SEM	%CV	Contrast	
						1 vs 2 & 3	2 vs 3
	------ % of total fatty acid ------						
$C_{4:0}$	2.67	2.68	2.69	0.17	12.93	0.8976	0.9763
$C_{6:0}$	1.38	1.51	1.60	0.09	11.39	0.0465	0.1470
$C_{8:0}$	0.95	0.78	0.89	0.16	35.62	0.8087	0.3133
$C_{10:0}$	1.42	1.61	2.34	0.46	51.85	0.0527	0.6899
$C_{11:0}$	0.15	0.16	0.17	0.02	23.71	0.2388	0.3684
$C_{12:0}$	4.59	4.85	4.75	0.33	14.04	0.9282	0.4476
$C_{13:0}$	0.14	0.18	0.13	0.04	47.89	0.3858	0.2234
$C_{14:0}$	7.57	9.22	9.11	0.61	14.03	0.0128	0.1879
$C_{14:1}$	1.26	1.11	1.58	0.34	51.65	0.1920	0.6606
$C_{15:0}$	0.60	0.58	0.59	0.03	11.57	0.8183	0.5885
$C_{16:0}$	22.03	22.09	22.76	1.22	10.96	0.1577	0.9605
$C_{16:1}$	2.48	2.60	2.55	0.23	18.39	0.9903	0.6061
$C_{18:0}$	8.92	9.42	10.04	1.10	23.23	0.3706	0.6554
$C_{18:1n9t}$	4.08	2.73	4.20	1.04	56.64	0.3907	0.2090
$C_{18:1n9c}$	35.75	35.91	33.13	1.98	11.35	0.1317	0.9374
$C_{18:2n6t}$	0.35	0.29	0.26	0.05	31.75	0.1780	0.2827
$C_{18:2n6c}$	3.06	2.92	2.43	0.24	16.78	0.0119	0.5758
$C_{20:0}$	0.38	0.15	0.24	0.15	119.87	0.8380	0.1371
$C_{18:3n6}$	0.16	0.15	0.14	0.01	14.03	0.1001	0.7238
CLAa[1]	0.86	0.80	0.80	0.07	18.11	0.6598	0.4677
CLAb[2]	0.02	0.01	0.01	0.01	118.02	0.3195	0.0923
$C_{22:0}$	0.05	0.04	0.05	0.01	25.78	0.4986	0.2466
$C_{20:3n6}$	0.10	0.11	0.12	0.01	20.69	0.1436	0.5173
$C_{22:1n9}$	0.09	0.09	0.07	0.01	21.76	0.6977	0.0540
Short[3]	11.30	11.77	12.59	0.63	10.62	0.1029	0.2098
Medium[4]	33.91	35.60	36.57	1.55	8.77	0.1243	0.5398
Long[5]	54.78	52.63	50.84	1.58	6.00	0.0328	0.2732
Saturated	50.40	53.08	54.76	2.46	9.34	0.1149	0.5028
Unsaturated	49.60	46.92	45.24	2.46	10.42	0.1149	0.5028

[1] CLAa = *cis-9, trans-11 octadecadienoic acid*
[2] CLAb = *transs-10,cis-12 octadecadienoic acid*
[3] Short chains FA: ($C_{4:0} - C_{13:0}$)
[4] Medium chains FA: ($C_{14:0} - C_{17:0}$)
[5] Long chains FA: ($\geq C_{18:0}$)

Table 5 A. Effect of lactic acid bacteria supplement on milk fatty acid yield (g/d). (Chapter 5)

Item	Control (1)	*L. plantarum* (2)	*L. acidophilus* (3)	SEM	%CV	Contrast	
						1 vs 2 & 3	2 vs 3
	---------- g/day ----------						
$C_{4:0}$	13.38	15.12	15.85	1.83	24.77	0.3274	0.3530
$C_{6:0}$	6.95	8.59	9.52	1.05	25.02	0.0668	0.1331
$C_{8:0}$	4.97	4.49	5.41	1.19	47.98	0.5180	0.6948
$C_{10:0}$	6.95	9.05	13.83	2.97	59.93	0.0352	0.4892
$C_{11:0}$	0.72	0.89	1.03	0.13	29.77	0.0620	0.1820
$C_{12:0}$	22.08	27.14	27.95	2.38	18.50	0.0460	0.1205
$C_{13:0}$	0.62	1.07	0.76	0.26	62.68	0.7097	0.0959
$C_{14:0}$	37.25	51.98	53.89	5.22	21.88	0.0536	0.0105
$C_{14:1}$	5.99	6.23	9.52	2.05	56.46	0.0688	0.9073
$C_{15:0}$	2.87	3.28	3.43	0.28	17.42	0.1522	0.1492
$C_{16:0}$	108.60	125.50	135.57	15.58	25.28	0.1851	0.2907
$C_{16:1}$	11.98	14.89	15.10	1.76	25.11	0.2877	0.1128
$C_{18:0}$	45.04	53.94	60.73	8.43	31.68	0.1395	0.3039
$C_{18:1n9t}$	18.21	15.17	22.71	4.53	48.49	0.1405	0.5108
$C_{18:1n9c}$	174.13	203.52	197.77	18.16	18.93	0.5758	0.1212
$C_{18:2n6t}$	1.56	1.62	1.50	0.13	16.64	0.4449	0.6485
$C_{18:2n6c}$	15.19	16.83	15.00	2.13	27.20	0.5898	0.4519
$C_{20:0}$	1.74	0.83	1.36	0.69	105.42	0.8981	0.2023
$C_{18:3n6}$	0.77	0.87	0.83	0.08	18.59	0.8522	0.1952
CLAa[1]	4.09	4.43	4.68	0.49	22.41	0.3352	0.5033
CLAb[2]	0.06	0.05	0.04	0.02	91.08	0.7729	0.4727
$C_{22:0}$	0.25	0.25	0.30	0.05	35.32	0.1824	0.9989
$C_{20:3n6}$	0.50	0.62	0.73	0.09	28.95	0.0379	0.2108
$C_{22:1n9}$	0.46	0.44	0.52	0.05	22.72	0.1144	0.7160
Short[3]	55.66	66.36	74.35	7.46	22.78	0.0340	0.2967
Medium[4]	166.69	201.89	217.51	21.87	22.39	0.0344	0.4835
Long[5]	269.13	298.69	299.39	27.92	19.31	0.2303	0.9802
Saturated	249.42	301.06	325.77	34.25	23.45	0.0433	0.4791
Unsaturated	242.06	265.88	265.48	25.87	20.07	0.3043	0.9878

[1] CLAa = *cis-9, trans-11 octadecadienoic acid*
[2] CLAb = *transs-10,cis-12 octadecadienoic acid*
[3] Short chain FA: ($C_{4:0} - C_{13:0}$)
[4] Medium chain FA: ($C_{14:0} - C_{17:0}$)
[5] Long chain FA: ($\geq C_{18:0}$)

Table 6 A. Effect of lactil acid becteria supplement on fatty acid per liter of milk. (Chapter 5)

