Original Article

Milk Production and Mammary Extraction of Nutrients by Late Lactating Cross-bred Saanen Goats Supplemented with Betaine in Diet

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Abstract

The objective of this study was to investigate the effect of supplementary betaine in diet on milk secretion, milk composition, mammary extraction of nutrients and relevant parameters in late lactating cross-bred Saanen goats. Ten, multiparous, non-pregnant cross-bred Saanen goats of approximately 10 weeks postpartum were used for the study. The animals were divided into two groups of five animals each. Animals in the experimental group received diet containing 4 g betaine per kg of concentrate diet for four weeks, while the control group received similar concentrate diet without betaine as concurrent control. Results showed that milk yields of betaine supplemented animals increased in both treatment and post-treatment periods by approximately 18% of the pretreatment level. Mean values of 4% fat corrected milk (FCM) were significantly higher ($p < 0.05$) in the betaine supplemented animals (1.23 kg/d) than the control animals (0.98 kg/d). Roughage DMI of the animals receiving betaine supplementation decreased significantly ($p < 0.05$) during treatment and post-treatment periods. Concentrations of milk fat during betaine supplementation were significantly higher ($p < 0.05$) than during pre-treatment and post-treatment periods. A significant increase in arterial plasma concentration of acetate ($p < 0.05$), arterio-venous concentration difference and mammary extraction ratio of acetate tended to increase ($0.05 < p < 0.10$), while β-hydroxybutyrate, triglyceride and glucose were not affected by dietary betaine supplementation. Betaine supplementation had no effects on plasma volume, blood volume, extracellular fluid volume, intracellular fluid volume and volume of total body water as compared with those of the control animals. Plasma concentrations of IGF-I and thyroxin were not affected by supplementary betaine in diet. These results suggest that dietary betaine supplementation increases milk yield in late lactating cross-bred Saanen goats which is due to an elevation of the plasma acetate level in supplying more source of acetate utilization by peripheral tissues, causing the mammary gland to meet more proportion of other nutrients demand for milk synthesis.

Keywords: betaine supplementation, cross-bred Saanen goats, late lactation, mammary extraction of nutrients

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บทคัดย่อ

การเพิ่มผลผลิตน้ำนมและสัดส่วนการใช้สารอาหารโดยต่อมน้ำนมในระยะท้ายของการให้นมของแพะนมลูกผสมซาอะเนนที่ได้รับอาหารเสริมบีเทน

หนึ่งนุช สายปิ่น [1] สมชาย จันทร์ผ่องแสง [2] ณรงค์ศักดิ์ ชัยบุตร [1]*

จุดประสงค์ของการศึกษาเพื่อดูผลของการให้อาหารเสริมบีเทนต่อการขับน้ำนม ส่วนประกอบน้ำนมสัดส่วนการใช้สารอาหารโดยต่อมน้ำนมและพารามิเตอร์ที่เกี่ยวข้องในระยะท้ายของการให้นมในแพะนมลูกผสมซาอะเนน การทดลองใช้แพะนมลูกผสมที่อยู่ในระยะท้ายของการให้นมจำนวน 10 ตัว แบ่งการทดลองออกเป็น 2 กลุ่ม กลุ่มละ 5 ตัว ในกลุ่มทดลองสัตว์จะได้รับอาหารเสริมบีเทนในปริมาณ 4 กรัมต่อกก.ของอาหารข้นติดต่อกันเป็นเวลา 4 สัปดาห์ในระยะทดลอง ส่วนกลุ่มควบคุมจะได้รับอาหารข้นเช่นเดียวกันแต่ไม่มีส่วนผสมของบีเทน ผลการทดลองแสดงให้เห็นว่าสัตว์ที่ได้รับอาหารเสริมบีเทนหลั่งน้ำนมเพิ่มขึ้นโดยเฉลี่ยประมาณร้อยละ 18 ในระยะทดลองและระยะหลังทดลอง เมื่อเทียบกับระยะก่อนทดลอง ปริมาณน้ำนมที่ปรับค่าไขมันนมร้อยละ 4 FCM เพิ่มขึ้นอย่างมีนัยสำคัญในสัตว์ที่ได้รับอาหารเสริมบีเทน ($p < 0.05$) สัตว์ที่ได้รับอาหารเสริมบีเทนกินอาหารหยาบลดลงอย่างมีนัยสำคัญ ($p < 0.05$) ในระหว่างการทดลองเมื่อเทียบกับก่อนทดลอง ค่าความเข้มข้นของไขมันนมระหว่างที่ให้อาหารเสริมบีเทนมีค่าสูงกว่าเมื่อเปรียบเทียบกับระยะก่อนและระยะท้ายของการให้อาหารเสริมบีเทนอย่างมีนัยสำคัญ ($p < 0.05$) ระดับความเข้มข้นของอะซีเตทในพลาสม่าเลือดแดงเพิ่มขึ้นอย่างมีนัยสำคัญทางสถิติ ส่วนความแตกต่างของความเข้มข้นของอะซีเตทระหว่างเลือดดำและเลือดแดงและสัดส่วนการใช้อะซีเตทโดยต่อมน้ำนมมีแนวโน้มเพิ่มขึ้น ($0.05 < p < 0.1$) แต่สารอาหารในร่างกายอย่าง เบต้าไฮดรอกซีบิวตาเรต ไตรกลีเซอไรด์และกลูโคสไม่ได้รับผลกระทบจากการให้อาหารเสริมบีเทน การให้อาหารเสริมบีเทนไม่มีผลต่อการเปลี่ยนแปลงปริมาณของเหลวในส่วนต่างๆ ของร่างกาย ทั้งปริมาณพลาสม่า ปริมาณเลือด ปริมาณของเหลวนอกเซลล์ ปริมาณของเหลวภายในเซลล์ และปริมาณน้ำทั้งหมดของร่างกาย การให้สารอาหารเสริมบีเทนไม่มีผลต่อระดับความเข้มข้นของฮอร์โมน IGF-1 และไทรอกซีนในพลาสมา จากผลของการศึกษานี้ชี้ได้ว่าการเพิ่มขึ้นของอัตราการหลั่งน้ำนมของแพะนมลูกผสมในขณะให้อาหารเสริมบีเทนเป็นผลจากการเพิ่มขึ้นของระดับอะซีเตทในพลาสม่าที่ถูกใช้เป็นแหล่งพลังงานอาหารที่เพิ่มขึ้นและถูกนำไปใช้ในส่วนต่างๆ ของร่างกายส่งผลทำให้ต่อมน้ำนมได้ใช้สารอาหารอื่นในสัดส่วนที่มากขึ้นเพื่อใช้ในการสังเคราะห์น้ำนมที่เพิ่มขึ้น

