ผลของการเสริม betaine ในอาหารต่อการทำงานของต่อมน้ำนมและของเหลวในร่างกายในระยะท้ายของการให้นมของแพะนมลูกผสมพันธุ์ซาเนน

นางสาวหนึ่งนุช สายปิ่น

สถาบันวิทยบริการ
จุฬาลงกรณ์มหาวิทยาลัย

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ลิขสิทธิ์ของจุฬาลงกรณ์มหาวิทยาลัย

EFFECTS OF BETAINE SUPPLEMENTATION ON MAMMARY FUNCTION AND BODY FLUID IN LATE LACTATING CROSSBRED SAANEN GOATS

Miss Nungnuch Saipin

สถาบันวิทยบริการ
จุฬาลงกรณ์มหาวิทยาลัย

A Thesis Submitted in Partial Fulfillment of the Requirements
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นางสาวหนึ่งนุช สายปิ่น : ผลของการเสริม betaine ในอาหารต่อการทำงานของต่อมน้ำนมและของเหลวในร่างกายในระยะท้ายของการให้นมของแพะนมลูกผสมพันธุ์ซาเนน (EFFECTS OF BETAINE SUPPLEMENTATION ON MAMMARY FUNCTION AND BODY FLUID IN LATE LACTATING CROSSBRED SAANEN GOATS) อ. ที่ปรึกษา: ศ.น.สพ.ดร. ณรงค์ศักดิ์ ชัยบุตร, อ. ที่ปรึกษาร่วม: รศ.น.สพ. สมชาย จันทร์ผ่องแสง 58 หน้า.

การทดลองครั้งนี้เพื่อศึกษาผลของการเสริม betaine ในอาหารต่อผลผลิตน้ำนม, ส่วนประกอบน้ำนม, และพารามิเตอร์อื่นๆที่เกี่ยวข้องกับการสังเคราะห์น้ำนมในระยะท้ายของการให้นมของแพะนมลูกผสมพันธุ์ซาเนนและกลไกที่ทำให้เกิดการทำหน้าที่ของเต้านมจากทั้งปัจจัยภายในและภายนอกต่อมน้ำนม ในการทดลองใช้แพะนมลูกผสมพันธุ์ซาเนนที่อยู่ในช่วง 11 สัปดาห์หลังคลอดจำนวน 10 ตัว แบ่งเป็น 2 กลุ่มๆละ 5 ตัว แพะในกลุ่มทดลองจะได้รับอาหารข้นและเสริมด้วย betaine ในขนาด 4 ก. ต่ออาหาร 1 กก. เป็นระยะเวลานาน 4 สัปดาห์ ขณะที่แพะนมในกลุ่มควบคุมได้รับอาหารข้นชนิดเดียวกันแต่ปราศจากสารเสริมอาหาร

ผลการทดลองพบว่าแพะกลุ่มที่ได้รับ betaine เสริมในอาหารมีการกินได้วัตถุแห้งของอาหารหยาบลดลงอย่างมีนัยสำคัญ ($P<0.05$) ทั้งในระหว่างการเสริม betaine และหลังจากหยุดให้สารเสริม ปริมาณน้ำนมมีแนวโน้มเพิ่มขึ้นประมาณ 1.11 กก./วัน และ 1.12 กก./วัน เมื่อเทียบกับระยะก่อนให้สารเสริมที่มีปริมาณน้ำนม 0.94 กก./วัน, ตามลำดับ ปริมาณน้ำนมที่ปรับค่าไขมันคิดเป็น 4% FCM พบว่าสูงขึ้นในกลุ่มทดลอง (1.23 กก./วัน) กว่ากลุ่มควบคุม (0.98 กก./วัน) อย่างมีนัยสำคัญ ($P<0.05$) ความเข้มข้นของไขมันนมและน้ำตาลแลคโตสในแพะกลุ่มที่ได้รับ betaine มีค่าสูงขึ้นอย่างมีนัยสำคัญ ($P<0.05$) กว่าระยะก่อนและหลังการให้สารเสริม ปริมาณ K^+ ในเลือดของกลุ่มทดลองพบว่ามีค่าลดลงตลอดการทดลองอย่างมีนัยสำคัญ ($P<0.05$) ในการทดลองครั้งนี้ไม่พบความแตกต่างอย่างมีนัยสำคัญในส่วนของความเข้มข้นของ Na^+, Cl^- ในเลือดและน้ำนม, ปริมาตรพลาสม่า, ปริมาตรเลือด, ปริมาตรน้ำนอกเซลล์, ปริมาตรน้ำในเซลล์และปริมาตรน้ำทั้งหมดในร่างกายระหว่างสัตว์ทดลองทั้งสองกลุ่ม ความเข้มข้นของ acetate ที่พบในเลือดแดงแม้ไม่มีความแตกต่างอย่างมีนัยสำคัญแต่พบว่าเพิ่มขึ้นประมาณ 45% หลังจากการเสริม betaine ในอาหาร รวมถึงค่าผลต่างของความเข้มข้นของ acetate ในเลือดแดงและดำระหว่างต่อมน้ำนมและเปอร์เซ็นต์ของการนำไปใช้โดยต่อมน้ำนมพบว่ามีค่าสูงขึ้นเมื่อเปรียบเทียบกับกลุ่มควบคุม

จากผลการทดลองสรุปได้ว่าการควบคุมการเพิ่มน้ำนมในแพะนมที่เสริมอาหารด้วย betaine ในระยะท้ายของการให้นมของแพะนมลูกผสมพันธุ์ซาเนนจะเป็นผลจากปัจจัยภายในต่อมน้ำนมมากกว่าจากปัจจัยภายนอก ในการใช้สารอาหารเพื่อการผลิตน้ำนม

ภาควิชา สรีรวิทยา ลายมือชื่อนิสิต .....

สาขาวิชา สรีรวิทยาการสัตว์ ลายมือชื่ออาจารย์ที่ปรึกษา .....

ปีการศึกษา 2549 ลายมือชื่ออาจารย์ที่ปรึกษาร่วม .....

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NUNGNUCH SAIPIN: EFFECTS OF BETAINE SUPPLEMENTATION ON MAMMARY FUNCTION AND BODY FLUID IN LATE LACTATING CROSSBRED SAANEN GOATS.

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An experiment was conducted to investigate the effects of dietary betaine supplementation on milk production, milk compositions and relevant other parameters to milk synthesis in late lactating crossbred Saanen goats. The mechanisms by which betaine supplementation on mammary function including intramammary factors and extramammary factors were carried out. Ten, multiparous, non pregnant crossbred Saanen goats in late lactation approximately 11 weeks postpartum were divided into two groups of five animals each. The experimental animals were received diet supplemented with 4 g betaine per kg of the concentrate diet for four weeks, while the control animals were received the similar concentrate diet without betaine as placebo for concurrent control.

The results showed that animals receiving betaine supplementation decreased significantly ($P<0.05$) roughage DMI during treatment and post-treatment periods. Milk yield showed the trend to increase in both treatment and post-treatment period by averaged 1.11 kg/d and 1.12 kg/d as compared with pretreated value 0.94 kg/d, respectively. The 4% fat corrected milk (FCM) was greater ($P<0.05$) for the betaine supplemented animals (1.23 kg/d) than those of the controls (0.98 kg/d). The concentration of milk fat and lactose of betaine supplemented animals in treatment period were significantly ($P<0.05$) higher than those of pretreatment and post-treatment periods. After betaine supplementation, the plasma K^+ concentration decreased significantly ($P<0.05$) during treatment and post-treatment periods. No statistically different were apparent for plasma and milk electrolytes concentrations, body fluid compartments for plasma volume, blood volume, extracellular fluid, intracellular fluid and total body water in both control and betaine supplemented animals. The arterial plasma concentration for acetate showed no significant increase by approximately 45% after betaine supplementation. The arterio-venous concentration difference of plasma acetate and the extraction ratio of acetate across the mammary gland in betaine supplemented animals were higher than those of the control animals.

The present result suggested that the regulation of an increase in milk yield during betaine supplementation in late lactating crossbred Saanen goats is influenced more by the intramammary factors than by extramammary factors in association with the utilization of substrate for milk synthesis.

Department Veterinary Physiology Student's signature Nungnuch Saipin

Field of study Animal Physiology Advisor's signature

Academic year 2006 Co-advisor's signature

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CHAPTER I
INTRODUCTION

Goat is small ruminant, cud-chewing animals as cattle and sheep. Goats are primarily browsers, selectively eating a variety of shrubs. It is known that goat herds in tropical countries are used for both meat and milk. For dairy goats, low milk production in crossbred goats is still the main problem. Since a variety of genetic combination exists between dairy goat and rearing for meat of indigenous goat. However, many factors can affect milk production in dairy goat in the tropics. For examples, high environmental temperature, inadequate supply for foraging during summer month and a lower genetic potential for milk production of native goats. In the hot climate, the low quality of roughage and agricultural raw material causing goats are unable to get enough nutrients from browse alone to meet their needs (Haenlein, 1981; NRC, 1981). Several approach have been attempted to improve dairy goat productivity in the tropics. The important factor influencing production output from goats is genetic merit. Genetic merit from crossbreeding has the advantage of selecting for superior genetic producing ability but adaptation of animals to climate is still a big problem (Haenlein, 1981). A few data are available for studies changes of bodily functions, e.g. body fluid, plasma metabolites, hormones and mammary utilization of nutrients in dairy goats under tropical climates.

It has been known that a marked decrease in milk yield during starvation in goats has been noted (Chaiyabutr et al., 1980; 1981). Therefore, the maintenance for milk production, physiological state and incidence of negative energy balance should be performed in excess energy to dairy goat. The nutritional value is not only the factor limiting milk production of goats in tropical climates but the genetic potential for milk synthesis in crossbred dairy goats would be involved. Feed additive and supplementation would be the choice for increase in milk production.

It is known that milk synthesis is depend on an adequate nutrient in the blood supplying to the mammary gland. Body fluid distribution relating to mammary blood flow

to the mammary glands would be a determinant factor in the process of milk production (Chaiyabutr et al., 1997). The source of substrate retrieving from the diet would be another factor including a necessary supplementation. The major precursor substances such as acetate and ß-hydroxybutyrate have been well known to enroll in the mechanism of milk fat synthesis (Kronfeld, 1969). A number of evidence on the studies of several kinds of supplementation in diet for feeding and improve milk production has been reported, such as methionine (Waterman and Schultz, 1972; Shoca et al., 1994; Madsen et al., 2005) and choline (Erdman and Sharma, 1991; Hartwell et al., 2000; Piepenbrink and Overton, 2003; Pinotti et al., 2003; Banskalieva et al., 2005). Betaine is a quaternary ammonium compound which extensively used as feed additive in the diets of poultry (Sauderson and MacKinlay, 1990) and swine (Matthews et al., 2001). Although, an increase in milk production in goat supplementation of betaine in diet has been noted (Fernandez et al., 2004a; 2004b). A few data are available for the mechanism of action of supplemented betaine in crossbred dairy goat.