Item	Control (1)	*L. plantarum* (2)	*L. acidophilus* (3)	SEM	%CV	Contrast	
						1 vs 2 & 3	2 vs 3
	---------- mg/liter ----------						
$C_{4:0}$	723	787	870	132.1	24.8	0.1928	0.5211
$C_{6:0}$	375	447	523	81.8	25.2	0.0332	0.2195
$C_{8:0}$	269	234	297	47.6	48.3	0.4212	0.5934
$C_{10:0}$	375	471	759	132.8	60.9	0.0275	0.5637
$C_{11:0}$	39	47	56	11.7	30.0	0.0369	0.2733
$C_{12:0}$	1193	1413	1535	112.7	18.6	0.0498	0.1022
$C_{13:0}$	33	55	42	16.1	61.3	0.8145	0.1113
$C_{14:0}$	2013	2707	2961	417.4	21.9	0.0226	0.0229
$C_{14:1}$	324	325	523	101.3	57.8	0.0537	0.9947
$C_{15:0}$	155	171	188	28.4	17.4	0.0622	0.3007
$C_{16:0}$	5870	6536	7448	1114.9	25.3	0.1019	0.4360
$C_{16:1}$	647	447	829	244.2	25.1	0.1639	0.1886
$C_{18:0}$	2434	2809	3216	499.4	33.1	0.1571	0.4316
$C_{18:1n9t}$	984	790	1248	220.7	49.2	0.1082	0.4429
$C_{18:1n9c}$	9412	10600	10259	1357.1	19.9	0.7739	0.2502
$C_{18:2n6t}$	462	384	424	66.9	46.6	0.6616	0.3447
$C_{18:2n6c}$	821	876	824	64.3	27.3	0.8052	0.6345
$C_{20:0}$	94	74	43	27.7	61.2	0.8540	0.1905
$C_{18:3n6}$	5	6	10	8.8	73.1	0.0653	0.8489
CLAa[1]	221	231	258	18.5	22.4	0.5445	0.3867
CLAb[2]	3	2	3	1.3	91.6	0.8653	0.3827
$C_{22:0}$	13	13	16	2.7	34.9	0.1069	0.8833
$C_{20:3n6}$	27	32	40	3.8	28.8	0.0185	0.3087
$C_{22:1n9}$	25	23	29	1.7	22.8	0.0518	0.5572
Short[3]	3008	3456	4085	1517.2	22.9	0.0244	0.2818
Medium[4]	9010	10515	11951	1726.5	22.4	0.0443	0.2156
Long[5]	14546	15555	16447	616.6	19.3	0.2954	0.5090
Saturated	13482	15680	17899	2381.2	23.5	0.0511	0.2477
Unsaturated	13082	13846	14584	1021.9	20.1	0.3627	0.5884

[1] CLAa = *cis-9, trans-11 octadecadienoic acid*
[2] CLAb = *transs-10,cis-12 octadecadienoic acid*
[3] Short chain FA: ($C_{4:0} - C_{13:0}$)
[4] Medium chain FA: ($C_{14:0} - C_{17:0}$)
[5] Long chain FA: ($\geq C_{18:0}$)

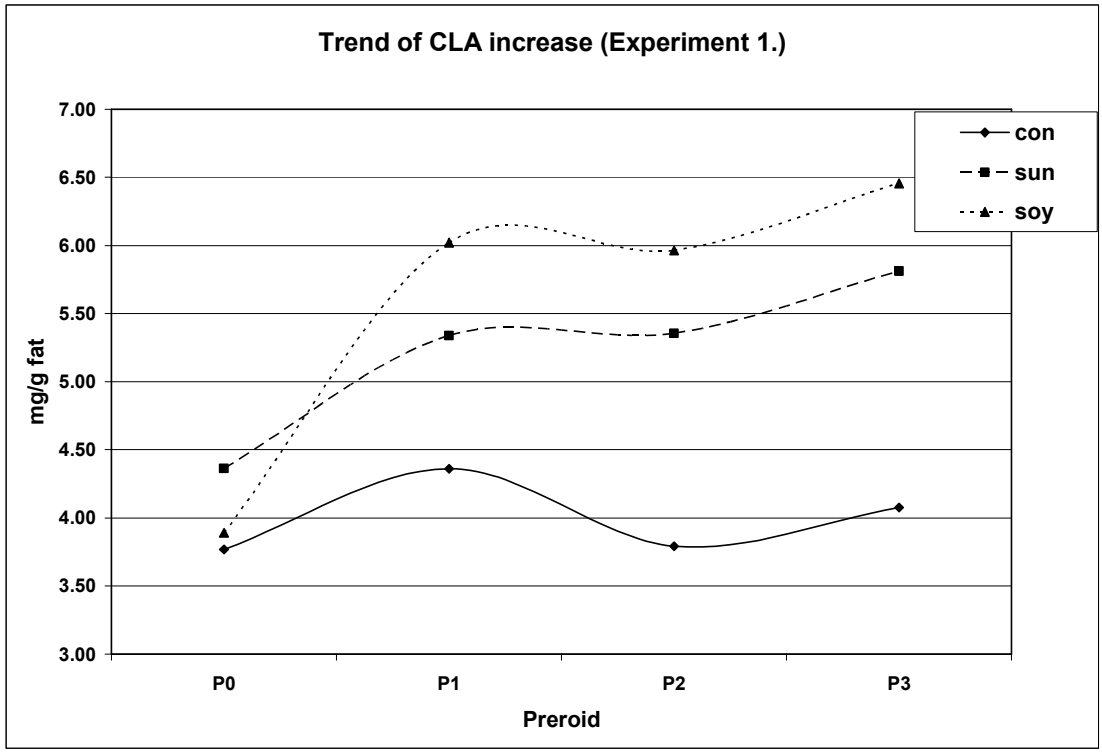

Figure 1A. Trend of CLA increase (Experiment 1.) (Chapter 4)