คำสำคัญ: การเสริมอาหารบีเทน แพะนมลูกผสมซาอาเนน ระยะท้ายการให้นม สัดส่วนการใช้สารอาหารโดยต่อมน้ำนม

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Introduction

Goat herds in tropical countries are used for both meat and milk. For dairy goats, low milk production is still the main problem of dairy goat farming. Low genetic potential for milk production of a variety of genetic combination exists between dairy goat and rearing for meat of indigenous goat. In addition to animal genetics, other factors can affect milk production of dairy goat in the tropics, for example, inadequate supply for foraging during dry summer months can generate a drop in milk yield. The mechanism of milk yield is known to be based on three main levels of regulation, which are arterial flow of substrates in the mammary gland, extraction of substrates by the mammary gland and secretory activity of the mammary epithelial cells (Chaiyabutr et al., 1997). Feed restriction is known to affect milk production in dairy goats with changes in both extramammary and intramammary factors (Chaiyabutr et al., 1980; 1981). Water intake has been shown to decrease during feed restriction and it consequently contributes to decrease in plasma volume (Chaiyabutr et al., 1980; Dahlborn, 1987) leading to reduced mammary blood flow, which could be an alternative explanation for the drop in milk secretion.

Lactation makes demands on the body to supply substrates for milk synthesis within the mammary gland. It is known that the supply of milk precursors may impose a limit on milk secretion directly on the mammary gland or indirectly via hormonal control. Feed additive and supplementation will be the choice for increase in milk production under the conditions of an inadequate of foraging in dairy goat. A number of studies about different types of dietary supplementation in goats have been reported such as methionine (Madsen et al., 2005), choline (Banskalieva et al., 2005) and betaine (Fernandez et al., 2004). Betaine is a natural compound with methyl donor properties as an osmoprotectant which is synthesized by a variety of plants and organism. It is also extensively used as feed additive in diets of poultry (Saunderson and MacKinlay, 1990) and swine (Matthews et al., 2001). Utilization of betaine in ruminant has been shown to convert to acetate by ruminal microbial activity (Mitchell et al., 1979), which will be a source for *de novo* synthesis of both short and medium chain fatty acids in milk fat (Moore and Christie, 1979). Although the supplementation of

betaine in diet has been noted to increase both milk yield and milk fat in goat (Fernandez et al., 2004) and dairy cattle (Peterson et al. 2012), few data are available in cross-bred dairy goat for the mechanism of action of supplementary betaine on mammary function. The mechanism behind this is still unclear for the regulation of milk yield and the utilization of nutrients in the mammary gland during betaine supplementation in the diet. The aim of this study was, therefore, to determine the effects of betaine supplementation in the diet on milk secretion relating to changes in the extraction of plasma acetate, β-hydroxybutyrate, triglycerides and glucose across the mammary gland. In addition, alterations in body fluids and plasma hormone levels for IGF-I and thyroxin were measured.