Betaine is known as glycine betaine which is a water soluble natural compound, occurred by methyl-properties donation and has a sweet taste. Betaine metabolism in ruminant has been shown to convert to acetate by ruminal microbial activity (Mitchell et al., 1979). The plasma acetate concentration is a source of milk fat synthesis *de novo* in the mammary glands for short chain and some part of the medium chain fatty acids (Moore and Christie, 1979). The higher milk production and the level of milk fat in goats after feeding with betaine have been noted (Fernandez et al., 2004b). Betaine has also been shown to protect cells from osmotic stress in continuing regular metabolic activities (Kidd et al., 1997). Although betaine has been reported on supporting of milk production, the effect of betaine on regulation of body fluids relating to mammary uptake of substrates for milk synthesis cannot be ruled out.

The objective of this study was therefore, undertaken;

i) To study the changes in milk yield, milk compositions, body fluids, plasma metabolites and hormone level in animals feeding with betaine supplementation in late lactation of crossbred Saanen goats.

ii) To find out any amount levels of plasma metabolites, milk yield and milk compositions which are important for mammary utilization of substrate during lactation in animals feeding with betaine supplementation.

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CHAPTER II
BACKGROUND INFORMATION

Mechanism of milk synthesis.

Milk is synthesized in the mammary gland from raw materials which are transported via the bloodstream to the secretory cells of the alveolus (Kronfeld, 1969). Milk production is under influences of extramammary and intramammary factors. The extramammary factors such as hormone, body fluids distribution, mammary blood flow, and environmental temperature. Substrates utilization in mammary gland is referred to an intramammary factor. One of the factors, which limit the milk production of tropical dairy goats, is an inadequate supply for the forage. It is known that values of body water content have been used as an index of the nutritional status of the animals. The rate of water turnover has been show to be related to the food and water intake and metabolism of the animals (Macfarlane and Howard, 1970). The function of lactating mammary gland depends upon an adequate supply of nutrients and hormonal stimuli from blood to sustain milk synthesis. Mammary blood flow and body fluids distribution are determinants for the supply of nutrients for milk synthesis (Chaiyabutr et al., 1980; Davis and Collier, 1985; Lacasse and Prossert, 2003). Milk compositions are composed of both major constituents for example protein, lactose, fat and minor constituents.

Milk protein is made up of a number of specific proteins, casein, ß-lactoglobulin, and α-lactalbumin. Casein being the most important component. The three possible sources of blood precursors of the milk protein are synthesized in golgi vesicles within the mammary cell e.g. peptide, plasma proteins, and free amino acids (Schmidt, 1971).

Lactose is a disaccharide that is made up of glucose and galactose molacule. Lactose is the primary carbohydrate in milk. The synthesis of lactose is apparently in golgi apparatus of the mammary epithelial cells. Milk is in osmotic equilibrium with the blood and is controlled by lactose, K, Na, Cl. Lactose concentration regulates the volume of milk (Clunie and Hill, 1967; Kronfeld, 1969; Peaker, 1975; Frimawaty and Manalu, 1999).

Milk fat is composed of a complex mixture of lipids. Triglycerides are the major type of lipid in milk fat. Triglycerides are composed of three fatty acids covalently bound to a glycerol molecule by ester bounds. The precursors of milk fat that are taken up by the mammary gland for milk fat synthesis e.g. acetate, ß-hydroxybutyrate, and triglycerides. Fatty acids in carbon atom chain length C_4-C_{14} are synthesized in the mammary cells. The carbon atom chain length of C_{16} and greater of fatty acids are performed as a result of rumen hydrogenation and are transported directly in the blood. Milk fat is secreted from mammary epithelial cells as fat globules to the apical membrane of the epithelial cells (Kronfeld, 1969; Hansen et al., 1984).

The structure of betaine.

Betaine or trimethylglycine is a quarternary ammonium compound, which is discovered in juice of sugar beet (*Beta vulgaris*). Betaine function is very close to choline, folic acid, B_{12} and methionine that known as “methyl donors” (Barak and Tuma, 1983). Structure of betaine closely relates to choline, but choline (tetramethylglycine) has four attached methyl groups in which betaine has three. Choline will become betaine (trimethylglycine) after donating one of methyl groups. If betaine donates one of methyl groups, it will become dimethylglycine (Barak and Tuma, 1983).

Betaine is also known as N-trimethylglycine, glycine betaine, glycocoll betaine, oxyneurine and lycine. The molecular formula of betaine is $C_5H_{11}NO_2$, chemical formula is $(CH_3)_3N^+$-CH_2COO^- and the molecular weight is 117.15 daltons. Betaine is very soluble in water and has a sweet taste and widely distributed in plants and animals. Both betaine and betaine in form of betaine hydrochloride are available in dietary supplements (Finkelstein et al., 1971; Dupuy, 1978; Barak and Tuma, 1983; Mar et al., 1995; Mar and Zeisel, 1999).

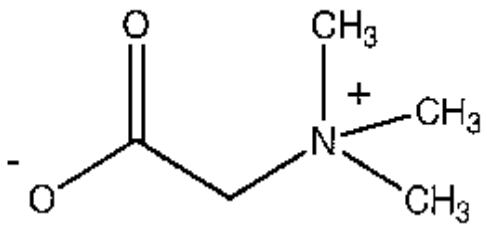

Figure 1: The chemical structure of betaine (Barak and Tuma, 1983).

Mechanism of betaine metabolism.

The present review summarizes the potential nutritional and physiological functions of betaine as a feed additive in relation to performance in livestock production. Betaine, the trimethyl derivative of the amino acid glycine, is a metabolite of animal tissues. Betaine is the product of choline oxidation or it originates from nutritional sources. Over the past decades, numerous studies have been carried out to investigate the potential effects of betaine supplementation on animal performance. Due to its chemical structure, betaine shows the characteristics of a dipolar zwitterion resulting in osmoprotective properties. Promoting effects on the intestinal tract against osmotic stress occurring during diarrhea or coccidiosis have been reported following betaine supplementation in pigs (Wray-Cahen et al., 2004) and poultry (Patricia et al., 1998). There is also some evidence that dietary betaine may improve the digestibility of specific nutrients. As a product of choline oxidation, betaine is involved in transmethylation reactions of the organism. Betaine as a methyl donor provides its labile methyl groups for the synthesis of several metabolically active substances such as creatine and carnitine (Daily et al., 1998).

Supplementation with betaine may decrease the requirement for other methyl donors such as methionine and choline. Methionine can be catabolized in the body to homocysteine, which intern could be resynthesized back to methionine. Betaine actively participates in methionine metabolism by donating methyl groups for the remethylation of homocysteine to methionine.

The two enzymes involved in resynthesis of methionine are betaine-homocysteine methyltransferase and 5-methyltetrahydrofolate homocysteine methyltransferase (Figure 2). There are also some evidences for enhanced methionine availability after dietary supplementation of betaine resulting in improved animal performance (Puchala et al., 1995).

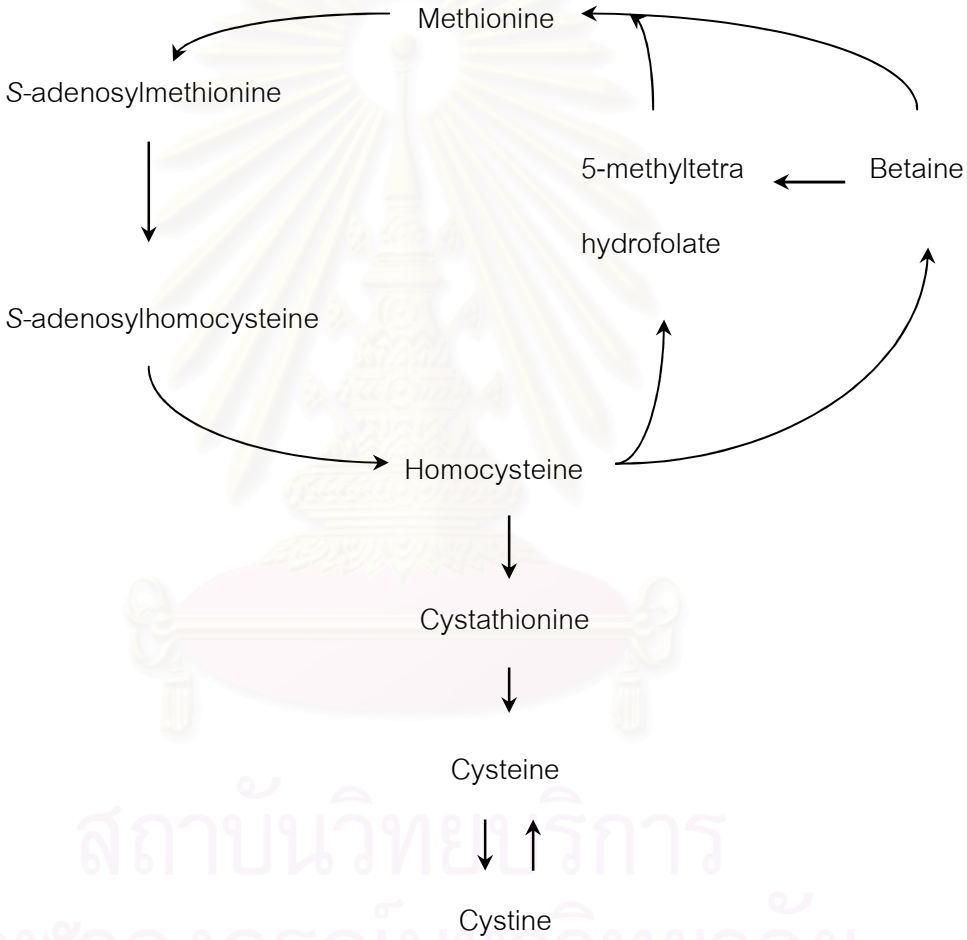

Figure 2: Contribution of betaine in methionine metabolism (Puchala et al., 1995)

Betaine is often referred as a lipotropic factor because of its ability to assist the liver to process fats or lipid. A lipotropic agent is defined as a substance which prevents

the fat deposition in the liver or accelerates its removal (Fernandez et al., 1998; Fernandez et al., 2000; Wray-Cahen et al., 2004).

Dietary betaine may reduce carcass fat in growing pigs, and it also improves animal growth performance by promoting utilization of dietary protein under the confinement conditions (Wray-Cahen et al., 2004). Betaine supplementation prevents accumulation of extramuscular fat, resulting in higher value of carcass. However, concentration of neutral lipid in intramuscular fat of lambs that being fed with enriched betaine diets is lower than those fed with the basal diet (Fernandez et al., 1998). The assumption is a generalized effect of betaine causes low fat in all lambs tissue. Neutral lipids are mainly composed by triglycerides, which are the principal part of depot lipids in meat system.

Supplementation with choline and betaine could partly replace methionine in use as a methyl group donor and thereby increase its availability for protein synthesis to elevate meat or milk production of goats (Banskalieva et al., 2005). The study of Sugiyama et al. (1998) has been shown that choline and betaine can substitute for *S*-adenosylmethionine as a methyl donor for the direct methylation of phosphatidylethanolamine to affect phosphatidylcholine and phosphatidylethanolamine concentrations. In addition, betaine and choline methyl groups may increase the synthesis of carnitine (Daily et al., 1998). Thereby Banskalieva et al. (2005) have concluded that betaine can influence fat metabolism in the goat liver (Figure 3).