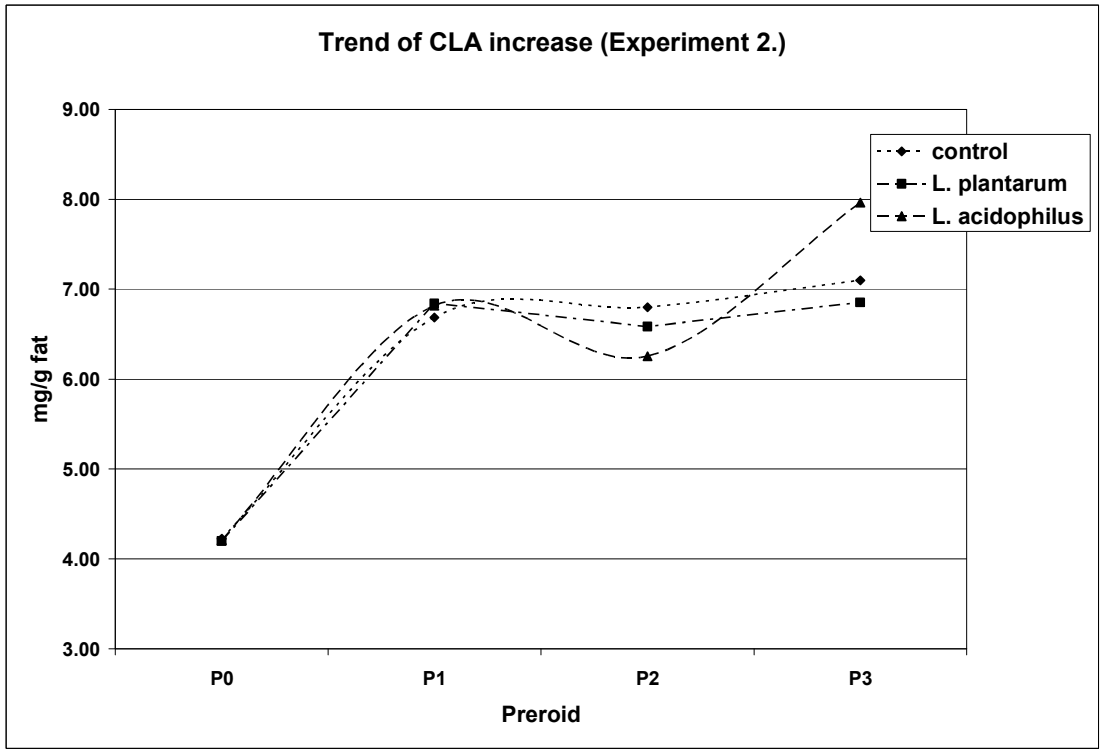

Figure 2A. Trend of CLA increase (Experiment 2.) (Chapter 5)

APPENDIX B

1. Energy Evaluation of Feedstuffs by NRC (2001) (Experiment 1 and 2)

Energy from NFC

tdNFC = 0.98 x (100 - [NDF_N + CP + EE + Ash]) x PAF

tdNFC (Silage Ex1) = 0.98 x (100-[58.64+7.57+1.36+15.37]) x 1 = 16.72
tdNFC (Silage Ex2) = 0.98 x (100-[65.58+9.25+2.08+10.94]) x 1 = 11.91
tdNFC (Conc. Ex1 Control) = 0.98 x (100-[39.88+21.52+3.81+7.51]) x 1
= 26.73
(Conc. Ex 1 Sunflower oil) = 0.98 x (100-[36.90+20.61+5.79+7.35]) x 1
= 28.75
(Conc. Ex 1 Soybean oil) = 0.98 x (100-[35.87+20.62+5.81+7.21]) x 1
= 29.89
tdNFC (Conc. Ex2) = 0.98 x (100-[35.71+20.31+6.31+7.87]) x 1
= 29.21

Energy from Protein

$tdCP_f$ (Silage)= CP x $exp^{[-1.2 \times (ADICP/CP)]}$

$tdCP_f$ (Silage Ex1) = 7.57 x $exp^{[-1.2 \times (2.93/7.57)]}$ = 4.75
$tdCP_f$ (Silage Ex2) = 9.25 x $exp^{[-1.2 \times (3.26/9.25)]}$ = 6.06

$tdCP_c$ (Conc.)= CP x [1 - (0.4 x (ADICP/CP))]

$tdCP_c$ (Conc. Ex1 Control) = 21.52 x [1 - (0.4 x (5.33/21.52))]
= 19.39
(Conc. Ex 1 Sunflower oil) = 20.61 x [1 - (0.4 x (4.61/20.61))]
= 18.76
(Conc. Ex 1 Soybean oil) = 20.62 x [1 - (0.4 x (4.65/20.62))]
= 18.76
$tdCP_c$ (Conc. Ex2) = 20.31 x [1 - (0.4 x (4.96/20.31))]
= 18.33

Energy from Fat

FA = EE - 1.0
tdFA = FA Note: If EE < 1, then FA = 0

tdFA (Silage Ex1) = 1.36-1 = 0.36
tdFA (Silage Ex2) = 2.08-1 = 1.08
tdFA (Conc. Ex1 Control) = 3.81-1 = 2.81
(Conc. Ex 1 Sunflower oil) = 5.79-1 = 4.79
(Conc. Ex 1 Soybean oil) = 5.81-1 = 4.81
tdFA (Conc. Ex2) = 6.31-1 = 5.31

Energy from NDF

tdNDF = 0.75 x (NDF_N - Lignin) x [1 - (Lignin/NDF_N)$^{0.667}$]

tdNDF (Silage Ex1) = 0.75 x (58.64 – 5.29) x [1-(5.29/58.64)$^{0.667}$]
= 31.96

tdNDF (Silage Ex2) = 0.75 x (65.58 – 7.59) x [1-(5.59/65.58)$^{0.667}$]
= 33.16

tdNDF (Conc. Ex1 Control) = 0.75 x (39.88 – 5.16) x [1-(5.16/39.88)$^{0.667}$]
= 19.39

(Conc. Ex 1 Sunflower oil) = 0.75 x (36.90 – 4.60) x [1-4.60/36.90)$^{0.667}$]
= 18.18

(Conc. Ex 1 Soybean oil) = 0.75 x (35.87 – 4.59) x [1-(4.59/35.87)$^{0.667}$]
= 17.50

tdNDF (Conc. Ex2) = 0.75 x (35.71 – 3.97) x [1-(3.97/35.71)$^{0.667}$]
= 18.30

Total digestible nutrient, (TDN)

TDN_{1X} (%) = tdNFC + tdCP + (tdFA x 2.25) + tdNDF – 7

TDN_{1X} (%) (Silage Ex1) = 16.72 + 4.75 + (0.36 x 2.25) + 31.96 - 7
= 47.26

TDN_{1X} (%) (Silage Ex2) = 11.91 + 6.06 + (1.08 x 2.25) + 33.16 - 7
= 46.56

TDN_{1X} (%) (Conc. Ex1 Control) = 26.73 + 19.39 + (2.81 x 2.25) + 19.39 - 7
= 64.82

(Conc. Ex 1 Sunflower oil) = 28.75 + 18.76 + (4.79 x 2.25) + 18.18 - 7
= 69.48

(Conc. Ex 1 Soybean oil) = 29.89 + 18.76 + (4.81 x 2.25) + 17.50 - 7
= 69.96

TDN_{1X} (%) (Conc. Ex2) = 29.21 + 18.33 + (5.31 x 2.25) + 18.30 - 7
= 70.79

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

DE_{1X}(Mcal/kg) (Silage Ex1) = [(16.72/100) x 4.2] + [(31.96/100) x 4.2] + [(4.75/100) x 5.6] + [(0.00/100) x 9.4] – 0.3
= 2.09 Mcal/kg

DE_{1X}(Mcal/kg) (Silage Ex2) = [(11.91/100) x 4.2] + [(33.16/100) x 4.2] + [(6.06/100) x 5.6] + [(0.58/100) x 9.4] – 0.3
= 2.16 Mcal/kg

DE_{1X}(Mcal/kg) (Conc. Ex1 Control) = [(26.73/100) x 4.2] + [(19.39/100) x 4.2] + [(19.39/100) x 5.6] + [(2.31/100) x 9.4] – 0.3
= 33.2 Mcal/kg