Materials and Methods

***Animals and managements*:** Ten, non-pregnant, cross-bred, (> 87.5% Saanen gene) dairy goats were used for the experiment. They were divided into two groups of five animals each. The control group as concurrent control animals did not receive any betaine supplement throughout period of study. Animals in the experimental group were given similar feeding, except that the treatment period, given additional betaine in the concentrated mixture (4 g/kg diet) lasted 30 days. The goats were fed concentrate once a day at 15.00 pm, while Pangola hay *ad libitum* and water were freely available. The amount of either concentrate or Pangola hay offered and refused were measured and recorded daily and DMI was calculated on days of each experimental period. The chemical composition of the diet is presented in Table 1. Each goat was kept in a pen which was placed indoor with environmental temperature of 29-36^{0}C. The experimental study of each goat started at day 70 post-partum from the transition of mid to late lactation. The animal was milked by hand once a day in the morning. The experimental procedures were approved by the ethic committee on animal use and care of Faculty of Veterinary Science, Chulalongkorn University. These guidelines were formulated to comply with international standards in accordance with the principles and guidelines of the National Research Council of Thailand.

Experimental design: The experiment in each group was divided into 3 periods; namely the pre-treatment period (day 70 to day 84 post-partum), the treatment period (day 84 to day 115 post-partum) and post-treatment period (days 116 to days 130 post-partum). The animals in the experimental group were given the concentrated diet containing betaine (0.4 g%) throughout 30 days of the treatment period, while during the pre-treatment and the post-treatment periods betaine was not supplemented in the diet. Parameter measurements of each group were performed on specified day of the pretreatment period (day 84 post-partum), the treatment period (day 115 post-partum) and the post-treatment period (day 130 post-partum). Milk secretion was recorded by hand milking once daily about 6.00 am and milk composition was determined every week. On each experimental period, determinations of plasma volume (PV), volume of extracellular fluid (ECF), volume of intracellular fluid (ICF), total body water (TBW), plasma metabolites, plasma hormones and mammary extraction of nutrients across the mammary gland were carried out.

Determinations of milk composition, plasma and milk osmolality: Milk sample (60 ml) was preserved with 0.3 ml of 0.02% (w/v) bronopol (2-Brom-2- nitro-1, 3-propandiol) and kept at 4^{0}C for determinations of milk fat, milk protein and lactose concentrations using Milkoscan (Milko-Scan 133B; A/S N. Foss Electric, Hillerod, Denmark). Values of daily milk yield and the concentration of milk fat were used for calculation of 4% fat corrected milk (4% FCM) according to the following formulae: 4% FCM = 0.4 x kg milk + 15x kg milk fat. Plasma and milk osmolality were determined using osmometer (model 3D3, Advanced instrument, Massachusetts, USA).

Determinations of plasma volume (PV), extracellular fluid (ECF), intracellular fluid (ICF) and total body water (TBW): On each specified days, a catheter was inserted into the jugular vein for injection of solution in each goat. The solution containing 3 ml of 0.5% Evans blue dye (T-1824), 5 ml of 10% sodium thiocyanate solution (NaSCN) and 5 ml of 15 % urea solution was injected via the jugular vein catheter for determinations of plasma volume (PV), extracellular fluid volume (ECF) (Medway and Kare, 1959) and total body water (TBW) (Chiba et al., 1990), respectively. By dye dilution technique, blood samples were collected from the contralateral jugular vein into heparinized tubes (25 IU/ml blood) at 20, 30, 40 and 50 min after dye injection. Concentration of dye in plasma at zero time was determined by using a semi-logarithmic concentration on time extrapolation.

Determinations of plasma metabolites concentration: At each specified day, plasma samples were collected simultaneously from milk vein and ear vein for determinations of plasma metabolites concentration. The plasma concentration of acetate was assayed by acetic acid UV-method (Cat No 10148261035, r-Biopharm, USA), the plasma concentration of ß-hydroxybutyrate was assayed by D-3-Hydroxybutyric acid colorimetric method (Cat No 10907979035, r-Biopharm, USA) and the plasma concentration of triglyceride was determined by enzymatic colorimetric test (Triglyceride liquicolor^{mono} SU-TRIMR, Germany). The plasma concentration of glucose was determined by glucose liquicolor enzymatic colorimetric test (SU-GLL Q2, Germany).

Table 1 Chemical compositions of experimental diet (% 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	-	0.4

Measurement of the arterio-venous (A-V) concentration differences and percentage of mammary extraction of substrates across the mammary gland: In each period of experiment, the arterio-venous (A-V) concentration differences of plasma acetate, ß-hydroxybutyrate, triglycerides and glucose across the mammary gland were calculated from the concentration of plasma samples from ear vein (A) and milk vein (V). The percentage of mammary extraction of substrates across the mammary gland was calculated by dividing the arterio-venous concentration differences (A-V) with the arterial plasma concentration (A).