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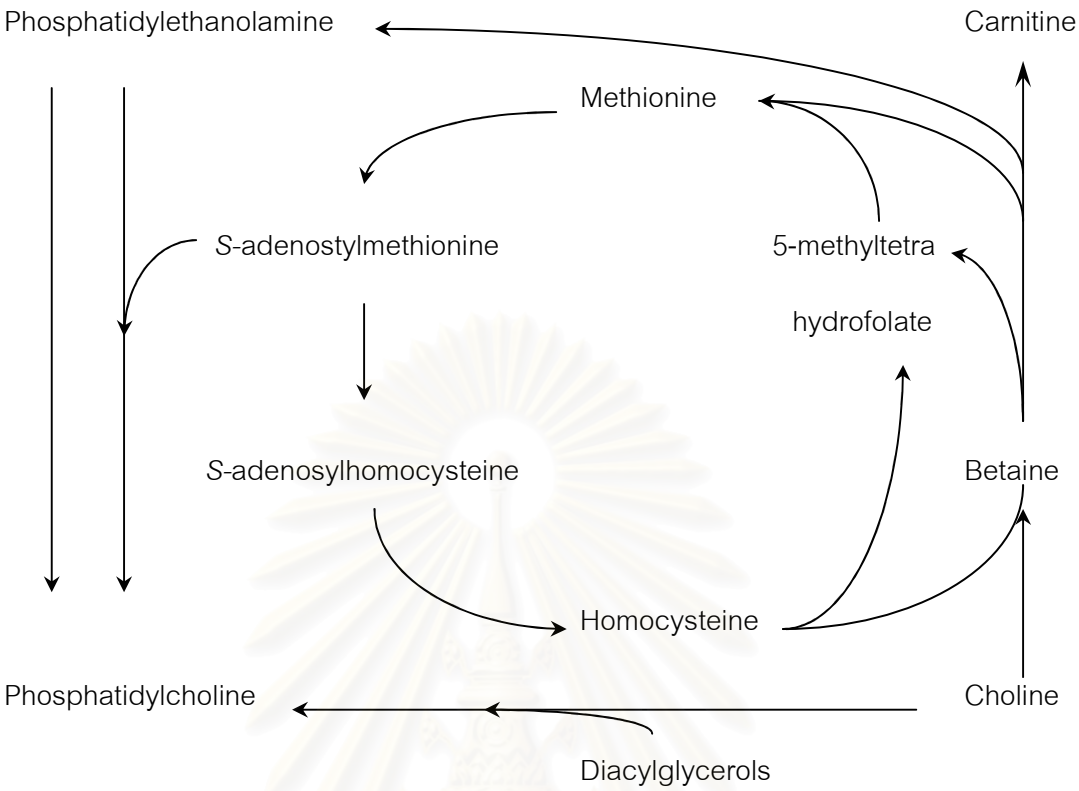

Figure 3: Metabolic pathways in ruminants involving choline and betaine (Banskalieva et al., 2005)

It has been known that the first stage of liver damaged due to alcohol drinking is the accumulation of fat in the liver, the condition of fatty degeneration is called steatosis. The study of Barak et al. (1996) has been carried out the effect of betaine on hepatic methionine metabolism eliciting by short-term ethanol feeding in rats. It has shown that dietary betaine supplementation enhanced methionine metabolism and S-adenosylmethionine generation in both control group and ethanol-fed rats.

There is widely known that hepatotoxic in mammalian species including human is caused by chloroform infection. Kim et al. (1998) studied the effects of singly administration of betaine on hepatotoxicity of chloroform in mice, the result has been demonstrated that betaine has either potential or inhibited action on the chloroform hepatotoxicity.

Organic osmolyte effect of betaine

The osmoprotective effect of betaine has been demonstrated as cytoprotective effect in rat liver. Wettstein and Haussinger (1997) studied the influence of osmolarity and osmolytes by investigating a rat's liver perfusion model of warm ischemia. Livers were perfused with different medium osmolarities for 60 to 90 minutes in the oxygen absence condition, then another 90 minutes of reoxygenation. The conclusion was that the warm ischemia-reoxygenation injury in rat liver was aggravated by hyperosmolarity and attenuated by hypo-osmolarity. The osmolyte betaine has shown a protective effect, presumable by inhibition of Kupffer cell activation.

An osmoprotective effect on plasma electrolytes has been study by Meury (1988) for the accumulation of glycine betaine to a high internal concentration by *Escherichia coli* cells in high osmolarity medium restores. The experimental results support the view that cell adaptation to high osmolarity involves a decrease in the initiation frequency of DNA replication via a stringent response. In contrast, glycine betaine transport and accumulation could suppress the stringent response within 1-2 min and restore a higher initiation frequency. High osmolarity also triggers the cells to lengthen, perhaps via an inhibition of cellular division. Glycine betaine also reverses this process. It is inferred that turgor could control DNA replication and cell division in two separate ways. Glycine betaine action is not mediated by K^+ since the internal level of K^+ is not modified significantly following glycine betaine accumulation.

In many mammalian cells, betaine accumulates actively under hypertonic condition. This process has widespread importance in cell volume regulation (Schliess and Haussinger, 2002) such as a particularly high accumulation in the inner medullar of the mammalian kidney (Garcia and Burg, 1991; Zhang et al., 1996; Weik et al., 1998; Regehr et al., 1999).

In case of water interaction with glycine betaine was studied by Sironi et al. (2001), which considered appropriate an investigation of the hydration properties of glycine betaine. The interaction of water with glycine betaine that osmoprotectants is one of the most effective and widespread in nature. Its effects on structure and dynamics of the surrounding water are compared with those exerted by two other

remarkable bioprotectants: the disaccharide trehalose and the quaternary ammonium compound TMAO (trimethylamine-*N*-oxide).

According to the betaine transport mechanism across animals plasma membrane could explain by the results of Dellow et al. (1999) that glycine betaine is one of organic osmolytes actively accumulated intracellularly in the renal medullar. Glycine betaine is freely filtered in the kidney with most being reabsorbed from the glomerular filtrate via active Na^+ dependent transports in the proximal tubule. The study of Kettunen et al. (2001a; 2001b) also showed that betaine transport in broiler chick intestine involves both a Na^+ dependent and a Na^+ independent component. Betaine in the diet increased the Na^+ dependent component of betaine uptake, and thus the total uptake of betaine. The Na^+ independent component remained unaffected by the dietary betaine supplementation.

Betaine is able to improve nutrient utilization of animal under stress. After provide the dietary supplementation at a level of 0.15%, there is an enhancement in the anticoccidial activity of the ionophore. Since betaine may act as an osmoprotectant, it improves the integrity and function of the infected intestinal mucosa (Patricia et al., 1998).

The study of effects of feed grade betaine on animals performance and carcass characteristics including feed intake and body weight has been shown that top-dressing feed grade betaine to the diet had no effect on feed intakes of cattle (Loest et al., 2000; 2001; 2002) and pigs (Matthews et al., 2001).

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Betaine degradation in the rumen

The study of Loest et al. (2001) was conducted to evaluate the *in vitro* degradation of betaine sources by rumen microbes. The degradation of betaine was slower with the high roughage diet than the grain diet. Ruminal microbes can degrade betaine, which some of the betaine remained after 24 hours. It has been suggested that some betaine would escape ruminal degradation and thus pass to the small intestine (Loest et al., 2001).

The study in betaine metabolism has shown that betaine is metabolized in the rumen. *In vitro* experiment, which methyl-labeled betaine showed that when betaine was incubated with rumen contents collected from a mature cow. There was a progressively decrease in amounts of betaine. The decreasing is associated with an increasing recovery of radioactivity in acetate and CO_2. For *in vivo* testing, betaine involved digestion trials in rumen by using radiolabelled carbon in sheep and found 85% of betaine lost in rumen. Inside rumen, betaine is converted to acetate by microbes, and passed into the blood where it is used in the metabolism (Mitchell et al., 1979).

Milk production response to betaine administration.

The biosynthesis of lactose, fat and protein are highly characteristic of lactation and may influence the rate of milk secretion. Acetate and probably including ß-hydroxybutyrate, is utilized and taken up in the blood for synthesis of short chain fatty acid (C_4-C_{14} fatty acid) which are precursors for milk synthesis. All of the short chain and some part of the medium chain fatty acid that up to C_{16} being synthesized *de novo* in the udder from acetate and ß-hydroxybutyrate in milk fat (Moore and Christie, 1979). Milk yield and fat corrected milk (FCM) of dairy cows have generally increased in response to feeding rumen protected choline and betaine during the transitional period (Erdman and Sharma, 1991; Hartwell et al., 2000; Piepenbrink and Overton, 2003; Pinotti et al., 2003).

The study of betaine supplementation in dairy cows after 42-days of lactation showed that no differences were observed in average daily milk production (kg/cow/d), feed consumption (kg/cow/d) or yield of milk protein (kg/cow/d) and butterfat (kg/cow/d), as comparison with for the control animals (Kellems, 2002).

The effect of betaine on fatty acids profile in goat milk fat has been reported by Fernandez et al. (2004b) who showed the characteristics fatty acids pattern of goat milk that Caprylic ($C_{8:0}$), Capric ($C_{10:0}$), Luaric ($C_{12:0}$), Caproleic ($C_{10:1}$) and Linolenic ($C_{18:3}$) were significantly higher in betaine group than in control one. The increased rates of secretion of C_4 - C_{16} fatty acids are probably a result of increased availability of substrate

for mammary fatty acids synthetase. As the C_{18} fatty acids would be largely of dietary origin, it might be argued that the diminished secretion of the C_{18} fatty acids is the result of some effect of increased acetate concentration on the concentration of the plasma triglyceride or free fatty acid fraction. Fernandez et al. (2004a; 2004b) concluded that oral supplementation with betaine (4g/kg) increased greater milk production from the third month and the higher level of milk fat on the fifth month during late lactation in goats because betaine increased the short chain fatty acid and linolenic acid. Moreover, the study of feeding betaine on milk production performance in goats in the fourth month showed that the goats fed with betaine diet have higher milk yield than the ones fed with control diet (Fernandez et al., 2004a; 2004b). There is a similarity of the incorporation between level of betaine to diet in Fernandez's experiment and Sanchez's as 4g/kg (Fernandez et al., 2000) and 2g/kg diet (Sanchez et al., 2001). The effect of feeding betaine on milk compositions in goats is no significant differences in the chemical compositions of milk in the fourth month between betaine supplemented goats and complemented ones. However, the percentage of fat is numerically higher in the betaine feeding group than the controlling one (Fernandez et al., 2004a; 2004b).

Effect of betaine on plasma metabolites.

Choline, betaine and methionine are the three main dietary sources of methyl groups in animal diet, and also consider being essential components in the regulation of methylation processes (Banskalieva et al., 2005). It has been shown that choline has a variety of functions in mammalian tissue. The most significant function is as a component of the predominant phospholipids that phosphatidylcholine contain in the membranes of all cells in the body. Choline is the direct precursor to betaine in methyl group metabolism. Choline or betaine supplementation has focused on the role in lipid metabolism because phosphatidylcholine is required for synthesis and release of very low density lipoproteins (VLDL) by liver. According to the studied of Yao and Vance (1990) it was shown that choline deficiency in rats have increased in liver triglyceride content.