Conc. Ex 1 Sunflower oil) = [(28.75/100) x 4.2] + [(18.18/100) x 4.2] + [(18.76/100) x 5.6] + [(4.29/100) x 9.4] – 0.3

= 3.74 Mcal/kg

Conc. Ex 1 Soybean oil) = [(29.87/100) x 4.2] + [(17.50/100) x 4.2] + [(18.76/100) x 5.6] + [(4.31/100) x 9.4] – 0.3

= 3.76 Mcal/kg

DE_{1X}(Mcal/kg) (Conc. Ex2) = [(29.21/100) x 4.2] + [(18.30/100) x 4.2] + [(18.33/100) x 5.6] + [(4.81/100) x 9.4] – 0.3

= 3.85 Mcal/kg

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

Discount (Silage Ex1) = [47.26-([(0.18 x 47.26) – 10.3] x 2)] /47.26 = 1.08
Discount (Silage Ex2)= [46.56-([(0.18 x 46.56) – 10.3] x 2)] /46.56 = 1.08
Discount (Conc. Ex1 Control) = [64.82 - ([(0.18 x 64.82) - 10.3] x 2)]/64.82
= 0.96
(Conc. Ex 1 Sunflower oil) = [69.48 - ([(0.18 x 69.48) - 10.3] x)]/69.48
= 0.94
(Conc. Ex 1 Soybean oil) = [69.96 - ([(0.18 x 69.96) - 10.3] x 2)]/69.69
= 0.93
Discount (Conc. Ex2) = [70.79 - ([(0.18 x 70.79) - 10.3] x 2)]/70.79
= 0.93

DE_P (Mcal/kg) = DE_{1X} x Discount

DE_P (Silage Ex1) = 2.09 x 1.08 = 2.25 Mcal/kg
DE_P (Silage Ex2) = 2.16 x 1.08 = 2.33 Mcal/kg
DE_P (Conc. Ex1 Control) = 3.32 x 0.96 = 3.18 Mcal/kg
(Conc. Ex 1 Sunflower oil) = 3.74 x 0.94 = 3.49 Mcal/kg
(Conc. Ex 1 Soybean oil) = 3.76 x 0.93 = 3.51 Mcal/kg
DE_P (Conc. Ex2) = 3.85 x 0.93 = 3.58 Mcal/kg

ME_P(Mcal/kg) = $[(1.01 \times DE_P) - 0.45] + [0.0046 \times (EE - 3)]$

ME_P (Silage Ex1) = [(1.01x2.25)-0.45]+[0.0046 x (1.36-3)] = 1.82 Mcal/kg
ME_P (Silage Ex2) = [(1.01x2.33)-0.45]+[0.0046 x (2.08-3)] = 1.91 Mcal/kg
ME_P (Conc. Ex1 Control) = [(1.01x3.18)-0.45]+[0.0046 x (3.81-3)]
= 2.76 Mcal/kg
(Conc. Ex 1 Sunflower oil) = [(1.01x3.49)-0.45]+[0.0046 x (5.79-3)]
= 3.10 Mcal/kg
(Conc. Ex 1 Soybean oil) = [(1.01x3.51)-0.45]+[0.0046 x (5.81-3)]
= 3.11 Mcal/kg
ME_P (Conc. Ex2) = [(1.01x3.58)-0.45]+[0.0046 x (6.31-3)]
= 3.18 Mcal/kg

NE_{Lp}(Mcal/kg) = [(0.703 x ME_P) – 0.19] + [((0.0097 x ME_P)+ 0.19)/97) x (EE - 3)]

NE_{Lp} (Silage Ex1) = [(0.703 x 1.82) – 0.19] + [((0.0097 x 1.82)+0.19)/97] x (1.37-3)]
= 1.09 Mcal/kg

NE_{Lp} (Silage Ex2) = [(0.703 x 1.91) – 0.19]+[((0.0097 x 1.91) + 0.19)/97)x(2.08-3)]
= 1.15 Mcal/kg

NE_{Lp} (Conc. Ex1 Control)
= [(0.703 x 2.76) – 0.19] + [((0.0097 x 2.76) + 0.19)/97) x (3.81 - 3)]
= 1.75 Mcal/kg

(Conc. Ex 1 Sunflower oil)
= [(0.703 x 3.10) – 0.19] + [((0.0097 x 3.10)+0.19)/97) x (5.79 - 3)]
= 1.99 Mcal/kg

(Conc. Ex 1 Soybean oil)
= [(0.703 x 3.11) – 0.19] + [((0.0097 x 3.11 + 0.19)/97) x (5.81 - 3)]
= 2.00 Mcal/kg

NE_{Lp} (Conc. Ex2)
= [(0.703 x 3.18) – 0.19] + [((0.0097 x 3.18) + 0.19)/97)x(6.31 - 3)]
= 2.05 Mcal/kg

2. Calculation of Requirement for Energy and Protein of dairy cow by NRC, 2001 (Experiment 1) (Example)

2.1 Energy Requirement

Group 1. Lactating Cow 450.94 kgLW (97.86 $kg^{0.75}$) 3.5 BSC loss 0.078 kg/d, produced 17.36 kg.milk/d, Milk: 3.79fat%, 2.68%CP

$$\mathbf{NE_{LR} = NE_{LM}+NE_{LG}+NE_{LL}}$$

NE_{LM}(Mcal/kg) = 0.08 x (Live Weight)$^{0.75}$
= 0.08 x (450.94)$^{0.75}$
= 7.82 Mcal/day

NE_{LG}(Mcal/kg) = Reserve Energy x 0.82

Reserve Energy = (Proportion of empty body fat x 9.4) + (Proportion of empty Body protein x 5.5)

Proportion of empty body fat = 0.037683 x BCS(9)

Proportion of empty body protein = 0.20086 – [0.0066762 x BCS(9)]

BCS(9) = ((Dairy BCS – 1) x 2) + 1
= ((3.5 – 1) x 2) + 1
= 6

Proportion of empty body fat = 0.037683 x 6
= 0.226098

Proportion of empty body protein = 0.20086 – (0.0066762 x 6)
= 0.1608288

Reserve Energy = (0.226098 x 9.4) + (0.1608288 x 5.5)
= 3.02

NE_{LG}(Mcal/kg loss) = 3.02 x 0.82 x 0.21730 (kg/d)
= -0.58 Mcal/day

NE_{LL}(Mcal/kg) = (0.0929xFat%)+(0.0547xCP%) + 0.192
= [(0.0929x3.79)+(0.0547x2.68)+0.192] x 17.83 (kg milk/d)
= 12.37 Mcal/day

NE_{LR} = 7.82 – 0.58 + 12.34
= 19.61 Mcal/day

Table 1 B. The estimates of the partitioning of NE_{intake} (Mcal/d) (Experiment 1.)