Determinations of plasma levels of insulin-like growth factor I (IGF-I) and thyroxine (T4): Venous plasma sample from jugular vein was collected on each specified day of the pretreatment period (day 84 post-partum) and the treatment period (day 115 post-partum) for determinations of plasma concentration of hormone IGF-I using Immulite analyzer by Chemiluminescence immunoassay and plasma concentration of thyroxin (T4) using Elecsys 2010 analyzer by Electrochemiluminescence method.

***Statistical analysis*:** All data are presented as the mean ± SD. Statistical analyses were performed using repeated measures ANOVA and interactions of data among the experimental periods in the same group using Duncan's multiple range test as post hoc. Significant differences of values between the control group and the betaine supplemented group using unpaired *t*-test. Statistical differences of hormonal levels between pre-treatment and treatment periods in the same group were analyzed by paired *t*-test. Statistical significance was indicated as $p < 0.05$ and trends were declared at $0.05 < p < 0.10$.

Results

Effects of betaine supplementation on dry matter intake (DMI) of roughage and concentrate, total DMI/milk yield, and body weight : The data in Table 2 show that the mean values of roughage intake of the betaine supplemented animals were significantly lower ($p < 0.05$) in both treatment and post-treatment periods in comparison to the pretreatment period. The values of total dry matter intake per milk yield were not significantly different between the control and treated groups. During experiment, the betaine supplemented animals showed no changes in body weights.

Effects of betaine supplementation on milk yield and milk composition : Effects of betaine supplementation on milk yield and milk composition are shown in Fig 1 and Table 3. The milk yield of the betaine supplemented animals showed an increase in both treatment and post-treatment periods by approximately 18% of the pre-treatment level. During the treatment period, the milk yield of the betaine supplemented animals were higher than that of the control animals which were apparent in the first week of betaine supplementation (Fig 1). The values of 4% fat corrected milk (FCM) in the betaine supplemented animals significantly increased ($p < 0.05$) by approximately 38% in comparison to values of FCM in the pre-treatment and post-treatment periods. The

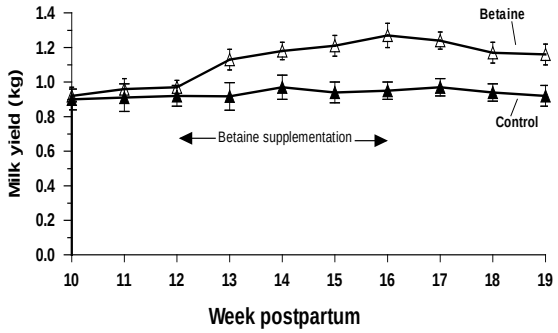

Figure 1 Milk yield of control and betaine supplemented animals

Table 2 Effects of betaine supplementation on dry matter intake (DMI) of roughage and concentrate, total DMI/milk yield and body weight

	Pre-treatment	Treatment	Post-treatment	*P*-value[1]
DMI-Roughage (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]	$p < 0.006$
DMI-Concentrate (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
Total DMI/milk yield				
Control	1.14 ± 0.15	1.03 ± 0.09	1.15 ± 0.10	0..235
Betaine	1.09 ± 0.30	0.81 ± 0.32	0.93 ± 0.32	0.395
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	0.829

Values are presented as mean ± SD.
[1] Statistical significance of interaction effects among periods of treatments by ANOVA
[a,b] Mean values within a row indicated with different superscripts are significantly different ($p < 0.05$).

Table 3 Effects of betaine supplementation on milk yield and milk compositions

		Pre-treatment	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
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]	$p < 0.007$
Milk compositions:					
Milk 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]	$p < 0.039$
Milk 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
Milk Lactose (g%)	Control	4.52 ± 0.32	4.62± 0.29	4.32 ± 0.15	0.722
	Betaine	4.42 ± 0.43	4.34 ± 0.22	4.33 ± 0.24	0.883
Milk osmolality (mOsm/kg)					
	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

Values are presented as mean ± SD.
[1]Statistical significance of interaction effects among periods of treatments by ANOVA
[a,b]Mean values within a row indicated with different superscripts are significantly different ($p < 0.05$)
Comparison of *P*-values of control group versus betaine-treated group using unpaired *t*-test, * $p < 0.05$; NS, not significant.

Table 4 Effects of betaine supplementation on concentrations of arterial plasma (A) venous plasma (V), A-V differences and mammary extraction ratio of acetate, ß-hydroxybutyrate (ß-HBA), triglyceride and glucose

