กลไกการควบคุมการผลิตน้ำนมในแพะลูกผสมที่เลี้ยงภายใต้สภาพเขตร้อนชื้น:

ผลของอาหารที่มีแร่ธาตุประจุบวกสูง

นายเหวียน เทียต

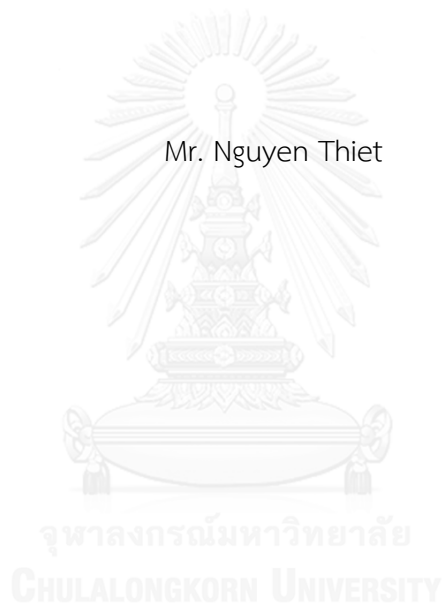วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรดุษฎีบัณฑิต

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THE CONTROL MECHANISMS OF MILK PRODUCTION IN CROSSBRED GOAT

UNDER TROPICAL CONDITION: EFFECTS OF HIGH DIETARY

CATION AND ANION DIFFERENCE

Mr. Nguyen Thiet

A Dissertation Submitted in Partial Fulfillment of the Requirements

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iv

เหวียน เทียต : กลไกการควบคุมการผลิตน้ำนมในแพะลูกผสมที่เลี้ยงภายใต้สภาพเขตร้อนชื้น: ผลของอาหารที่มีแร่ธาตุประจุบวกสูง (THE CONTROL MECHANISMS OF MILK PRODUCTION IN CROSSBRED GOAT UNDER TROPICAL CONDITION: EFFECTS OF HIGH DIETARY CATION AND ANION DIFFERENCE) อ.ที่ปรึกษาวิทยานิพนธ์หลัก: รศ. ดร. สัมพันธ์ ธรรมเจริญ, อ.ที่ปรึกษาวิทยานิพนธ์ร่วม: ศ. ดร. ณรงค์ศักดิ์ ชัยบุตร, ศ. ดร. สมชาย จันทร์ผ่องแสง, 98 หน้า.