Item	Control	Sunflower oil	Soybean oil
$NE_{L\ intake}$	22.63	23.99	24.57
NE_{LM}	7.82	7.84	7.76
NE_{LL}	12.37	12.00	12.54
NE_{LG}	-0.58	0.68	-0.31
NE_{LR}	19.16	20.52	20.00
Efficiency	0.80	0.93	0.84

2.2 Protein Requirement

Group 1. Lactating Cow 450.94 kgLW (97.86 $kg^{0.75}$) 3.5 BSC loss 0.078 kg/d, produced 17.36 kg.milk/d, Milk: 3.79fat%, 2.68%CP

MP_R = $MP_M + MP_G + MP_L$

MP_M(g/d) = $MP_u + MP_{sh} + MP_{MFP}$

MP_u = UPN/0.67

UPN(g/d) = 2.75 x (Live weight)$^{0.5}$

MP_u = [2.75 x ($450.94^{0.5}$)]/0.67

= 87.02

MP_{sh} = SPN/0.67

SPN = 0.2 x (Live weight)$^{0.6}$

MP_{sh} = [0.2 x ($450.94^{0.6}$)]/0.67

= 11.66

MP_{MFP} = MFP – (bacteria + bacterialdebris in Cecum, large intestine + keratinized Cell + others)

MFP(g/d) = 30 x Dry matter intake (kg.)

MP_{MFP} = [(DMI(kg) x 30) – 0.50((Bact MP/ 0.8) – Bact MP)] + Endo MP/0.67

When;

Endo MP(g/d) = 0.4 x 1.9 x DMI (kg) x 6.25

= 0.4 x 1.9 x 15.04 x 6.25

= 71.42

Bact MP(g/d) = 0.64 MCP

MCP = 0.85 $gRDP_{req}$

RDP_{req}(Corn silge) = 0.15294 x $TDN_{Act\ ual}$

TDN_{Act} Total (Silage) = DMI(kg) x %TDN x 1000

RDP_{req}(Silage) = 0.15294 x (5.55 kg x 0.4726x 1000)

= 401.05 g/d

RDP_{req}(Conc.) = 0.15294 x TDN_{Act} Total

TDN_{Act} Total (Conc.) = DMI(kg) x %TDN x 1000

RDP_{req}(Conc.) = 0.15294 x (9.46 kg x 0.6482 x 1000)

= 937.82 g/d

RDP_{req} = RDP_{req}(Silage) + RDP_{req}(Conc.)

= 401.01+ 937.82

= 1338.83 g/d

MCP = 0.85 x 1338.83

= 1138.21

MP_{Bact}(g/d) = 1138.21 x 0.64

= 728.45

MP_{End} (g/d) = 0.4 x (1.9 x 15.04 x 6.25)

= 71.42

MP_{MFP} (g/d) = [(DMI(kg) x 30) – 0.50((Bact MP/ 0.8) – Bact MP)] + Endo MP/0.67

		=	[(15.04 x 30) – 0.50((728.45/0.8) – 728.45)] + 71.42/0.67
		=	466.63
	MP_M(g/d)	=	$MP_u + MP_{sh} + MP_{MFP}$
		=	87.2 + 11.66 + 466.63
		=	565.31 g/d
	MP_G(g/d)	=	NP_g/EffMP_NP_g
	NP_g(g/d)	=	SWG x (268 – (29.4 x (RE/SWG)))
When;			
	RE(Mcal)	=	$0.0635 \times EQEBW^{0.75} \times EQEBG^{1.097}$
	EQEBW	=	0.891 x EQSBW
	EQSBW	=	SBW x (478/MSBW)
	SBW	=	Shrunk body weight
		=	0.96 x BW
		=	0.96 x 450.94 kgLW
		=	432.90 kgLW
	MSBW	=	Mature shrunk body weight
		=	500 kgLW
	EQSBW	=	432.90 x (478/500)
		=	413.85 kgLW
	EQEBW	=	0.891 x 413.85
		=	368.74 kgLW
	EQEBG	=	0.956 x SWG
		=	0.956 x 0.42
		=	0.40 kgLW
	RE (Mcal/d)	=	$0.0635 \times 368.74^{0.75} \times 0.40^{1.097}$
		=	2.05 Mcal/d
	NP_g	=	0.42 x (268 – (29.4 x (2.05/0.42)))
		=	52.59 g/d
	EffMP_NP_g	=	(83.4 – (0.114 x EQSBW))/100
		=	(83.4 – (0.114 x 368.74))/100
		=	0.36
	MP_G(g/d)	=	52.59/0.36
		=	152.75 g/d
	MP_L (g/d)	=	(Yprotn/0.67) x 1000
	Yprotn (kg/d)	=	Milk production (kg/d) x (Milk TP/100)
		=	17.83 (kg/d) x (2.68/100)
		=	0.477 kg/d
	MP_L (g/d)	=	(0.48/0.67) x 1000
		=	713.42 g/d
	MP_R (g/d)	=	565.31 + 152.75 + 713.42
		=	1431.37 g/d
	MP_{req}	=	$MP_{Bact} + MP_{RUP} + MP_{Endo}$
	MP_{RUP}	=	MP_{req} – (MPBact + MPEnd)
		=	1431.37 – (728.45 + 71.42)
		=	631.49 g/d
	$0.8RUP_{req}$	=	total digest RUP
	0.66 x Total digest RUP		= MP_{RUP}

Total digest RUP = 631.49/0.66
= 956.8
Total digest RUP = $0.8RUP_{req}$
RUP_{req} = 956.8/0.8
= 1196.00
CP_{req} = RDP_{req} + RUP_{req}
= 1338.83 + 1196.00
= 2535.07 g

By Feed;

RDP_{sup} (Silage) = Total DMFed x1000xDiet CP x CP_RDP
= 5.55 x 1000 x (7.56/100) x 0.45
= 189.01 g/d
RDP_{sup} (Conc.) = Total DMFed x1000xDiet CP x CP_RDP
= 9.46 x 1000 x (21.52/100) x 0.66
= 1343.62 g/d
RDP_{sup} = RDP_{sup}(Silage) + RDP_{sup}(Conc.)
= 189.01 + 1343.62
= 1532.64 g/d
CPTotal (Silage) = Total DMFed x 1000 x Diet CP
= 5.55 x 1000 x (7.56/100)
= 420.03 g/d
CPTotal (Conc.) = Total DMFed x 1000 x Diet CP
= 9.46 x 1000 x (21.56/100)
= 2035.79 g/d
CPTotal = CPtotal (Silage) + CPtotal (Conc.)
= 420.03 + 2035.79
= 2455.82 g/d
RUP_{sup} = CPTotal - RDP_{sup}
= 2485.82 – 1532.64
= 923.19 g/d

Table 2 B. The estimated supply of RDP and RUP (g/cow daily) (Experiment 1.)