		Pre-treatment	Treatment	Post-treatment	*P*-value[1]
Acetate (A) (mM) :	Control	0.77 ± 0.24	0.91 ± 0.11	0.89 ± 0.27	0.477
	Betaine	0.64 ± 0.35[a]	0.93 ± 0.21[ab]	1.07 ± 0.18[b]	$p < 0.039$
A-V difference (mM):	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	$0.05 < p < 0.065$
Extraction ratio (%) :	Control	79.6 ± 5.9	77.5 ± 3.5	80.2 ± 4.4	0.716
	Betaine	82.4 ± 7.1	85.4 ± 2.6	90.1 ± 6.3	$0.05 < p < 0.067$
ß-HBA (A) (mM) :	Control	0.67 ± 0.43	0.62 ± 0.11	0.62 ± 0.17	0.930
	Betaine	0.64 ± 0.18	0.60 ± 0.17	0.65 ± 0.16	0.853
A-V difference (mM):	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
Extraction ratio (%) :	Control	66.60 ± 9.59	73.29 ± 10.72	67.32 ± 19.82	0.604
	Betaine	60.29 ± 20.69	76.66 ± 13.93	66.42 ± 16.42	0.113
Triglyceride (A) (mM) :	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
A-V difference (mM) :	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
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
Glucose (A) (mM) :	Control	3.05 ± 0.25	3.28 ± 0.46	3.00 ± 0.40	0.374
	Betaine	3.09 ± 0.45	2.98 ± 0.18	2.89 ± 0.71	0..550
A-V difference (mM) :	Control	1.01 ± 0.22	1.28 ± 0.35	1.00 ± 0.41	0.268
	Betaine	1.09 ± 0.30	0.87 ± 0.40	0.89 ± 0.51	0.204
Extraction ratio (%) :	Control	33.28 ± 7.56	38.50 ± 7.47	32.26 ± 13.47	0..358
	Betaine	36.40 ± 9.65	30.19 ± 14.01	31.50 ± 19.54	0.336

Values are presented as mean ± SD.
[1]Statistical significance of interaction effects among periods of treatments by ANOVA
[a,b]Mean values within a row indicated with different superscripts are significantly different ($p < 0.05$)

value of 4% FCM in the betaine supplemented animals was also significantly higher ($p < 0.05$) than that of the control animals. The concentration of milk fat of the betaine supplemented animals significantly increased ($p < 0.05$) in the treatment period, while the concentrations of milk protein, lactose and milk osmolarity did not significantly change.

Effects of betaine supplementation on concentrations of arterial plasma (A), venous plasma (V), arteriovenous concentration differences (A-V differences) and mammary extraction ratio of acetate, ß-hydroxybutyrate (ß-HBA), triglyceride and glucose : The data of the arterial concentration (A), the arteriovenous concentration differences and the mammary extraction ratio of nutrients in the control and betaine supplemented animals are shown in

Table 4. The concentration of acetate in arterial plasma significantly increased ($p < 0.05$) during the betaine supplementation. The A-V concentration differences of plasma acetate and the extraction ratio by the mammary gland showed significant increase ($0.05 < p < 0.10$) during betaine supplementation. The A-V concentration differences and the mammary extraction ratio of plasma acetate of the betaine supplemented animals were higher than those of the control animals. The concentrations of arterial plasma, the A-V concentration differences and the mammary extraction ratio of ß-hydroxybutyrate, triglyceride and glucose were not affected by the betaine supplementation throughout the periods of study.

Effects of betaine supplementation on plasma volume (PV), blood volume (BV), total body water (TBW), extracellular fluid (ECF), intracellular fluid (ICF), packed cell volume (PCV) and plasma osmolality: The data of body fluid compartments during betaine supplementation are shown in Table 5. The values of PV, BV, TBW, ECF, ICF, PCV and plasma osmolality were not affected by the betaine supplementation throughout the periods of study.

Effects of betaine supplementation on plasma concentrations of insulin like growth factor (IGF-I) and thyroxin (T4) of crossbred Saanen goats: The data in Table 6 show that the plasma concentrations of IGF-I and T4 were not affected by the betaine supplementation throughout the periods of study.

Table 6 Effects of betaine supplementation on plasma concentrations of IGF-I and T4

	Pre-treatment	Treatment	*P*-value[1]
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.
Comparison of *p*-values of control group versus betaine-treated group using unpaired *t*-test, NS, not significant
Comparison of *P*-values of treatment versus pretreatment of both groups using paired *t*-test, NS, not significant

Table 5 Effects of betaine supplementation on plasma volume (PV), blood volume (BV), total body water (TBW), extracellular fluid (ECF), intracellular fluid (ICF), plasma osmolality and pack cell volume (PCV)