การศึกษาวิจัยครั้งนี้ดำเนินการเพื่อศึกษาผลของอาหารที่มีส่วนประกอบระดับอิออนประจุบวกและลบต่างกันสูง (Dietary Cation and Anion Difference, DCAD) ในแพะนมที่เลี้ยงภายใต้สภาวะแวดล้อมอุณหภูมิสูง (High ambient temperature, HTa) ต่อผลผลิตน้ำนม ความสัมพันธ์กับการตอบสนองทางสรีรวิทยาประกอบด้วย การเปลี่ยนแปลงรูปแบบของการกินอาหารระหว่างวัน และรูปแบบของมื้ออาหาร การทำงานของกระเพาะหมักรูเมนและการย่อยได้ของสารอาหาร และความสมดุลของน้ำและส่วนของสารน้ำในร่างกายในแพะนมพันธุ์ผสมช่วงตั้งท้องใกล้คลอดจำนวน 10 ตัว ถูกใช้สำหรับการทดลองโดยแบ่งออกเป็น 2 กลุ่ม กลุ่มละ 5 ตัว อาหารสำหรับการทดลองคือ อาหารควบคุม DCAD (Control, 22.8 mEq/100g DM) และอาหารที่มีปริมาณ DCAD สูง (DCAD, 39.1 mEq/100g DM) ส่วนประกอบของทั้งสองสูตรประกอบด้วยเศษเหลือจากต้นข้าวโพดหมัก (corn stover by product silage) 44% และอาหารข้น (concentrate) 56% ในระหว่างช่วงการทดลองเริ่มจากสัปดาห์ที่ 2 ถึงสัปดาห์ที่ 8 หลังคลอด (PP-2 และ PP-8) แพะจะได้รับอาหารแบบเต็มที่ 2 ครั้งต่อวันและให้น้ำกินตลอดเวลา ในช่วงการทดลอง สภาวะแวดล้อม อุณหภูมิร่างกายที่วัดจากทวารหนัก (rectal temperature, Tr) และอัตราการหายใจ (respiration rate, RR) บ่งชี้ว่าแพะที่เลี้ยงภายใต้สภาวะ HTa (average THI = 85.2) และอยู่ในระยะที่มีความเครียดจากความร้อน โดยเฉพาะเปอร์เซ็นต์ของการเปลี่ยนแปลง Tr ในกลุ่ม DCAD น้อยกว่ากลุ่มควบคุม ในช่วงเวลา 9.00 และ 13.00 นาฬิกา อาหารสูตร DCAD สูงมีแนวโน้มการกินอาหารเพิ่มขึ้นและรูปแบบของมื้ออาหารเมื่อเทียบกับอาหารสูตรควบคุม ปริมาณการกินอาหารในรูปวัตถุแห้งต่อน้ำหนักตัว (dry matter intake/body weight, DMI/BW) มีแนวโน้มสูงตลอดการทดลองและมีค่าสูงกว่ากลุ่มควบคุมอย่างมีนัยสำคัญทางสถิติ (P<0.05) ในช่วง PP-8 อย่างไรก็ตามปริมาณน้ำนมและองค์ประกอบน้ำนมในทั้งสองกลุ่มไม่แตกต่างกัน การเพิ่มขึ้นของ DMI/BW ในช่วง PP-8 เกิดจากการเพิ่มขนาดและระยะเวลาของมื้ออาหาร และสอดคล้องกับการย่อยที่ดีขึ้นภายในกระเพาะรูเมน อย่างไรก็ตามระดับของเลปติน (leptin) ในพลาสม่าของกลุ่ม DCAD มีค่าสูงกว่ากลุ่มควบคุม ในช่วงเวลา 9.00 และ 16.00 นาฬิกา ความเข้มข้นของโปตัสเซียมไอออน คลอไรด์ไอออนและออสโมลาริตี้ในพลาสม่าไม่ได้รับผลกระทบจากการให้อาหาร DCAD แต่ระดับความเข้มข้นของโซเดียมไอออน และผลต่างของประจุบวกและลบ (cation and anion difference, CAD) ในกลุ่ม DCAD จะเพิ่มสูงขึ้นในช่วงเวลา 16.00 นาฬิกา แพะในกลุ่ม DCAD ดื่มน้ำในปริมาณมากกว่ากลุ่มควบคุม อย่างไรก็ตามอัตราการขับปัสสาวะและระดับความเข้มข้นของฮอร์โมนแอนติไดยูเรติก (antidiuretic hormone, ADH) ในพลาสม่าไม่แตกต่างกันเมื่อเปรียบเทียบระหว่างกลุ่ม ผลดังกล่าวทำให้ดุลย์ของน้ำเพิ่มสูงขึ้นในกลุ่ม DCAD ในช่วงเวลา 24 ชั่วโมง ไม่พบความแตกต่างของปริมาตรพลาสม่าและปริมาตรเลือดจากการให้ DCAD แต่ปริมาณน้ำนอกเซลล์มีแนวโน้มที่จะเพิ่มขึ้น เป็นผลให้ปริมาณน้ำทั้งหมดของร่างกายเพิ่มสูงขึ้น ผลการศึกษาในครั้งนี้แสดงให้เห็นว่า แพะนมที่ได้รับอาหารที่มีปริมาณ DCAD สูงมีแนวโน้มที่จะเพิ่ม DMI/BW จากการเพิ่มขึ้นของขนาดมื้ออาหาร และระยะเวลามื้ออาหาร การเพิ่มของ DMI/BW เป็นผลจากการทำงานของระบบทางเดินอาหารที่ทำงานดีขึ้น โดยไม่ขึ้นกับผลของการทำงานของฮอร์โมนเลปติน อีกทั้งปริมาณ DCAD ขนาดสูงจะเพิ่มปริมาณน้ำและความสมดุลของน้ำในร่างกาย ผลลัพธ์เหล่านี้มีส่วนทำให้กระบวนการปรับตัวแบบการระเหยเพื่อระบายความร้อนและช่วยในการชะลอการเพิ่มขึ้นของ Tr ภายใต้สภาวะ HTa