Supplementation with choline or betaine could partly replace methionine in methyl donated group, and increase the availability of protein synthesis to elevate meat or milk production (Fernandez et al., 1998; 2000; 2004a; 2004b). However, effect of betaine and choline supplementation on body fluid, plasma volume, plasma osmolality and electrolytes are not consistent. For example, Banskalieva et al. (2005) reported neither betaine nor choline had affected blood flow, packed cell volume, hemoglobin concentration or oxygen consumption in goats. Also betaine affected plasma triacylglycerides (TG) concentration were increased by betaine with 9% dietary crude protein. The effects of dietary crude protein level (9% and 15% DM) and supplementation with ruminally protect betaine or choline on plasma concentrations of ammonia nitrogen, non-esterified fatty acids (NEFA), triacylglycerols (TG), cholesterol and net fluxes of oxygen across the portal-drained viscera (PDV) and liver.

An experiment of Matthews et al. (2001) was conducted to determine the effect of dietary betaine on growth and plasma metabolites. The result showed that plasma concentrations of urea nitrogen, total protein, albumin, triglycerides and high density lipoproteins (HDL) were not affected, but plasma total cholesterols and non-esterified fatty acids (NEFA) were increased in pigs fed betaine. The study of Huang et al. (2006) was conducted to investigate the effect of dietary betaine supplementation on carcass characteristics, hormones, growth factor and lipid metabolism. The effect of betaine supplementation in finishing pigs showed no remarkable influence of betaine on plasma triacylglycerol levels.

The experiment by Puchala et al. (1995) about the effect of betaine on plasma methionine and relate metabolite showed the difference in utilization of betaine in Angora and Alpine kids. Plasma triglyceride levels did not change by single injection of betaine (0.2 g/kg BW).

Effect of betaine on hormonal changes.

Milk production reaches a peak, and then declines as lactation progresses. Involution of the mammary glands in goats may be compared to other mammals by the loss of DNA during involution (Anderson and Wahab, 1990). The decrease in number of the mammary cell during lactation may account for the large decreasing in milk yield after peak in lactating period (Wilde et al., 1997). A number of studies were reported about increase the rate of milk production within the mammary gland and provides the necessary nutrients in support of enhance rate of milk synthesis by growth hormone or the variation forms or the synthetically derivative hormone such as recombinant bovine somatotropin (rbST) (Burton et al., 1994). Analysis of milk from intracellular constituents, such as glucose has proved useful. Changes in the concentration of glucose in milk have been found to correlate significantly with changes in milk production under a variety of situations such as feed restriction (Chaiyabutr et al., 1981). The nutrient partitioning response to bovine somatotropin (bST) treatment that support increase milk yield particularly concerns the preferential oxidation of fatty acids and the sparing of glucose by peripheral tissues, the increase substrate utilization by the mammary gland may in turn provide a stimulus for increasing feed intake or high quality diet feeding (Bauman and Vernon, 1993).

It has been known that growth hormone causes growth of almost all tissues of the body that are capable of growing. It promotes increases in size and number of the cells. The normal concentration of growth hormone in the plasma is not too much. However, the values often increase after depletion of body stores of protein or carbohydrate (Guyton, 1991). Supplementation with betaine could partly replace methionine in methyl donated group, which may increase the availability of protein synthesis to elevate meat or milk production (Fernandez et al., 1998; 2000; 2004a; 2004b; Banskalieva et al., 2005). So this factor may relate to production of growth hormone. An experiment of Huang et al. (2006) was conducted to investigate the effect of dietary betaine supplementation on carcass characteristics, hormones, growth factor and lipid metabolism in finishing pigs. The result showed that serum growth hormone (GH), insulin-like growth factor I (IGF-I), free triiodothyronine (FT3), free thyroxine (FT4)

and insulin levels were significantly increased with betaine treatment. Meanwhile, serum free fatty acids concentration in betaine supplemented pigs was higher compared to controls but there was no marked difference for serum triacylglycerol.

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CHAPTER III
MATERIALS AND METHODS

1. Animal and diets

Ten healthy crossbred goats (> 87.5% Saanen gene) were used for study in the late lactating period. They were randomely divided into two groups of 5 animals each.

Animals in the control group were fed with concentrate and Pangola hay as roughage. Animals in the experimental group were given similar diet, which was supplemented with betaine in concentrate diet (4g/kg) throughout experimental periods. Goats were fed concentrate diet once a day at 15.00 pm. Pangola hay and water were freely available. The chemical compositions of feed are presented in table 1. Daily feed refusals were weighed and sample of diet was taken for chemical analysis. The goat was kept in small pens individual, which was placed indoor with an environmental temperature at 29-36 °C.

The experimental period was started at day 70 postpartum during decline of peak yield from the transition of mid to late lactation. The milk sample was taken by hand milking once daily and recorded milk yield by weighing. Milk samples were collected and analyzed for milk compositions in every two weeks.

Table 1: Chemical compositions of feed components (% on dry matter basis)

	roughage	concentrate
Dry matter	88.06	90.94
Crude protein	3.29	16.20
Acid detergent fiber	28.50	23.63
Neutral detergent fiber	71.50	62.04
Betaine	0	0.4

2. Experimental protocol

The experimental protocol of this experiment is shown in Figure 4. Three periods of measurements were carried out during the course of experiment; the first period was performed as a pretreatment. In the treatment period, animals were given betaine supplementation daily (4g/kg diet) for 4 weeks during 12th to 16th weeks postpartum. Post treatment period was carried out for another 2 weeks after treatment period without betaine supplementation (18th weeks postpartum). Animals in the control group were normal fed without betaine in diet, which carried out in a similar period as the concurrent control.

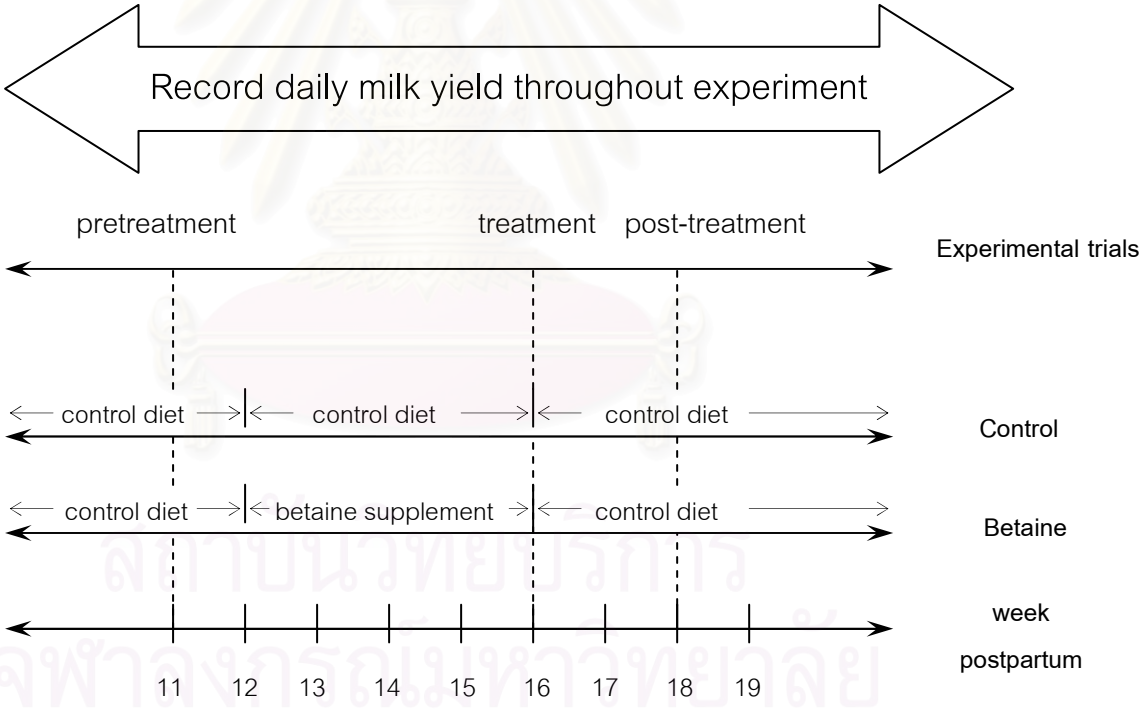

Figure 4: The experimental protocol of this experiment

3. Determinations of milk compositions and milk osmolality.

Milk secretion was recorded by hand milking once daily about 6.00 am and milk compositions were determined in every two weeks. Approximately 60 ml of milk sample was stored in the refrigerator at 4 °C with preservative of 0.2 ml of 2% w/v of 2-bromo-2-nitropropane-1, 3 diol. Milk samples were used to determine for the milk fat concentration by the Gerber method (Clunie and Hill, 1967), milk protein concentration by Milkoscan and lactose concentration according to a previous reported by Teles et al. (1978).

Milk osmolality was determined using osmometer (model 3D3, Advanced instrument, Massachusetts, USA). Aqueous phase of milk was used for determination of milk electrolytes concentration of Na^+ and K^+ by centrifugation at 4,000 rpm, 4°C, for 15 min to separate milk fat and aqueous phase. The 50 μl of aqueous milk was transfer into the test tube and mixed well with 10 ml of the diluents. The determination for the Na^+ and K^+ concentration were performed by the flame photometer (410C, Corning, England). Milk Cl^- concentration were measured by chloride analyzer, respectively (925, Corning, England).

4. Determinations of plasma volume (PV), extracellular fluid (ECF), intracellular fluid (ICF) and total body water (TBW).

The jugular vein was cathetered for dye injection. Plasma volume (PV) was determined using dye dilution technique by intravenous injection of 3 ml of 0.5% Evans blue dye (T-1824) via the jugular vein. Determination for extracellular fluid (ECF) was performed by intravenous injection of 5 ml of 10% sodium thiocyanate solution (NaSCN) and total body water (TBW) also was performed by intravenous injection of 5 ml of 15 % urea solution via the jugular vein.

Blood samples were collected from the contralateral jugular vein into heparinised tubes (25 iu/ml blood). Blood samples were obtained at 30, 40 and 50 minutes after dye injection. The blood samples were centrifuged to collect plasma for

determination of the concentration of Evans blue dye, sodium thiocyanate (Medway and Kare, 1959) and urea (Chiba et al., 1990) by using spectrophotometer.

Plasma volume (PV) was calculated from the equation:

$$PV = \frac{\text{Amount of Evans blue dye injected (mg)}}{\text{Concentration of Evans blue dye at zero time concentration (mg/L)}}$$

Plasma volume as percentage of body weight (%PV) was calculated from the plasma volume (PV) and body weight (BW) as equation:

$$\%PV = \frac{PV \times 100}{BW}$$

Extracellular fluid (ECF) was calculated as equation:

$$ECF = \frac{\text{Amount of sodium thiocyanate injected (mg)}}{\text{Concentration of sodium thiocyanate at zero time concentration (mg/L)}}$$

Percentage of extracellular fluid (%ECF) was calculated from the extracellular fluid (ECF) and body weight (BW) as equation:

$$\%ECF = \frac{ECF \times 100}{BW}$$

Total body water (TBW) was calculated as equation:

$$TBW = \frac{\text{Amount of urea injected (mg)}}{\text{Concentration of urea at zero time concentration (mg/L)}}$$

Percentage of total body water (%TBW) was calculated from the total body water (TBW) and body weight (BW) as equation:

$$\%TBW = \frac{TBW \times 100}{BW}$$

Blood volume (BV) was calculated from the plasma volume (PV) and packed cell volume (PCV) as equation:

$$BV = \frac{PV}{1-PCV}$$

Percentage of blood volume (%BV) was calculated from the percentage of plasma volume (%PV) and pack cell volume (PCV) as equation:

$$\%BV = \frac{BV \times 100}{BW}$$

Intracellular fluid (ICF) was calculated from extracellular fluid (ECF) and total body water (TBW) as equation:

$$ICF = TBW - ECF$$

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Percentage of intracellular fluid (%ICF) was calculated from ICF and body weight (BW) as equation:

$$\%ICF = \frac{ICF \times 100}{BW}$$

5. Determinations of plasma osmolality and plasma electrolytes concentration.

On the specified day of measurement, plasma samples were collected to measure plasma osmolality by using freezing point depression by osmometer (model 3D3, Advanced instrument, Massachusetts, USA).