	Pre-treatment	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
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
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
BV *(%BW)*				0.726
Control	7.19 ± 1.16	7.09 ± 0.95	6.71 ± 1.04	
Betaine	6.72 ± 0.64	6.33 ± 0.97	6.00 ± 0.50	0.340
TBW (L)				0.609
Control	22.34 ± 4.11	22.88 ± 6.52	24.28 ± 7.81	
Betaine	25.37 ± 2.54	25.90 ± 1.90	26.75 ± 4.73	0.456
TBW (%BW)				0.393
Control	55.92 ± 3.57	57.16 ± 4.69	59.90 ± 3.25	
Betaine	59.92 ± 0.93	60.38 ± 2.97	60.06 ± 3.12	0.956
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
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	0.249
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
ICF (%BW)				
Control	33.29 ± 3.66	34.01 ± 1.76	38.07 ± 3.27	0.702
Betaine	34.02 ± 1.08	36.49 ± 4.76	37.76 ± 2.47	0.237
PCV (%)				
Control	26.00 ± 2.83	25.00 ± 3.16	24.80 ± 4.32	0.612
Betaine	26.40 ± 2.88	24.80 ± 1.92	23.40 ± 2.70	0.322
Plasma osmolality (mOsm/kg)				0.219
Control	283.00 ± 3.61	285.40 ± 1.34	278.00 ± 7.48	
Betaine	284.80 ± 5.63	283.20 ± 4.97	286.40 ± 3.58	0.606

Values are presented as mean ± SD.
[1] Statistical significance of interaction effects among periods of treatments by ANOVA
[a,b] Mean values within a row indicated with different superscripts are significantly different ($p < 0.05$).

Discussion

During the dietary betaine supplementation in cross-bred dairy goats, milk yield and 4% FCM increased by approximately 17.6% and 38%, respectively. These results were in agreement with other studies for the effect of dietary betaine supplementation on an increase in milk yield in dairy cattle (Loest et al., 2002) and goats (Fernandez et al., 2004). In the present study, the increase in milk yield occurred in the absence of changes in total dry matter intake, but the roughage intake significantly decreased during the betaine supplementation. The marked increase in the concentration of plasma acetate was apparent during betaine supplementation by approximately 45%. This result confirmed previous findings (Mitchell et al., 1979; Loest et al., 2002) in ruminants that betaine is rapidly degraded to convert to acetate by ruminal microbial fermentation, then is transported into the blood for metabolism. An elevation of plasma acetate level during dietary betaine supplementation may affect energy balance since acetate serves as a source of energy (Jarettt and potter, 1950). Acetate is involved in mammary gland metabolism in either a source for the *de novo* synthesis of both short and medium chain fatty acids in milk fat or generation of cellular ATP and NADPH (Hansen et al., 1984). The decrease in the roughage intake accompanying with an elevation of the plasma acetate level during dietary betaine supplementation will be the compensatory mechanism for the regulation of volatile fatty acids production in the rumen. More complex processes probably occur in the rumen between the fermentation of dietary roughage and degradation of supplementary betaine in diet. Digestion of roughage in ruminants is known to produce both acetate and methane production. Effects of microbial digestion on increased methane production in the rumen of ruminants fed unprotected sources of betaine were reported by Mitchell et al. (1979) and Neill et al. (1978). The betaine was cleavaged by microbes to produce both acetate and trimethylamine in the rumen, where trimethylamine was degraded by ruminal microorganisms resulting in methane production (Neill et al., 1978). An adjusted downward roughage intake probably occurred to reduce methane gain during betaine supplementation. The mechanism of these changes remains to be further elucidated.

Milk secretion is known to be affected principally by the availability of the concentration of nutrients in arterial blood and its rate of uptake from circulating nutrients by the mammary cell. In the present study, the arteriovenous concentration differences and the extraction of plasma acetate across the mammary gland of betaine supplemented animals were higher than those of the control animals. Thus, an increase in milk yield during dietary betaine supplementation could be achieved in part by increasing the plasma acetate level, and so nutrient partitioning of absorbed acetate to distribute more energy for body utilization to mammary gland for milk synthesis. However, these results are not consistent with the conclusion of Rook (1979), who stated that there was no direct relationship between the rate of milk synthesis and the plasma acetate concentration.

In the present study, there were no changes in arterial plasma concentrations, arteriovenous concentration differences across the mammary gland and the mammary extraction of β-hydroxybutyrate, triglyceride and glucose during the dietary betaine supplementation. No alteration of the plasma β-hydroxybutyrate level indicates that the metabolism of butyrate across the ruminal wall was affected by betaine supplementation although circulating ß-hydroxybutyrate arises mainly from rumen butyrate in the fed animals (Leng and West, 1969). An increase in hepatic ketogenesis from mobilization of fat reserved for the energy requirements (Schultz, 1974) was not suspected to occur during supplementary betaine. The arterial plasma triglyceride concentration was not affected by betaine supplementation in the present study, which was in agreement with the study in Angora and Alpine kids (Puchala et al., 1995). However, the value of extraction ratio of plasma triglyceride by the mammary gland of betaine supplemented animal was slightly higher than that of the control animals. It is known that the origin of milk fat synthesis involves both the uptake of circulating triglycerides and synthesis *de novo* within the mammary gland of long chain fatty acids. Therefore, the high milk fat concentration in betaine supplemented animals was derived partly from the source of the circulating lipids and plasma acetate taken up by the mammary gland. These results were in agreement with the experiment of Fernandez et al. (2004) in goats, which oral supplementation with betaine increased milk fat during late lactation. Increased milk fat secretion due to betaine supplementation, possibly relates to an interaction of betaine with lipid metabolism via the role of carnitine synthesis. The methyl group from betaine has been shown to increase the synthesis of carnitine and influence the secretion of milk fat (Daily et al., 1998; Fernandez et al., 2000; Wray-Cahen et al., 2004).