ภาควิชา	สรีรวิทยา	ลายมือชื่อนิสิต	_____
สาขาวิชา	สรีรวิทยาการสัตว์	ลายมือชื่อ อ.ที่ปรึกษาหลัก	_____
ปีการศึกษา	2559	ลายมือชื่อ อ.ที่ปรึกษาร่วม	_____
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KEYWORDS: BODY FLUIDS / CATION AND ANION / DAIRY GOATS / HIGH AMBIENT TEMPERATURE / MEAL PATTERN / MILK YIELD

NGUYEN THIET: THE CONTROL MECHANISMS OF MILK PRODUCTION IN CROSSBRED GOAT UNDER TROPICAL CONDITION: EFFECTS OF HIGH DIETARY CATION AND ANION DIFFERENCE. ADVISOR: ASSOC. PROF. SUMPUN THAMMACHAROEN, Ph.D., CO-ADVISOR: PROF. NARONGSAK CHAIYABUTR, Ph.D., PROF. SOMCHAI CHANPONGSANG, 98 pp.

The present study was carried out to evaluate the effect of high dietary cation and anion difference (DCAD) rations in dairy goats fed under high ambient temperature (HTa) on milk production in relation to physiological responses, including diurnal variations in eating and meal patterns, ruminal function and nutrition digestibility, water balance and body fluid compartments. Ten crossbred dairy goats during peri-parturition period were selected and divided into two groups of five animals each. Experimental diets were control DCAD (control, 22.8 mEq/100 g DM) and high DCAD (DCAD, 39.1 mEq/100 g DM). The composition of two diets consisted of 44% corn stover by-product silage and 56% concentrate. During the experimental period from the 2nd to 8th weeks of postpartum (PP-2 and PP-8), goats were fed twice daily either with the control or DCAD total mix ration with free access to water. The environmental conditions, rectal temperature (Tr) and respiratory rate (RR) in the present experiment indicated that goats were fed under HTa conditions (average peak THI = 85.2) and were in the stage of heat stress. The percentage change of Tr from DCAD group was lower than control group between 09:00 h and 13:00 h. High DCAD apparently increased eating and meal patterns compared with the control. Dry matter intake/body weight (DMI/BW) tended to increase throughout experiment and significantly higher than in animals fed with high DCAD at PP-8 (P<0.05), but milk yield and composition were similar between groups. An increase in DMI from DCAD group at PP-8 mainly came from increase in meal size and duration in accordance with the improvement of ruminal function and nutrition digestibility. However, the plasma leptin concentration from DCAD was higher than those from control. The concentrations of plasma K^+, Cl^- and osmolality was not affected by DCAD at 09:00 h and 16:00 h, but plasma Na^+ level and cation and anion difference (CAD) from DCAD increased at 16:00 h. Goats in DCAD group drank more water than control. However urine volume and plasma ADH concentration were not different between groups. As a result, apparent water balance was higher from DCAD group during 24 h. There were no effects of DCAD on plasma and blood volumes, but tended to increase in extracellular fluid and thereby increasing total body water. The results from current study indicate that dairy goats fed with high DCAD tended to increase DMI/BW by increasing meal size, meal duration. An increase in DMI/BW apparently came from an improving gastrointestinal tract function and was independent from the action of leptin. In addition, high DCAD resulted greater in total body water and apparent water balance. These results have contributed the process of adaptation for evaporative cooling and would be useful in slowing down the elevation in Tr under HTa.

Department:	Veterinary Physiology	Student's Signature	....
Field of Study:	Animal Physiology	Advisor's Signature	....
Academic Year:	2016	Co-Advisor's Signature	....
		Co-Advisor's Signature	....

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CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABREVIATIONS

AA	Amino acids
ADF	Acid detergent fiber
BV	Blood volume
BW	Body weight
Ca	Calcium
CAD	Cation and anion difference
Cl	Chloride
CP	Crude protein
DCAD	Dietary cation and anion difference
Diff WI-Uex	Difference between water intake and urinary excretion
DM	Dry matter
DMI	Dry matter intake
ECF	Extracellular fluid
HT_a	High ambient temperature
K	Potassium
mEq	miliequivalent
Na	Sodium
NDF	Neutral detergent fiber
OM	Organic matter
P	Phosphorus
PCV	Packed cell volume
PP-2	Second week of post-partum
PP-4	Fourth week of post-partum
PP-8	Eighth week of post-partum
PV	Plasma volume
RH	Relative humidity
RR	Respiration rate