The concentrations of Na^+ and K^+ in plasma were determined by flame photometer (410C, Corning, England), and the Cl^- in plasma was determined by chloride analyzer (925, Corning, England).

6. Determinations of plasma metabolites concentration.

Plasma samples were collected from both milk vein and ear artery at each specified day of measurement for determination of the plasma metabolites concentration. The plasma concentration for acetate was assayed by acetic acid UV-method (Cat. No. 10 148 261 035, r-biopharm, USA) and plasma concentration for ß-hydroxybutyrate was assayed by D-3-Hydroxybutyric acid colorimetric method (Cat. No. 10 907 979 035, r-biopharm, USA). The plasma concentration for glucose was determined by glucose liquicolor emzymatic colorimetric test (SU-GLL Q2, Germany) and the plasma concentration for triglyceride was determined by enzymatic colorimetric test (triglyceride liquicolor^{mono} SU-TRIMR, Germany).

7. Determinations of the arterio-venous (A-V) concentration difference and percentage of mammary extraction of substrates across the mammary gland.

Substrates used in this study were acetate, ß-hydroxybutyrate, glucose and triglyceride. The extraction of substrate across the mammary gland was calculated from the concentration of plasma samples from milk vein and ear artery blood in each period of experiment by dividing the mammary arterio-venous concentration difference [A-V (mmol/l)] with arterial plasma concentration (A).

The equation for extraction ratio by the mammary gland of each substrate was determined as follow:

$$\text{Extraction (\%)} = \frac{[(A-V)] \times 100}{A}$$

8. Determinations of insulin-like growth factor I (IGF-I) and thyroxine (T4) hormone level.

Plasma samples were collected from jugular vein was cathetered for determination of IGF-I hormone level by Chemiluminescence immunoassay using Immulite analyzer and T4 hormone level was determined by Electrochemiluminescence method using Elecsys 2010 analyzer.

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9. Data analysis.

Data was reported as the mean ± SD. Experiments measuring was designed to compare data from the control and experiment groups using the unpaired *t*-test. The interaction of the periods in the same group were using Repeated measures ANOVA and Duncan's Multiple Range test. The differences of hormonal levels between the periods of study in the same group using pair *t*-test. The statistical significance assessed at level $P<0.05$.

CHAPTER IV
RESULTS

Dry matter intake and body weight in the controls and betaine supplemented animals (Table 2).

The data in Table 2 show that dry matter intake of roughage of betaine supplemented animals were significantly lower ($P<0.05$) in either the treatment (0.55 ± 0.10 kg/d) or post-treatment periods (0.59 ± 0.09 kg/d) as compared with the pretreatment period (0.75 ± 0.17 kg/d). The dry matter intake of concentrate and total dry matter intake per milk yield were not significantly different among groups and periods of experiments. Body weights were not affected by betaine supplementation in concentrate diet in both groups.

Milk yield and milk compositions in the controls and betaine supplemented animals (Table 3, Figure 5).

Effects of betaine supplementation on milk yield and milk compositions are shown in Figure 5 and Table 3. No significant changes ($P>0.05$) in milk yield were apparent throughout periods of studies in the control group. There were no significant differences ($P>0.05$) of milk yield as lactation advanced in the control group among periods of the treatment (0.98 ± 0.09 kg/d) and the post-treatment (0.97 ± 0.15 kg/d) as compared with the pretreatment value (0.97 ± 0.12 kg/d). Milk yield of betaine supplemented animals showed no significant increases either the treatment period (1.11 ± 0.27 kg/d) or the post-treatment period (1.12 ± 0.29 kg/d) as compared with the pretreatment period (0.94 ± 0.18 kg/d). During the treatment period, milk yield of betaine supplemented animals was higher than those of the control animals which was apparent in the first week after feeding with betaine supplementation (Figure 5).

The 4% fat corrected milk (FCM) of the betaine supplemented animals increased significantly ($P<0.05$) in the treatment period (1.23 ± 0.23 kg/d) which were higher than the pretreatment (0.89 ± 0.12 kg/d) and post-treatment period (0.95 ± 0.27 kg/d) by approximately 38% after betaine administration. The FCM of betaine supplemented animals was also significantly higher ($P<0.05$) than that of the control animals at the treatment period.

No significant differences were found on milk compositions for the concentrations of milk protein, solid not fat (SNF) and total solids (TS) among the periods of studies in both control animals and betaine supplemented animals.

However, the concentration of milk fat of betaine supplemented animals in treatment period (4.35 ± 0.84 g%) were significantly higher ($P<0.05$) than those of the pretreatment period (3.46 ± 0.93 g%) and the post-treatment period (3.33 ± 0.32 g%).

The concentration of lactose of betaine supplemented animals in the treatment period (4.94 ± 0.22 g%) were significantly higher ($P<0.05$) than those of the pretreatment period (4.22 ± 0.43 g%) and the post-treatment period (4.33 ± 0.24 g%).

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Table 2: Effects of betaine supplementation on dry matter intake (DMI) of roughage and concentrate, total DMI/milk yield, and body weights of crossbred Saanen goats.

		Pretreatment	Treatment	Post-treatment	*P*-value[1]
Roughage DMI (kg/d)					
	Control	0.76 ± 0.08	0.68 ± 0.09	0.72 ± 0.09	0.161
	Betaine	0.75 ± 0.17[a]	0.55 ± 0.10[b]	0.59 ± 0.09[b]	0.006
	P-value	NS	NS	NS	
Concentrate DMI (kg/d)					
	Control	0.38 ± 0.10	0.34 ± 0.08	0.35 ± 0.10	0.381
	Betaine	0.36 ± 0.08	0.35 ± 0.08	0.34 ± 0.08	0.564
	P-value	NS	NS	NS	
Total DMI/milk yield ratio					
	Control	1.14 ± 0.15	1.03 ± 0.09	1.15 ± 0.10	0.094
	Betaine	1.09 ± 0.30	0.81 ± 0.32	0.93 ± 0.32	0.071
	P-value	NS	NS	NS	
Body weight (kg)					
	Control	39.20 ± 9.78	40.00 ± 10.65	40.60 ± 11.67	0.398
	Betaine	42.40 ± 4.83	43.00 ± 4.36	44.40 ± 6.39	0.074
	P-value	NS	NS	NS	

Values are presented as mean ± SD.

[1] *P*-values from the measurements of different values among periods of experimental are evaluated by Repeated measures ANOVA

[a, b] Mean values with different superscripts within a row are significantly different (P<0.05).

The significant differences of values between the control group and betaine supplemented group using unpair *t*-test; * P<0.05, NS=not significant.

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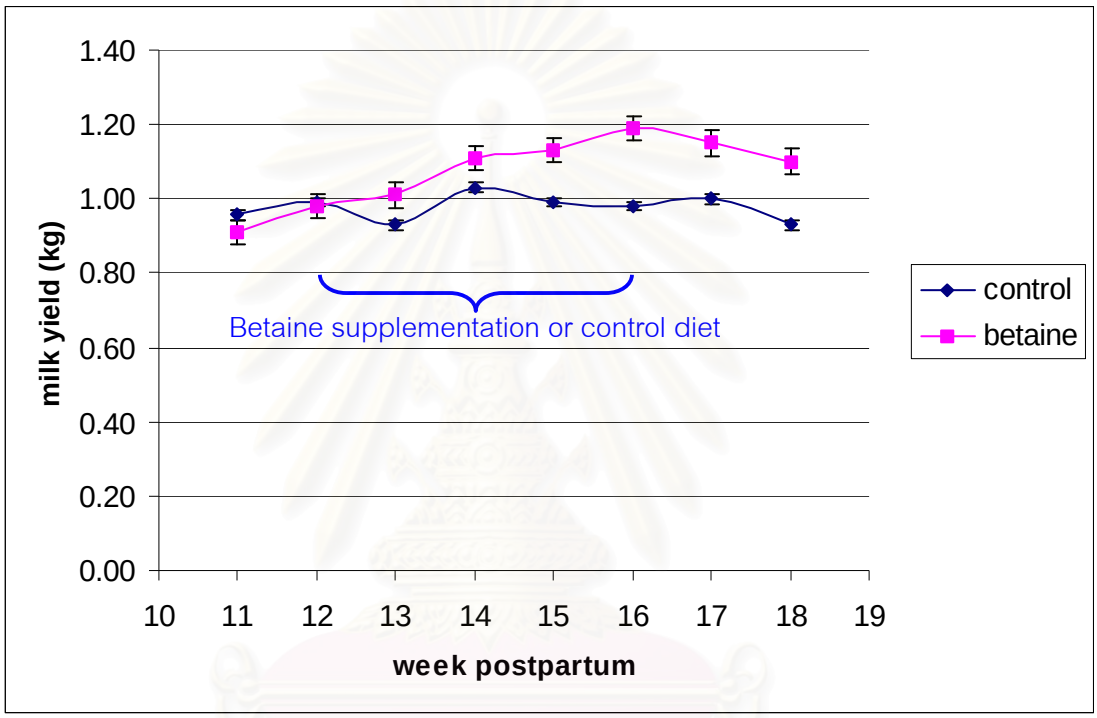

Figure 5: Milk yield of the controls and betaine supplemented animals.

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Table 3: Effects of betaine supplementation on milk yield and milk compositions of goats.

		Pretreatment	Treatment	Post-treatment	*P*-value[1]
Milk yield (kg/d)					
	Control	0.97 ± 0.12	0.98 ± 0.09	0.97 ± 0.15	0.890
	Betaine	0.94 ± 0.18	1.11 ± 0.27	1.12 ± 0.31	0.138
	P-value	NS	NS	NS	
4% FCM (kg/d)					
	Control	1.03 ± 0.11	0.98 ± 0.05	0.89 ± 0.18	0.256
	Betaine	0.89 ± 0.12[a]	1.23 ± 0.23[b]	0.95 ± 0.27[a]	0.007
	P-value	NS	*	NS	
Milk compositions:					
Fat (g%)					
	Control	3.96 ± 0.25	3.96 ± 0.59	3.68 ± 0.71	0.481
	Betaine	3.46 ± 0.93[a]	4.35 ± 0.84[b]	3.33 ± 0.32[a]	0.039
	P-value	NS	NS	NS	
Protein (g%)					
	Control	3.12 ± 0.87	3.09 ± 0.43	2.90 ± 0.38	0.745
	Betaine	3.21 ± 0.83	3.64 ± 0.64	3.22 ± 0.66	0.625
	P-value	NS	NS	NS	
Lactose (g%)					
	Control	4.52 ± 0.32	4.16 ± 0.39	4.23 ± 0.15	0.052
	Betaine	4.22 ± 0.43[a]	4.94 ± 0.22[b]	4.33 ± 0.24[a]	0.003
	P-value	NS	NS	NS	
SNF					
	Control	8.34 ± 1.06	8.37 ± 0.98	7.84 ± 0.50	0.349
	Betaine	8.82 ± 0.86	8.60 ± 0.63	8.25 ± 0.72	0.489
	P-value	NS	NS	NS	
TS					
	Control	12.30 ± 0.89	12.30 ± 0.87	11.51 ± 0.97	0.284
	Betaine	12.28 ± 1.69	12.87 ± 0.72	11.40 ± 1.19	0.167
	P-value	NS	NS	NS	

Values are presented as mean ± SD.