	Control	**Sunflower oil**	**Soybean oil**
MP_R	1431.37	1401.93	1461.14
MCP	1138.21	1144.95	1189.91
RDP_{req}	1339.07	1347.00	1399.90
RDP_{sup}	1532.64	1447.83	1450.84
RDP deficit/surplus	193.57	100.82	59.64
RUP_{req}	1196.00	1139.68	1194.60
RUP_{sup}	923.19	859.70	905.53
RUP deficit/surplus	-272.82	-279.79	-288.83

3. Calculation of Requirement for Energy and Protein of dairy cow by NRC, 2001 (Experiment 2) (Example)

3.1 Energy Requirement

Group 1. Lactating Cow 456.44 kgLW (98.75 $kg^{0.75}$) 3.5 BSC gain 0.018 kg/d, produced 18.49 kg.milk/d, Milk: 3.27 fat%, 2.69%CP

$$\mathbf{NE_{LR} = NE_{LM}+NE_{LG}+NE_{LL}}$$

NE_{LM}(Mcal/kg) = 0.08 x (Live Weight)$^{0.75}$
= 0.08 x (456.44)$^{0.75}$
= 7.89 Mcal/day

NE_{LG}(Mcal/kg) = Reserve Energy x 0.82

Reserve Energy = (Proportion of empty body fatx 9.4)+(Proportion of empty Body protein x 5.5)

Proportion of empty body fat = 0.037683 x BCS(9)

Proportion of empty body protein = 0.20086 – [0.0066762 x BCS(9)]

BCS(9) = ((Dairy BCS – 1) x 2) + 1
= ((3.5 – 1) x 2) + 1
= 6

Proportion of empty body fat = 0.037683 x 6
= 0.226098

Proportion of empty body protein = 0.20086 – (0.0066762 x 6)
= 0.1608288

Reserve Energy = (0.226098 x 9.4) + (0.1608288 x 5.5)
= 3.02

NE_{LG}(Mcal/kg gain) = 0.68 Mcal/day

NE_{LL}(Mcal/kg) = (0.0929 x Fat%)+(0.0547xCP%)+ 0.192
= [(0.0929 x 3.27) + (0.0547 x 2.69) + 0.192] x 18.49 (kg milk/d)
= 11.91 Mcal/day

NE_{LR} = 7.89 + 0.68 + 11.91
= 20.48 Mcal/day

Table 3 B. The estimates of the partitioning of NE_{intake} (Mcal/d) (Experiment 2.)

Item	Control	*L. plantarum*	*L. acidophilus*
$NE_{L\ intake}$	25.72	26.15	25.76
NE_{LM}	7.89	7.85	7.76
NE_{LL}	11.91	12.55	12.48
NE_{LG}	0.68	-1.21	-0.58
NE_{LR}	20.48	19.81	19.65
Efficiency	0.71	0.62	0.66

3.2 Protein Requirement

Group 1. Lactating Cow 456.44 kgLW (98.75 $kg^{0.75}$) 3.5 BSC gain 0.018 kg/d, produced 18.49 kg.milk/d, Milk: 3.27 fat%, 2.69%CP

MP_R = $MP_M + MP_G + MP_L$

MP_M(g/d) = $MP_u + MP_{sh} + MP_{MFP}$

MP_u = UPN/0.67

UPN(g/d) = 2.75 x (Live weight)$^{0.5}$

MP_u = [2.75 x ($456.44^{0.5}$)]/0.67

= 87.56

MP_{sh} = SPN/0.67

SPN = 0.2 x (Live weight)$^{0.6}$

MP_{sh} = [0.2 x ($456.44^{0.6}$)]/0.67

= 11.75

MP_{MFP} = MFP – (bacteria + bacterialdebris in Cecum, large intestine + keratinized Cell + others)

MFP(g/d) = 30 x Dry matter intake (kg.)

MP_{MFP} = [(DMI(kg) x 30) – 0.50((Bact MP/ 0.8) – Bact MP)] + Endo MP/0.67

when;

Endo MP(g/d) = 0.4 x 1.9 x DMI (kg) x 6.25

= 0.4 x 1.9 x 14.9 x 6.25

= 70.78

Bact MP(g/d) = 0.64 MCP

MCP = 0.85 $gRDP_{req}$

RDP_{req}(Corn silge) = 0.15294 x $TDN_{Act\ ual}$

TDN_{Act} Total (Silage) = DMI(kg) x %TDN x 1000

RDP_{req}(Silage) = 0.15294 x (5.3 kg x 0.465x 1000)

= 379.92 g/d

RDP_{req}(Conc.) = 0.15294 x TDN_{Act} Total

TDN_{Act} Total (Conc.) = DMI(kg) x %TDN x 1000

RDP_{req}(Conc.) = 0.15294 x (9.56 kg x 0.708 x 1000)

= 1035.17 g/d

RDP_{req} = RDP_{req}(Silage) + RDP_{req}(Conc.)

= 379.92 + 1035.17

= 1415.14 g/d

MCP = 0.85 x 1415.14

= 1202.86

MP_{Bact}(g/d) = 1202.86 x 0.64

= 769.83

MP_{End} (g/d) = 0.4 x (1.9 x 14.9 x 6.25)

= 70.78

MP_{MFP} (g/d) = [(DMI(kg) x 30) – 0.50((Bact MP/ 0.8) – Bact MP)] + Endo MP/0.67

		=	[(14.9 x 30) – 0.50((769.83/0.8) – 769.83)] + 70.78/0.67
		=	447.03
	MP_M(g/d)	=	$MP_u + MP_{sh} + MP_{MFP}$
		=	87.56 + 11.75 + 447.03
		=	555.75 g/d
	MP_G(g/d)	=	NP_g/EffMP_NP_g
	NP_g(g/d)	=	SWG x (268 – (29.4 x (RE/SWG)))
When;			
	RE(Mcal)	=	0.0635 x $EQEBW^{0.75}$ x $EQEBG^{1.097}$
	EQEBW	=	0.891 x EQSBW
	EQSBW	=	SBW x (478/MSBW)
	SBW	=	Shrunk body weight
		=	0.96 x BW
		=	0.96 x 456.44 kgLW
		=	438.18 kgLW
	MSBW	=	Mature shrunk body weight
		=	500 kgLW
	EQSBW	=	438.18 x (478/500)
		=	418.90 kgLW
	EQEBW	=	0.891 x 418.90
		=	373.24 kgLW
	EQEBG	=	0.956 x SWG
		=	0.956 x 0.63
		=	0.60 kgLW
	RE (Mcal/d)	=	0.0635 x $373.24^{0.75}$ x $0.60^{1.097}$
		=	3.18 Mcal/d
	NP_g	=	0.63 x (268 – (29.4 x (3.18/0.63)))
		=	75.96 g/d
	EffMP_NP_g	=	(83.4 – (0.114 x EQSBW))/100
		=	(83.4 – (0.114 x 373.24))/100
		=	0.36
	MP_G(g/d)	=	75.96/0.36
		=	214.31 g/d
	MP_L (g/d)	=	(Yprotn/0.67) x 1000
	Yprotn (kg/d)	=	Milk prod.(kg/d) x (Milk TP/100)
		=	18.49 (kg/d) x (2.69/100)
		=	0.497 kg/d
	MP_L (g/d)	=	(0.497/0.67) x 1000
		=	734.44 g/d
	MP_R (g/d)	=	555.75 + 214.31 + 734.44
		=	1504.50 g/d
	MP_{req}	=	$MP_{Bact} + MP_{RUP} + MP_{Endo}$
	MP_{RUP}	=	MP_{req} – (MPBact + MPEnd)
		=	1504.50 – (769.83 + 70.78)
		=	663.89 g/d
	$0.8RUP_{req}$	=	total digest RUP
	0.66 x Total digest RUP	=	MP_{RUP}