The present study showed no significant change in the concentration of milk lactose during betaine supplementation. Lactose is an osmotic regulator of milk volume with the osmotic movement of water to golgi vesicles during lactose formation. As such, the lactose concentration in milk remains relatively stable even during high milk yield. In the present study, supplementary betaine in the diet had no effects on the plasma glucose concentration, arterio-venous differences and the mammary extraction of glucose. These results have revealed an interesting process between glucose precursor and lactose synthesis. It is proposed that supplementary betaine in diet increases milk yield with a high lactose yield (lactose concentration x milk secretion) whilst a higher concentration of plasma acetate during betaine supplementation provides more source of acetate utilization by peripheral tissues. It might reduce required energy expenditure for the utilization of glucose by peripheral tissues, thereby permitting more proportion of glucose to the mammary gland for lactose synthesis. Although the goats were milked once a day in the present study, down-regulated lactose synthesis in response to the once a day milking

has been noted (Guinard-Flament, et al., 2006). However, the effect of supplementary betaine in diet on the mechanism of lactose synthesis has still not been fully elucidated. Question that arises whether an increase in milk secretion in betaine treated goats is a function of betaine supplementation per se or of the associated changes in energy balance needs to be further studied.

It has been known that body fluid is used as vehicle of substrates in distribution to the mammary gland and for evaporative cooling during heat dissipation. The present study showed no alterations of total body water (TBW), plasma volume (PV), blood volume (BV), volume of extracellular fluid (ECF) and intracellular fluid (ICF) during the betaine supplementation. No changes in packed cell volume (PCV) were apparent during the betaine supplementation, which was in agreement with the study in betaine supplemented meat goat by Banskalieva et al. (2005). These results indicate that betaine supplementation does not change the body fluid distribution in cross-bred dairy goat. Betaine has been known to act as an organic osmolyte in cell volume regulation in organ (Garcia and Burg, 1991). The accumulation of betaine inside the cells may change the surrounding tonicity and movement of water across the cell (Kettunen et al., 2001). Betaine indirectly affects water movement which reduces the energy expenditure of body cells on ion pumps to regulate their water balance. The mechanism by which betaine exerts its effect as an osmoprotectant is still not clear. However, the question remains unsolved for the increase in milk yield during betaine supplementation in cross-bred dairy goats in the tropic, whether the role betaine works by reducing the energy required by animals to cool themselves, leaving more energy for the process of milk production.

The arterial concentration of nutrients could affect the site of uptake into the secretory cell, while mammary blood flow could lead to an increase in nutrients supply to sustain milk synthesis (Davis and Collier, 1985). To clarify this mechanism in the present study, it will be necessary to demonstrate the effect of specific hormone stimulating milk secretion. In the present study, the increase in milk secretion during betaine supplementation could not simply attribute to the effect of either the circulating IGF-I or thyroxine (T4), which maintained similar levels throughout the experimental study. Thus, the secondary responses for the role of IGF-I on the mammary blood flow were not expected to occur in the betaine supplemented animals. Although the increase in the mammary blood flow is attributed to the action of circulating IGF-I, which has been noted in either cattle (Chaiyabutr et al., 1997) or goat (Prosser et al., 1988), these results are contrast with the study in finishing pigs by Huang et al. (2006), which betaine supplementation for 42 days could affect the IGF-I level and increased lipolysis. Therefore, the supplementary betaine in the diet of cross-bred dairy goats might not be expected to promote the activity of growth factors for IGF-I and T4, a galactopoietic hormone, in the present study.

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References

Banskalieva V, Puchala R, Goetsch AL, Luo J and Sahlu T 2005. Effects of ruminally protected betaine and choline on net flux of nutrients across the portal-drained viscera and liver of meat goat wethers consuming diets differing in protein concentration. Small Ruminant Res. 57: 193-202.

Chaiyabutr N, Faulkner A and Peaker M 1980. Effects of starvation on the cardiovascular system, water balance and milk secretion in lactating goats. Res Vet Sci. 28: 291-295.

Chaiyabutr N, Faulkner A and Peaker M 1981. Changes in the concentrations of the minor constituents of goat milk during starvation and on refeeding of the lactating animal and their relationship to mammary gland metabolism. Brit J Nutri. 45: 149-157.

Chaiyabutr N, Komolvanich S, Sawangkoon S, Preuksagorn S and Chanpongsang S 1997. The regulation of body fluid and mammary circulation during late pregnancy and early lactation of crossbred Holstein cattle feeding on different types of roughage. J Anim Physiol Anim Nutri. 77: 167-179.