[1] *P*-values from the measurements of different values among periods of experimental are evaluated by Repeated measures ANOVA

[a, b] Mean values with different superscripts within a row are significantly different (P<0.05).

The significant differences of values between the control group and betaine supplemented group using unpair *t*-test; * P<0.05, NS=not significant.

Milk fat yield, milk protein yield, and milk lactose yield in the controls and betaine supplemented animals (Table 4).

The data in Table 4 show that milk fat yield of betaine supplemented animals in the treatment period (50.25 ± 9.82 g/d) was significantly (P<0.05) higher than those of the pretreatment period (32.92 ± 5.43 g/d) and the post-treatment period (34.90 ± 9.46 g/d). Milk fat yield of the control animals showed no differences throughout periods of studies. There were no significant differences of milk protein yield throughout experimental periods in both control animals and betaine supplemented animals.

Table 4: Comparison for milk fat yield, milk protein yield, and milk lactose yield in the controls and betaine supplemented animals.

	Pretreatment	Treatment	Post-treatment	*P*-value[1]
Milk fat yield (g/d)				
Control	41.10 ± 5.10	38.82 ± 3.57	33.82 ± 6.91	0.174
Betaine	32.92 ± 5.43[a]	50.25 ± 9.82[b]	34.90 ± 9.46[a]	0.003
P-value	NS	NS	NS	
Milk protein yield (g/d)				
Control	31.74 ± 6.44	30.66 ± 6.02	27.38 ± 8.11	0.271
Betaine	31.52 ± 10.24	42.78 ± 11.42	34.16 ± 12.22	0.217
P-value	NS	NS	NS	
Milk lactose yield (g/d)				
Control	47.20 ± 5.74	41.04 ± 5.26	39.62 ± 8.88	0.216
Betaine	48.26 ± 8.17	50.66 ± 15.69	46.20 ± 15.99	0.683
P-value	NS	NS	NS	

Values are presented as mean ± SD.

[1] *P*-values from the measurements of different values among periods of experimental are evaluated by Repeated measures ANOVA

[a, b] Mean values with different superscripts within a row are significantly different (P<0.05).

The significant differences of values between the control group and betaine supplemented group using unpair *t*-test; * P<0.05, NS=not significant.

Milk electrolytes and milk osmolality in the controls and betaine supplemented animals (Table 5).

The data in Table 5 show that no significant differences were observed in the concentration of milk Na^+, K^+ and Cl^- including of Na^+ / K^+ ratio among periods of studies in both groups.

Milk osmolality of betaine supplemented animals was also showed no significant differences as compared with the control animals among periods of studies.

The concentration of plasma electrolytes and plasma osmolality in the controls and betaine supplemented animals (Table 6).

The data in Table 6 show that betaine supplementation had no effects on the concentration of plasma Na^+ and Cl^-. The concentration of plasma K^+ in betaine supplemented animals decreased significantly ($P<0.05$) from the value at the pretreatment period by 4.86 ± 0.36 mmol/L to 4.44 ± 0.21 mmol/L and 4.34 ± 0.21 mmol/L at the treatment and the post-treatment periods, respectively.

There were no significant differences of plasma osmolality throughout experimental periods in both the control animals and betaine supplemented animals.

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Table 5: Effects of betaine supplementation on the concentration of milk electrolytes and milk osmolality of crossbred Saanen goats.

	Pretreatment	Treatment	Post-treatment	*P*-value[1]
Na^+ (mmol/L)				
Control	34.60 ± 11.33	32.60 ± 4.51	36.40 ± 1.67	0.709
Betaine	32.20 ± 3.70	37.80 ± 9.78	38.75 ± 4.71	0.135
P-value	NS	NS	NS	
K^+ (mmol/L)				
Control	43.36 ± 4.99	43.58 ± 1.44	43.74 ± 1.89	0.984
Betaine	45.68 ± 2.99	40.28 ± 3.61	41.70 ± 3.17	0.062
P-value	NS	NS	NS	
Cl^- (mmol/L)				
Control	56.80 ± 7.16	57.40 ± 3.21	57.00 ± 5.00	0.961
Betaine	53.60 ± 6.43	53.60 ± 5.27	56.60 ± 7.80	0.761
P-value	NS	NS	NS	
Na^+ / K^+ ratio				
Control	0.83 ± 0.41	0.75 ± 0.11	0.83 ± 0.04	0.839
Betaine	0.71 ± 0.12	0.96 ± 0.35	0.94 ± 0.16	0.118
P-value	NS	NS	NS	
Milk osmolality ($mOsm/kgH_2O$)				
Control	279.60 ± 4.93	273.60 ± 13.74	278.00 ± 2.83	0.467
Betaine	281.40 ± 5.68	276.40 ± 7.70	279.40 ± 4.77	0.189
P-value	NS	NS	NS	

Values are presented as mean ± SD.

[1] *P*-values from the measurements of different values among periods of experimental are evaluated by Repeated measures ANOVA

[a, b] Mean values with different superscripts within a row are significantly different (P<0.05).

The significant differences of values between the control group and betaine supplemented group using unpair *t*-test; * P<0.05, NS=not significant.

Table 6: Effects of betaine supplementation on the concentration of plasma electrolytes and plasma osmolality of crossbred Saanen goats.

		Pretreatment	Treatment	Post-treatment	*P*-value[1]
Na^+ (mEq/L)					
	Control	145.60 ± 3.51	141.00 ± 7.31	144.80 ± 0.84	0.325
	Betaine	145.20 ± 2.05	145.00 ± 2.92	145.40 ± 3.21	0.977
	P-value	NS	NS	NS	
K^+ (mEq/L)					
	Control	4.46 ± 0.43	4.16 ± 0.38	4.56 ± 0.36	0.185
	Betaine	4.86 ± 0.36[b]	4.44 ± 0.21[a]	4.34 ± 0.21[a]	0.011
	P-value	NS	NS	NS	
Cl^- (mEq/L)					
	Control	102.80 ± 3.11	101.20 ± 5.72	101.60 ± 4.16	0.854
	Betaine	103.80 ± 3.42	102.20 ± 6.91	101.60 ± 6.35	0.658
	P-value	NS	NS	NS	
Plasma osmolality (mOsm/kg)					
	Control	283.00 ± 3.61	285.40 ± 1.34	278.00 ± 7.48	0.219
	Betaine	284.80 ± 5.63	283.20 ± 4.97	286.40 ± 3.58	0.606
	P-value	NS	NS	NS	

Values are presented as mean ± SD.

[1] *P*-values from the measurements of different values among periods of experimental are evaluated by Repeated measures ANOVA

[a, b] Mean values with different superscripts within a row are significantly different (P<0.05).

The significant differences of values between the control group and betaine supplemented group using unpair *t*-test; * P<0.05, NS=not significant.

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Plasma volume (PV), blood volume (BV) and packed cell volume (PCV) in the controls and betaine supplemented animals (Table 7).

The data in Table 7 show that both absolute values and the relative values as percentage of body weight of plasma volume (PV) and blood volume (BV) showed no significant differences among periods of studies in both the control animals and the betaine supplemented animals. The packed cell volume (PCV) was not affected throughout periods of studies in both groups.

Total body water (TBW), extracellular fluid (ECF) and intracellular fluid (ICF) in the controls and betaine supplemented animals (Table 8).

The data in Table 8 show that both absolute values and relative values as percentage of body weight of total body water (TBW), extracellular fluid (ECF) and intracellular fluid (ICF) were not significantly affected ($P>0.05$) during betaine supplemented in concentrate diet. These parameters for body fluids were not different between the control animals and the betaine supplemented animals throughout periods of studies.

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Table 7: Effects of betaine supplementation on plasma volume (PV), blood volume (BV) and pack cell volume (PCV) of crossbred Saanen goats.

		Pretreatment	Treatment	Post-treatment	*P*-value[1]
PV (L)					
	Control	2.11 ± 0.77	2.13 ± 0.59	2.04 ± 0.41	0.931
	Betaine	2.11 ± 0.40	2.06 ± 0.23	2.01 ± 0.20	0.853
	P-value	NS	NS	NS	
PV (%BW)					
	Control	5.27 ± 0.75	5.41 ± 1.07	5.08 ± 0.97	0.788
	Betaine	4.93 ± 0.43	4.83 ± 0.77	4.86 ± 0.20	0.948
	P-value	NS	NS	NS	
BV (L)					
	Control	2.89 ± 1.20	2.81 ± 0.68	2.86 ± 0.83	0.938
	Betaine	2.87 ± 0.58	2.71 ± 0.35	2.65 ± 0.36	0.618
	P-value	NS	NS	NS	
BV (%BW)					
	Control	7.19 ± 1.16	7.09 ± 0.95	6.71 ± 1.04	0.726
	Betaine	6.72 ± 0.64	6.33 ± 0.97	6.00 ± 0.50	0.340
	P-value	NS	NS	NS	
PCV (%)					
	Control	26.00 ± 2.83	25.00 ± 3.16	23.80 ± 4.32	0.053
	Betaine	26.40 ± 2.88	24.80 ± 1.92	23.40 ± 2.70	0.091
	P-value	NS	NS	NS	

Values are presented as mean ± SD.

[1] *P*-values from the measurements of different values among periods of experimental are evaluated by Repeated measures ANOVA

[a, b] Mean values with different superscripts within a row are significantly different (P<0.05).

The significant differences of values between the control group and betaine supplemented group using unpair *t*-test; * P<0.05, NS=not significant.

Table 8: Effects of betaine supplementation on total body water (TBW), extracellular fluid (ECF) and intracellular fluid (ICF) of crossbred Saanen goats.

		Pretreatment	Treatment	Post-treatment	*P*-value[1]
TBW (L)					
	Control	22.34 ± 4.11	22.88 ± 6.52	24.28 ± 7.81	0.609
	Betaine	25.37 ± 2.54	25.90 ± 1.90	26.75 ± 4.73	0.456
	P-value	NS	NS	NS	
TBW (%BW)					
	Control	55.92 ± 3.57	57.16 ± 4.69	59.90 ± 3.25	0.393
	Betaine	59.92 ± 0.93	60.38 ± 2.97	60.06 ± 3.12	0.956
	P-value	NS	NS	NS	
ECF (L)					
	Control	9.33 ± 2.44	9.24 ± 2.68	8.93 ± 2.97	0.913
	Betaine	10.80 ± 0.64	10.28 ± 1.30	9.93 ± 1.40	0.337
	P-value	NS	NS	NS	
ECF (%BW)					
	Control	22.63 ± 4.01	23.15 ± 3.38	21.83 ± 2.22	0.852
	Betaine	25.90 ± 1.89	23.89 ± 3.08	22.30 ± 1.71	0.083
	P-value	NS	NS	NS	
ICF (L)					
	Control	13.01 ± 3.61	13.65 ± 3.95	15.35 ± 5.02	0.431
	Betaine	14.57 ± 2.00	15.62 ± 2.13	16.82 ± 3.48	0.176
	P-value	NS	NS	NS	
ICF (%BW)					
	Control	33.29 ± 3.66	34.01 ± 1.76	38.07 ± 3.27	0.079
	Betaine	34.02 ± 1.08	36.49 ± 4.76	37.76 ± 2.47	0.237
	P-value	NS	NS	NS	

Values are presented as mean ± SD.