	Total digest RUP	=	663.89/0.66
		=	1005.89
	Total digest RUP	=	$0.8RUP_{req}$
	RUP_{req}	=	1005.89/0.8
		=	1257.37
	CP_{req}	=	$RDP_{req} + RUP_{req}$
		=	1415.14 + 1257.37
		=	2672.50 g
By Feed;	RDP_{sup} (Silage)	=	Total DMFed x 1000xDiet CPx CP_RDP
		=	5.3 x 1000 x (9.24/100) x 0.66
		=	325.54 g/d
	RDP_{sup} (Conc.)	=	Total DMFed x1000xDiet CP x CP_RDP
		=	9.56 x 1000 x (20.25/100) x 0.62
		=	1199.89 g/d
	RDP_{sup}	=	RDP_{sup}(Silage) + RDP_{sup}(Conc.)
		=	325.54 + 1199.89
		=	1525.43 g/d
	CPTotal (Silage)	=	Total DMFed x 1000 x Diet CP
		=	5.34 x 1000 x (9.23/100)
		=	493.24 g/d
	CPTotal (Conc.)	=	Total DMFed x 1000 x Diet CP
		=	9.56 x 1000 x (20.25/100)
		=	1935.9 g/d
	CPTotal	=	CPTotal (Silage)+CPTotal (Conc.)
		=	493.42 + 1935.9
		=	2428.54 g/d
	RUP_{sup}	=	CPTotal - RDP_{sup}
		=	2428.54 – 1525.43
		=	903.11 g/d

Table 4 B. The estimated supply of RDP and RUP (g/cow daily) (Experiment 2.)

Item	Control	*L. plantarum*	*L. acidophilus*
MP_R	1504.50	1611.56	1460.53
MCP	1202.86	1225.23	1205.00
RDP_{req}	1415.14	1441.44	1417.65
RDP_{sup}	1525.43	1547.93	1527.57
RDP deficit/surplus	110.29	106.49	109.93
RUP_{req}	1257.37	1427.65	1171.19
RUP_{sup}	903.12	914.71	904.22
RUP deficit/surplus	-354.25	-512.49	-266.96

APPENDIX C

Table 1 C. Table of ANOVA (Fatty acid (Experiment 1.))

C4

Source	df	SS	MS	F value	Pr>F
Block	1	26.29	26.29	5.25	0.0336
Treatment	2	13.14	6.29	1.31	0.2928
Error	19	95.21	5.01	2.62	
Total	22	134.65			

$R^2 = 0.29$ %CV = 13.72

C6

Source	df	SS	MS	F value	Pr>F
Block	1	0.96	0.96	0.28	0.6042
Treatment	2	26.99	13.50	3.92	0.0375
Error	19	65.39	3.44		
Total	22	93.34			

$R^2 = 0.30$ %CV = 15.90

C8

Source	df	SS	MS	F value	Pr>F
Block	1	0.08	0.08	0.04	0.8439
Treatment	2	17.42	8.71	4.39	0.0271
Error	19	37.71	1.98		
Total	22	55.22			

$R^2 = 0.32$ %CV = 21.66

C10

Source	df	SS	MS	F value	Pr>F
Block	1	24.75	24.75	1.19	0.2888
Treatment	2	89.03	44.52	2.14	0.1449
Error	19	394.95	20.78		
Total	22	508.75			

$R^2 = 0.14$ %CV = 41.10

C12

Source	df	SS	MS	F value	Pr>F
Block	1	8.93	8.93	0.22	0.6416
Treatment	2	136.26	68.13	1.71	0.2081
Error	19	758.49	39.92		
Total	22	903.68			

$R^2 = 0.17$ %CV = 13.38

C14

Source	df	SS	MS	F value	Pr>F
Block	1	122.29	122.29	0.65	0.4309
Treatment	2	311.67	155.83	0.83	0.4531
Error	19	3586.76	188.77		
Total	22	4020.72			

$R^2 = 0.11$ %CV = 14.27

C16

Source	df	SS	MS	F value	Pr>F
Block	1	907.77	907.77	1.11	0.3050
Treatment	2	6171.82	3085.91	3.78	0.0416
Error	19	15519.88	816.83		
Total	22	22599.48			

$R^2 = 0.31$ %CV = 11.86

C18

Source	df	SS	MS	F value	Pr>F
Block	1	2327.12	23.27.12	10.68	0.0041
Treatment	2	4923.34	2461.66	11.29	0.0006
Error	19	4141.41	217.96		
Total	22	11391.86			

$R^2 = 0.64$ %CV = 15.51

C18:1n9t

Source	df	SS	MS	F value	Pr>F
Block	1	9.02	9.02	0.12	0.7317
Treatment	2	783.68	391.84	5.26	0.0152
Error	19	1414.75	74.46		
Total	22	2207.46			

$R^2 = 0.36$ %CV = 35.75

C18:1n9c

Source	df	SS	MS	F value	Pr>F
Block	1	1821.72	1821.72	1.34	0.2612
Treatment	2	11973.17	5986.58	4.41	0.0268
Error	19	25806.89	13.58.25		
Total	22	39601.78			

$R^2 = 0.35$ %CV = 17.27

C18:2n6c

Source	df	SS	MS	F value	Pr>F
Block	1	19.07	19.07	0.72	0.4057
Treatment	2	20.95	10.47	0.40	0.6776
Error	19	500.98	26.36		
Total	22	541.00			

$R^2 = 0.07$ %CV = 39.29

C18:3n6

Source	df	SS	MS	F value	Pr>F
Block	1	0.04	0.04	0.38	0.5443
Treatment	2	1.46	0.73	7.64	0.0037
Error	19	1.82	0.096		
Total	22	3.32			

$R^2 = 0.45$ %CV = 25.92

CLA

Source	df	SS	MS	F value	Pr>F
Block	1	0.06	0.06	0.02	0.8794
Treatment	2	16.62	8.31	3.20	0.0636
Error	19	49.41	2.60		
Total	22	66.10			

$R^2 = 0.25$ %CV = 31.02

Short chain FA

Source	df	SS	MS	F value	Pr>F
Block	1	77.56	77.56	0.55	0.4690
Treatment	2	1079.26	539.63	3.80	0.0410
Error	19	2699.40	142.07		
Total	22	3856.22			

$R^2 = 0.30$ %CV = 12.00

Medium chain FA

Source	df	SS	MS	F value	Pr>F
Block	1	2285.62	2285.62	1.29	0.2708
Treatment	2	10709.35	5354.68	3.01	0.0729
Error	19	33748.43	1776.23		
Total	22	46743.40			

$R^2 = 0.28$ %CV = 11.41

Long chain FA

Source	df	SS	MS	F value	Pr>F
Block	1	9706.14	9706.14	3.43	0.0795
Treatment	2	44565.59	22282.80	7.88	0.0032
Error	19	53727.67	2827.77		
Total	22	107999.4			

$R^2 = 0.50$ %CV = 14.95

Saturated FA

Source	df	SS	MS	F value	Pr>F
Block	1	265.15	265.15	0.09	0.7672
Treatment	2	3928.94	1964.47	0.67	0.524
Error	19	55861.11	2940.05		
Total	22	60055.21			

$R^2 = 0.07$ %CV = 10.09

Unsaturated FA

Source	df	SS	MS	F value	Pr>F
Block	1	1868.98	1868.98	0.94	0.3445
Treatment	2	21227.31	10613.65	5.34	0.0145
Error	19	37776.11	1988.22		
Total	22	60872.40			

$R^2 = 0.38$ %CV = 15.52

Table 2 C. Table of ANOVA (Fatty acid (Experiment 2.))