Chiba LI, Lewis AJ and Peo Jr ER 1990. Efficacy of the urea dilution technique in estimating empty body composition of pigs weighing 50 kilograms. J Anim Sci. 68: 372-383.

Daily III JW, Hongu N, Mynatt RL and Sachan DS 1998. Choline supplementation increases tissue concentrations of carnitine and lower body fat in guinea pigs. J Nutri Biochem. 9: 464-470.

Dahlborn K 1987. Fluid balance in food-deprived lactating goats drinking saline. Qt J Exp Physiol. 72: 593-600.

Davis SR and Collier RJ 1985. Mammary blood flow and regulation of substrate supply for milk synthesis. J Dairy Sci. 68: 1041-1058.

Fernandez C, Lopez-Saez A, Gallego L and de la Fuente JM 2000. Effect of source of betaine on growth performance and carcass traits in lambs. Anim Feed Sci Tech. 86: 71-82.

Fernandez C, Sanchez-Seiquer P, Sanchez A, Contreras A and de la Fuente JM 2004. Influence of betaine on milk yield and composition in primiparous lactating dairy goats. Small Ruminant Res. 52: 37-43.

Garcia PA and Burg MB 1991. Renal medullary organic osmolytes. Physiol Rev. 71: 1081-1115.

Guinard-Flament J, Delamaire E, Lemosque TS, Boutinaud M and Yolande D 2006. Changes in mammary uptake and metabolic fate of glucose with once-daily milking and feed

restriction in dairy cows. Reprod Nutr Dev. 5: 589–598.

Hansen HO, Grunnet I and Kundsen J 1984. Triacylglycerol synthesis in goat mammary gland. The effect of ATP, Mg^{2+} and glycerol 3-phosphate on the esterification of fatty acids synthesized *de novo*. Biochem J. 220: 513-519.

Jarrett IG and Potter BJ 1950. Metabolism of acetate and propionate in the ruminant. Nature (London): 166: 515-517.

Kettunen H, Peuranen S and Tiihonen K 2001. Betaine aids in the osmoregulation of duodenal epithelium of broiler chicks, and affects the movement of water across the small intestinal epithelium *in vitro*. Comp Biochem Physiol Part A. 129: 595-603.

Leng RA and West CE 1969. Undernutrition in grazing sheep. I. Changes in the composition of body, blood and rumen contents. Aust J Agri Res. 23(3): 483-497.

Loest CA, Titgemeyer EC, Drouillard JS, Coetzer CM, Hunter RD, Bindel DJ and Lambert BD 2002. Supplemental betaine and peroxide-treated feather meal for finishing cattle. J Anim Sci. 80: 2234-2240.

Madsen TG, Nielsen L and Nielsen MO 2005. Mammary nutrient uptake in response to dietary supplementation of rumen protected lysine and methionine in late and early lactating dairy goats. Small Ruminant Res. 56: 151-164.

Matthews JO, Southern LL, Higbie AD, Persica MA and Bidner TD 2001. Effects of betaine on growth, carcass characteristics, pork quality and plasma metabolites of finishing pigs. J Anim Sci. 79: 722-728.

Medway W and Kare MR 1959. Thiocyanate space in growing domestic fowl. Am J Physiol. 196: 873-875.

Mitchell AD, Chappell A and Knox KL 1979. Metabolism of betaine in the ruminant. J Anim Sci. 49: 764-775.

Moore JH and Christie WW 1979. Lipid metabolism in the mammary gland of ruminant in the mammary gland of ruminant. Prog Lipid Res. 17: 347-395.

Neill AR, Grime DW, and Dawson RMC 1978. Conversion of the choline methyl groups through trimethylamine into methane in the rumen. Biochem J. 170: 529-535.

Prosser EG, Fleet IR, Corps AN, Heap RB and Froesch ER 1988. Increased milk secretion and mammary blood flow during close arterial infusion of insulin like growth factor I (IGFI) into the mammary gland of the goat. J Endocr. 117 (Suppl) : 248.

Puchala R, Sahlu T, Herselman MJ and Davis JJ 1995. Influence of betaine on blood metabolites of Alpine and Angora kids. Small Ruminant Res. 18: 137-143.

Rook JAF 1979. The role of carbohydrate metabolism in the regulation of milk production. Proc Nutri Soc. 38: 309-314.

Saunderson CL and MacKinlay J 1990. Changes in body weight, compositions and hepatic enzyme activities in response to dietary methionine, betaine and choline levels in growing chicks. Brit J Nutri. 63: 339-349.

Schultz LH 1974. Ketosis. In: Lactation. BL Larson and VR Smith (eds). New York: Academic Press.

Wray-Cahen D, Fernandez-Figares I, Virtanen E, Steele NC and Caperna TJ 2004. Betaine improves growth, but does not induce whole body or hepatic palmitate oxidation in swine *Sus scrofa domestica*. Comp Biochem Physiol Part A. 137: 131-140.