[1] *P*-values from the measurements of different values among periods of experimental are evaluated by Repeated measures ANOVA

[a, b] Mean values with different superscripts within a row are significantly different (P<0.05).

The significant differences of values between the control group and betaine supplemented group using unpair *t*-test; * P<0.05, NS=not significant.

The concentration of arterial (A), venous (V), the A-V difference and the mammary extraction ratio of acetate in the controls and betaine supplemented animals (Table 9).

The data in Table 9 show that no significant differences (P>0.05) for the concentration of arterial and venous plasma acetate were found during betaine supplementation. The A-V difference of plasma acetate and the extraction ratio by the mammary gland were not affected during betaine supplementation and periods of the experiment in both groups.

In betaine supplemented animals the concentration of arterial plasma acetate were higher for the treatment period (0.93 ± 0.21 mmol/L) and the post-treatment period (1.07 ± 0.18 mmol/L) as compared with the pretreatment period (0.64 ± 0.35 mmol/L). Also the arterio-venous difference of plasma acetate concentration and the mammary extraction ratio in the betaine supplemented animals were higher than those of the control animals.

The concentration of arterial (A), venous (V), the A-V difference and the mammary extraction ratio of ß-hydroxybutyrate (ß-HBA) in the controls and betaine supplemented animals (Table 10).

The data in Table 10 show that no significant differences (P>0.05) for the concentration of arterial plasma ß-hydroxybutyrate (ß-HBA) were observed during betaine supplementation. The concentration of venous plasma ß-hydroxybutyrate of betaine supplemented animals were significantly lower (p<0.05) in the treatment period (0.10 ± 0.06 mmol/L) than the pretreatment period (0.23 ± 0.08 mmol/L) and post-treatment period (0.21 ± 0.09 mmol/L). The A-V difference of plasma ß-hydroxybutyrate and the extraction ratio by the mammary gland were not affected during betaine supplementation and periods of the experiment in both groups. However, the trend of the mammary extraction ratio in betaine supplemented animals at the treatment period (81.10 ± 13.93 %) was higher than the pretreatment period (60.29 ± 20.69 %).

Table 9: Effects of betaine supplementation on concentration of arterial plasma acetate (A), venous plasma acetate (V), the A-V difference, the mammary extraction ratio of crossbred Saanen goats. (n=5)

	Pretreatment	Treatment	Post-treatment	*P*-value[1]
Acetate (A) (mmol/L)				
Control	0.77 ± 0.24	0.91 ± 0.11	0.89 ± 0.27	0.477
Betaine	0.64 ± 0.35	0.93 ± 0.21	1.07 ± 0.18	0.070
P-value	NS	NS	NS	
Acetate (V) (mmol/L)				
Control	0.16 ± 0.09	0.20 ± 0.03	0.17 ± 0.04	0.521
Betaine	0.10 ± 0.02	0.13 ± 0.04	0.10 ± 0.06	0.266
P-value	NS	NS	NS	
A-V difference (mmol/L)				
Control	0.61 ± 0.16	0.71 ± 0.10	0.72 ± 0.25	0.534
Betaine	0.55 ± 0.35	0.79 ± 0.19	0.96 ± 0.20	0.065
P-value	NS	NS	NS	
Extraction ratio (%)				
Control	79.63 ± 5.96	77.53 ± 3.53	80.27 ± 4.39	0.716
Betaine	82.47 ± 7.09	85.41 ± 2.68	90.08 ± 6.33	0.067
P-value	NS	NS	NS	

Values are presented as mean ± SD.

[1] *P*-values from the measurements of different values among periods of experimental are evaluated by Repeated measures ANOVA

[a, b] Mean values with different superscripts within a row are significantly different (P<0.05).

The significant differences of values between the control group and betaine supplemented group using unpair *t*-test; * P<0.05, NS=not significant.

Table 10: Effects of betaine supplementation on concentration of arterial plasma ß-hydroxybutyrate (ß-HBA) (A), venous plasma ß-hydroxybutyrate (V), the A-V difference, the mammary extraction ratio of crossbred Saanen goats. (n=5)

	Pretreatment	Treatment	Post-treatment	*P*-value[1]
ß-HBA (A) (mmol/L)				
Control	0.67 ± 0.43	0.62 ± 0.11	0.62 ± 0.17	0.930
Betaine	0.64 ± 0.18	0.58 ± 0.17	0.65 ± 0.16	0.853
P-value	NS	NS	NS	
ß-HBA (V) (mmol/L)				
Control	0.22 ± 0.18	0.16 ± 0.05	0.20 ± 0.11	0.773
Betaine	0.23 ± 0.08[b]	0.10 ± 0.06[a]	0.21 ± 0.09[a]	0.031
P-value	NS	NS	NS	
A-V difference (mmol/L)				
Control	0.45 ± 0.26	0.46 ± 0.11	0.42 ± 0.17	0.912
Betaine	0.41 ± 0.24	0.48 ± 0.17	0.44 ± 0.18	0.848
P-value	NS	NS	NS	
Extraction ratio (%)				
Control	66.60 ± 9.59	73.29 ± 10.72	67.32 ± 19.82	0.604
Betaine	60.29 ± 20.69	81.10 ± 13.93	66.42 ± 16.42	0.113
P-value	NS	NS	NS	

Values are presented as mean ± SD.

[1] *P*-values from the measurements of different values among periods of experimental are evaluated by Repeated measures ANOVA

[a, b] Mean values with different superscripts within a row are significantly different (P<0.05).

The significant differences of values between the control group and betaine supplemented group using unpair *t*-test; * P<0.05, NS=not significant.

The concentration of arterial (A), venous (V), the A-V difference and the mammary extraction ratio of triglyceride in the controls and betaine supplemented animals (Table 11).

The data in Table 11 show that there were no significant differences ($P>0.05$) of arterial, venous and the A-V difference of plasma triglyceride concentration in either groups or periods of experiment. However, the mammary extraction ratio at the post-treatment period in the betaine supplemented animals (34.96 ± 15.46 %) were significant higher ($P<0.05$) than those of the control animals (20.86 ± 9.15 %).

The concentration of arterial (A), venous (V), the A-V difference and the mammary extraction ratio of glucose in the controls and betaine supplemented animals (Table 12).

The data in Table 12 show that there were no significant differences ($P>0.05$) of arterial, venous, the A-V difference and the mammary extraction ratio of plasma glucose concentration in both groups and periods of the experiment.

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Table 11: Effects of betaine supplementation on concentration of arterial plasma triglyceride (A), venous plasma triglyceride (V), the A-V difference, the mammary extraction ratio of crossbred Saanen goats. (n=5)

		Pretreatment	Treatment	Post-treatment	*P*-value[1]
Triglyceride (A) (mmol/L)					
	Control	0.61 ± 0.25	0.72 ± 0.31	0.52 ± 0.08	0.545
	Betaine	0.73 ± 0.21	0.78 ± 0.30	0.63 ± 0.29	0.600
	P-value	NS	NS	NS	
Triglyceride (V) (mmol/L)					
	Control	0.41 ± 0.11	0.52 ± 0.18	0.42 ± 0.10	0.392
	Betaine	0.57 ± 0.19	0.53 ± 0.17	0.41 ± 0.25	0.365
	P-value	NS	NS	NS	
A-V difference (mmol/L)					
	Control	0.20 ± 0.17	0.20 ± 0.19	0.10 ± 0.04	0.607
	Betaine	0.16 ± 0.07	0.27 ± 0.19	0.22 ± 0.11	0.304
	P-value	NS	NS	NS	
Extraction ratio (%)					
	Control	29.16 ± 15.26	23.58 ± 17.68	20.86 ± 9.15	0.665
	Betaine	21.62 ± 7.97	30.02 ± 14.49	34.96 ± 15.46	0.207
	P-value	NS	NS	*	

Values are presented as mean ± SD.

[1] *P*-values from the measurements of different values among periods of experimental are evaluated by Repeated measures ANOVA

[a, b] Mean values with different superscripts within a row are significantly different (P<0.05).

The significant differences of values between the control group and betaine supplemented group using unpair *t*-test; * P<0.05, NS=not significant.

Table 12: Effects of betaine supplementation on concentration of arterial plasma glucose (A), venous plasma glucose (V), the A-V difference, the mammary extraction ratio of crossbred Saanen goats. (n=5)

	Pretreatment	Treatment	Post-treatment	*P*-value[1]
Glucose (A) (mmol/L)				
Control	3.05 ± 0.25	3.28 ± 0.46	2.99 ± 0.40	0.374
Betaine	3.09 ± 0.45	2.78 ± 0.18	2.89 ± 0.71	0.550
P-value	NS	NS	NS	
Glucose (V) (mmol/L)				
Control	2.04 ± 3.26	2.00 ± 0.30	2.02 ± 0.45	0.988
Betaine	2.00 ± 0.44	2.04 ± 0.43	2.00 ± 0.84	0.949
P-value	NS	NS	NS	
A-V difference (mmol/L)				
Control	1.01 ± 0.22	1.28 ± 0.35	0.97 ± 0.41	0.268
Betaine	1.09 ± 0.30	0.74 ± 0.40	0.89 ± 0.51	0.204
P-value	NS	NS	NS	
Extraction ratio (%)				
Control	33.28 ± 7.56	38.50 ± 7.47	32.26 ± 13.47	0.358
Betaine	36.40 ± 9.65	26.56 ± 14.01	31.50 ± 19.54	0.336
P-value	NS	NS	NS	

Values are presented as mean ± SD.

[1] *P*-values from the measurements of different values among periods of experimental are evaluated by Repeated measures ANOVA

[a, b] Mean values with different superscripts within a row are significantly different (P<0.05).

The significant differences of values between the control group and betaine supplemented group using unpair *t*-test; * P<0.05, NS=not significant.

The concentration of plasma insulin-like growth factor I (IGF-I) and thyroxine (T4) in the controls and betaine supplemented animals (Table 13).

The data in Table 12 show that the concentration of plasma IGF-I and T4 were not different between control animals and animals treated with betaine.

Table 13: Effects of betaine supplementation on plasma concentration of IGF-I and T4 of crossbred Saanen goats.

		Pretreatment	Treatment	*P*-value[2]
IGF-I (ng/ml)				
	Control	113.23 ± 76.71	140.97 ± 89.48	NS
	Betaine	117.34 ± 58.05	152.88 ± 43.73	NS
	P-value	NS	NS	
T4 (µg/dl)				
	Control	8.32 ± 1.92	8.58 ± 1.78	NS
	Betaine	9.29 ± 2.07	8.91 ± 1.56	NS
	P-value	NS	NS	

Values are presented as mean ± SD.