C4

Source	df	SS	MS	F value	Pr>F
Block	1	1.89	1.89	0.30	0.5875
Treatment	2	9.49	4.74	0.76	0.4795
Error	20	124.52	6.22		
Total	23	135.90			

$R^2 = 0.08$ %CV = 11.04

C6

Source	df	SS	MS	F value	Pr>F
Block	1	0.19	0.19	0.12	0.7288
Treatment	2	27.78	13.89	8.75	0.0019
Error	20	31.75	1.59		
Total	23	59.72			

$R^2 = 0.46$ %CV = 9.93

C8

Source	df	SS	MS	F value	Pr>F
Block	1	10.39	10.39	1.58	0.2236
Treatment	2	5.43	2.72	0.41	0.6675
Error	20	131.71	6.59		
Total	23	147.53			

$R^2 = 0.11$ %CV = 34.52

C10

Source	df	SS	MS	F value	Pr>F
Block	1	54.66	54.66	0.74	0.3989
Treatment	2	349.90	174.95	2.38	0.1184
Error	20	1471.39	73.57		
Total	23	1875.95			

$R^2 = 0.22$ %CV = 56.17

C12

Source	df	SS	MS	F value	Pr>F
Block	1	62.15	62.15	2.30	0.1450
Treatment	2	73.23	36.62	1.36	0.2806
Error	20	540.46	27.02		
Total	23	675.84			

$R^2 = 0.20$ %CV = 12.92

C14

Source	df	SS	MS	F value	Pr>F
Block	1	38.33	38.33	0.34	0.5649
Treatment	2	1328.27	664.13	5.93	0.0095
Error	20	2238.03	111.90		
Total	23	3604.62			

$R^2 = 0.38$ %CV = 14.37

C16

Source	df	SS	MS	F value	Pr>F
Block	1	685.76	685.76	1.40	0.2510
Treatment	2	1301.81	650.91	1.33	0.2878
Error	20	9815.47	490.77		
Total	23	11803.05			

$R^2 = 0.17$ %CV = 11.75

C18

Source	df	SS	MS	F value	Pr>F
Block	1	737.15	737.15	2.33	0.1427
Treatment	2	868.40	434.20	1.37	0.2767
Error	20	6333.67	316.68		
Total	23	7939.22			

$R^2 = 0.20$ %CV = 22.05

C18:1n9t

Source	df	SS	MS	F value	Pr>F
Block	1	904.91	904.91	2.38	0.1385
Treatment	2	897.37	448.68	1.18	0.3277
Error	20	7602.68	380.13		
Total	23	9404.97			

$R^2 = 0.19$ %CV = 63.12

C18:1n9c

Source	df	SS	MS	F value	Pr>F
Block	1	56.24	56.24	0.05	0.8238
Treatment	2	1131.10	565.54	0.51	0.6072
Error	20	22110.72	1105.53		
Total	23	23298.07			

$R^2 = 0.05$ %CV = 11.19

C18:2n6c

Source	df	SS	MS	F value	Pr>F
Block	1	41.45	41.45	2.89	0.1049
Treatment	2	82.95	41.47	2.89	0.0791
Error	20	287.30	14.37		
Total	23	411.69			

$R^2 = 0.30$ %CV = 15.91

C18:3n6

Source	df	SS	MS	F value	Pr>F
Block	1	0.006	0.006	0.20	0.6579
Treatment	2	0.04	0018	0.59	0.5645
Error	20	0.62	0.30		
Total	23	0.67			

$R^2 = 0.06$ %CV = 13.82

CLAa

Source	df	SS	MS	F value	Pr>F
Block	1	4.08	4.08	2.73	0.1140
Treatment	2	0.26	0.13	0.09	0.9184
Error	20	29.91	1.49		
Total	23	34.25			

$R^2 = 0.13$ %CV = 17.78

Short chain FA

Source	df	SS	MS	F value	Pr>F
Block	1	198.26	198.26	1.60	0.2209
Treatment	2	1164.52	582.26	4.69	0.0214
Error	20	2483.25	124.16		
Total	23	3846.02			

$R^2 = 0.35$ %CV = 11.05

Medium chain FA

Source	df	SS	MS	F value	Pr>F
Block	1	354.74	354.74	0.37	0.5512
Treatment	2	6324.82	3162.41	3.28	0.0588
Error	20	19309.82	965.49		
Total	23	25989.38			

$R^2 = 0.26$ %CV = 10.35

Long chain FA

Source	df	SS	MS	F value	Pr>F
Block	1	177.45	177.45	0.28	0.6022
Treatment	2	541.76	270.88	0.43	0.6576
Error	20	12654.02	632.70		
Total	23	13373.24			

$R^2 = 0.05$ %CV = 5.63

Saturated FA

Source	df	SS	MS	F value	Pr>F
Block	1	1575.93	1575.93	0.78	0.3887
Treatment	2	15780.42	7890.21	3.89	0.0375
Error	20	40588.50	2029.43		
Total	23	57944.85			

$R^2 = 0.29$ %CV = 10.07

Unsaturated FA

Source	df	SS	MS	F value	Pr>F
Block	1	467.55	467.55	0.26	0.6173
Treatment	2	1006.61	503.30	0.28	0.7607
Error	20	36297.89	1814.89		
Total	23	37772.05			

$R^2 = 0.38$ %CV = 15.52

BIBLIOGRAPHY

Mr. Pipat Lounglawan was born on the 25th of June 1976 in Nakon Ratchasima, Thailand. He graduated with the Bachelor degree and Master degree from school of Animal Production Technology, Institute of Agricultural Technology, Suranaree University of Technology. After graduation, he obtained the scholarship from the Office of the Higher Education Commission, Ministry of Education, to presence a Doctor degree at the same university. He conducted the research in the topic of Factors affecting Conjugated linoleic acid content of cow's milk. The result of this project has been presented as oral presentation in the 5th National Symposium on Graduate Research 10th-11th October 2005 at Kasetsart University, Thailand. He also had opportunity to go train more CLA production by microbial techniques in Division of Applied Life Sciences, Graduate School of Agriculture, Kyoto University, Japan during July-August 2004.