[2] *P*-values from the measurements of different values among periods of experimental are evaluated by pair *t*-test

The significant differences of values between the control group and betaine supplemented group using unpair *t*-test; * P<0.05, NS=not significant.

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CHAPTER V
DISCUSSION

The effect of betaine supplementation to diet on concentrate dry matter intake and body weight of crossbred Saanen goats showed no significant differences during the treatment periods as compared with the control animals. These results confirm those of studies of feeding betaine supplementation in both cattle (Loest et al., 2001; 2002) and pigs (Matthews et al., 2001). However, the results of betaine supplementation had greater concentrate dry matter intake for finishing cattle (Loest et al., 2002). In the present study, roughage dry matter intake of the betaine supplemented animals showed significantly lower in either the treatment or post-treatment periods as compared with that of the pretreatment period. It is probably that the trend of an increase in arterial plasma acetate concentrations by approximately 45% after betaine supplementation may affect energy balance and depressing appetite (Clarenburg, 1992).

Betaine supplemented animals increased milk yield by approximately 17.6% and fat corrected milk (FCM) significantly increased by approximately 38% throughout the study periods in stepwise in a few days after supplementation. An increase in milk yield by average 0.17 kg per day after adding betaine at 4g/kg in the concentrate diet in the present results are agreed with the study of Fernandez et al. (2004a) for an increases in milk yield during supplemented betaine in the diet at the level of 4g/kg, and 2g/kg (Sanchez et al., 2001). At the post-treatment period, an increase in milk yield was still apparent which was higher than that of the treatment period after betaine supplementation ended. It is probably that a significant carry over effect of milk yield would relate osmoprotective properties of betaine preventing progressive loss of milk synthesis capacity of mammary epithelial cells as lactation advanced to late lactation (Zhang et al., 1996; Patricia et al., 1998; Weik et al., 1998; Regehr et al., 1999; Wray-Cahen et al., 2004).

Moreover, the present study showed the significantly increased in the concentration of lactose during the treatment period in betaine supplemented animals

as compared with the pretreatment and post-treatment period. The increased milk yield and lactose concentration might be explained by an increase in synthetic activity of secretory cell (Kleiber et al., 1995; Forsyth, 1996; Frimawaty and Manalu, 1999). Lactose is an osmotic regulator of milk during lactation. Lactose is a nonpermeable disaccharide, which cannot diffuse out of the golgi membrane or out of secretory vesicles membrane. This characteristic is important for milk synthesis because of the non-diffusible lactose resulting in water being drawn into the golgi. Therefore, water is drawn into the vesicles to balance the osmotic pressure (Clunie and Hill, 1967; Kronfeld, 1969; Peaker, 1975; Frimawaty and Manalu, 1999). It might be indicated that lactose synthesis would increase in betaine supplemented animals. An osmotic property of lactose for increase in milk volume would maintain the concentration of lactose throughout the periods of studies. However, mechanisms of lactose synthesis by betaine effects remain to be investigated.

The concentration of milk fat and milk fat yield showed significant increase by approximately 26% and 53%, respectively, in betaine supplemented animals at the treatment period. These results are similar to the experiment of Fernandez et al. (2004a) in goats, which oral supplementation with betaine increased milk fat during late lactation. An increase in the short chain fatty acid in milk during betaine supplementation was also noted. In ruminants, betaine has been shown to convert to acetate by ruminal microbial fermentation, and pass into the blood where it is used for their own metabolism (Mitchell et al., 1979; Loest et al., 2001). Confirmation these results by the present results that the trend of the arterial plasma acetate increased by averaged 45% after betaine administration and at the post-treatment period which increased by averaged 67% as compared with the pretreatment period after betaine supplementation ended. The arterio-venous concentration difference for plasma acetate and the extraction ratio across the mammary gland in betaine supplemented animals were higher than those of the control animals. It would be explained in light of the high energy and substrates demand for milk synthesis, because acetate involves in mammary gland metabolism in either *de novo* synthesis of short and medium chain fatty acids in milk or generation of cellular ATP and NADPH (Hansen et al., 1984). It indicates

that acetate would be partially redirected from oxidation to *de novo* fatty acids synthesis.

In the present study, the level of arterial plasma concentrations of ß-hydroxybutyrate did not affect during betaine supplementation. Circulating ß-hydroxybutyrate arises mainly from rumen butyrate in the fed animals (Leng and West, 1969). Therefore, the energy requirements resulting in an increase in hepatic ketogenesis from mobilization of fat reserve (Schultz, 1974) would not be apparent during supplemented betaine in the concentrate diet. However, the concentrations of plasma ß-hydroxybutyrate in milk vein were significant decreased in betaine supplemented animals during the treatment period. The arterio-venous concentrations difference and extraction ratio of ß-hydroxybutyrate across the mammary gland also increased in the treatment period by approximately 17% and 35%, respectively. It would be involved both the oxidation and *de novo* fatty acids synthesis.

According to the arterial plasma triglyceride concentrations, which were not affected by betaine supplementation. These results are similar to those reported that betaine could not affected plasma triglyceride concentrations in Angora and Alpine kids (Puchala et al., 1995) and finishing pigs (Matthews et al., 2001; Huang et al., 2006). However, in the present study, the extraction ratio of plasma triglycerides by the mammary gland at the post-treatment period in betaine supplemented animals was significantly higher than those of the control animals. This would be carrying over effects of betaine to increase milk fat concentrations especially long chain fatty acids during milk fat synthesis.

It has been reported that an interaction of betaine with the lipid metabolism by stimulating the oxidation catabolism of fatty acid occurs via the role of carnitine synthesis. The methyl group from betaine may increase the synthesis of carnitine and influence milk fat secretion (Daily et al., 1998; Fernandez et al., 2000; Wray-Cahen et al., 2004). Carnitine plays an important role shuttling the long chain fatty acids across the inner mitochondrial membrane for ß-oxidation and ATP production in peripheral tissue and any organ including mammary glands (Bremer, 1983; Clarenburg, 1992; Lamhonwah et al., 2005; Güllçin, 2006).

In the present experiment, no significant differences for other milk compositions were observed e.g. protein, solid not fat (SNF) and total solid (TS) between the controls and betaine supplemented animals. These results are similar to the studies in goats by Fernandez et al. (2004a; 2004b) that no effects of betaine supplementation on periods of lactation and milk compositions were apparent during 120 days of postpartum.

It is well known that various factors can affect changes in the concentrations of mineral in milk. For example, udder infection increases the concentration of milk sodium and chloride including an increase of the period of lactation (Judkins and Keener, 1963; Chaiyabutr et al., 1988). An opening of mammary tight junctions would be associated with increases in milk Na^+ and Cl^- concentrations, while the decrease in K^+ concentration, due to its leakage from milk to blood (Silanikove et al., 2000; Shamay et al., 2002). In the present study, the concentrations of milk Na^+, K^+, Cl^-, the ratio of Na^+ and K^+ in milk, milk osmolality showed no significant differences among periods of studies in both groups. These results indicate that betaine could not affect the tissue integrity in the mammary gland (Kharbanda et al., 2005).

Betaine acts as organic osmolytes that has widespread importance in the cell volume regulation (Garcia and Burg, 1991; Schliess and Haussinger, 2002). The adaptive mechanism involves a dramatic increase in the cytoplasmic concentrations of osmoprotective compounds. So betaine is able to increase the internal osmotic pressure without affecting vital cellular function (Heermann and Jung, 2004). The presence of betaine helped the epithelium to maintain water balance and the movement of water across the cell (Kettunen et al., 2001a; 2001b; Sironi et al., 2001). The present results showed no significant changes in body fluids of betaine supplemented animals both absolute values and the relative values for plasma volume (PV), blood volume (BV), extracellular fluid (ECF), intracellular fluid (ICF) and total body water (TBW). No changes in values of packed cell volume (PCV) were apparent among periods of studies in both controls and betaine supplemented animals. These results were agree with an experimental result of Banskalieva et al. (2005) which showed no significant differences in values of packed cell volume during betaine supplementation. These results indicate that maintenance of the balance distribution of the body fluid compartments occurred during betaine supplementation. Both control animals and betaine supplemented

animals were fed with the same concentrate which presumably maintained a similar level of osmolality in ruminal fluid throughout periods of the studies. This would not affect water intake and fluid distribution.

In present study, the concentrations of plasma Na^+, Cl^-, and plasma osmolality were no significant different throughout experimental periods in both controls and betaine supplemented animals. However, the concentration of plasma K^+ in betaine supplemented animals decreased significantly from those of values of the pretreatment. The decrease in the plasma K^+ concentration is probably due to intracellular uptake of K^+ for regulated cell volume and cellular adaptation following betaine accumulation. These results might be explained by the cellular absorption of betaine. An involvement of betaine both a Na^+ dependent and a Na^+ independent transporter has been noted in human kidney (Dellow et al., 1999) and broiler chick intestine (Kettunen et al., 2001a; 2001b). This would be expected to lead higher cell Na^+ concentration (Schiller et al., 2004). The stimulation of basolateral active Na-K exchange would operate pumping Na^+ out and pumping K^+ in (Beck et al., 1992). It is possible causing the decrease in the concentration of plasma K^+ in betaine supplemented animals.

A few data are available for the effect of betaine on milk production relating to hormonal level. In the present study, plasma insulin-like growth factor I (IGF-I) and thyroxine (T4) levels were not significantly different between the controls and betaine supplemented animals. These results are contrast with the results of Huang et al. (2006) in finishing pigs that betaine supplementation for 42 days can affect the IGF-I hormone level and lipid metabolism. The effects of betaine supplementation on monogastric animals were attributed to diminish endogenous fatty acid synthesis (lipogenesis) and increased fat degradation (lipolysis). This implicated effect of betaine on lipid metabolism in pig was nearly interrelated to its effect on hormone (Fernandez et al., 1998; Wray-Cahen et al., 2004; Huang et al., 2006). In ruminant, the mechanism of betaine metabolism in rumen is unlike; betaine is converted to acetate by the ruminal microorganism (Mitchell et al., 1979; Loest et al., 2001). Therefore, it might not be expected to promote the activity of growth factors such as IGF-I and T4 in the current study. It has been known that the blood level of IGF-I and T4 relating to mammary blood flow to the mammary gland and body fluid distribution (Chaiyabutr et al., 1997). In the

present study, an increase in milk yield did not relate to the levels of IGF-I and T4. This, betaine would affect on intramammary factors than extramammary factors in association the utilization of the substrates for milk synthesis.

Conclusion: The present result suggested that the regulation of an increase in the concentration of milk fat, milk lactose and milk fat yield during betaine supplementation in late lactating crossbred Saanen goats is influenced more by the intramammary factors than by extramammary factors in association with the utilization of substrate for milk synthesis. The changes in intramammary factors would be due to the nutritional uptake by mammary epithelium. Betaine could increase substrates in plasma especially the arterial plasma acetate concentration that is important source for milk synthesis. Betaine supplementation affects mammary function directly by the trend of an increase in arterial acetate concentration and extraction ratio of acetate that could improve substrates utilization of mammary glands for milk fat synthesis. No effect of betaine was apparent for the changes in body fluids or hormone level in relating to the mammary function.

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