SO_4^{2-}	Sulfate
Ta	Ambient temperature
TBW	Total body water
THI	Temperature humidity index
TMR	Total mix ration
Tr	Rectal temperature
UV	Urine volume
VFA	Volatile fatty acids
WI	Water intake

CHAPTER I

GENERAL INTRODUCTION

Goat plays an important role in the rural economy of many developing countries in Asia. Throughout the region, goats are very important in the milk and meat for people. For both dairy cattle and goat raised in tropical area including Thailand, the low milk yield is still the main problem. Factors such as environmental condition, nutrition, disease and lower genetic potential for milk production can influence on milk yield of dairy animals in tropical countries. In Thailand, dairy animals appear to confront with prolong high ambient temperature (HTa) condition. This condition could be characterized in part by altering animal behaviors such as body temperature, respiratory rate, eating behavior. Goats are considered more tolerant to HTa condition compared with dairy cows (Silanikove, 2000). Dairy goats and cattle under HTa similarly reduced feed intake (22 – 35%), but reduction of milk yield in dairy goats (3 – 13%) was much lower than in dairy cattle (27 – 33%) (Sano et al., 1985; Rhoads et al., 2009; Salama et al., 2014). Heat accumulation and production in dairy animals appear to be one of the major causes that decrease the production of milk. The reduction of milk yield under HTa condition may be in part due to the lower in feed intake and increase the demand for maintenance of body temperature (NRC, 2007; Rhoads et al., 2009). Because of the reduction of feed intake during HTa condition, intake of mineral elements is also decreased. Therefore, the increasing elemental levels in diet are strategy to meet mineral element requirements during high temperature environment (Sanchez et al., 1994). The current s aims to investigate the mechanisms by which the mineral elements influence the responses to HTa condition in dairy goat.

It has been known that K and Na requirement increased during HTa and DMI increased when these minerals were supplemented for lactating cows during high environmental condition (Schneider et al., 1986; West et al., 1987). In addition, high DCAD levels in diet increased the DMI of lactating cows during early and mid lactation (Delaquis and Block, 1995a). Furthermore, West et al. (1992) found that heat stress cows increased DMI when DCAD level increased from 12 to 46 mEq/100g DM.

However, the mechanisms underlying the increase in DMI did not fully understand when dairy animals supplemented with high DCAD during HTa. There are many factors which regulate the feed intake in ruminant such as characteristic of diet, environmental condition, hormones and physiological status of animal. In ruminant, the effect of high DCAD diet on DMI apparently came in part from the changes in ruminal pH, the ruminal microbial activities and the fermentation products (Tucker et al., 1988; Wildman et al., 2007a; Sharif et al., 2010b; Eriksson and Rustas, 2014). Additionally, leptin as adiposity hormone has been shown to involve in satiation in ruminant (Thammacharoen et al., 2014) and chronic heat stress have been shown to up regulate plasma leptin (Morera et al., 2012). Similar findings were reported by Accorsi et al. (2005) and Chanchai (2010) in dairy cows. It is possible that the mechanisms of high DCAD diet on increase of DMI may mediate through either the improvement of ruminal fermentation or alteration in satiation hormone. Thus, the second aim of present study is to investigate the mechanism by which the high DCAD diet increases DMI under HTa condition (Chapter V). The effects of high DCAD on both ruminal fermentation pattern and alteration in plasma leptin are included in the current study. Further, to describe the effect of high DCAD on DMI in behavioral context, the current study firstly measures the effects of high DCAD on meal patterns including meal size, meal frequency and inter-meal interval (Chapter IV). The latter information may in part describe the mechanism involved in regulation of feed intake and milk production.

It has been demonstrated that increasing dietary cation and anion difference (DCAD) by addition of cations such as sodium (Na^+) and potassium (K^+) increased milk yield (Tucker et al., 1988; Apper-Bossard et al., 2006). In addition to milk yield, dairy cows supplemented with high DCAD increased DMI in both cool and warm environments (West et al., 1991; Silanikove et al., 1998). However, the mechanisms underlying the increase in milk yield did not fully understand when dairy animals supplement with high DCAD during HTa condition. It has been known that milk production is affected by both internal and external factors e.g. substrates utilization by mammary gland, mammary blood flow, food and water intake, and environmental temperature. Body fluid homeostasis, substrates extraction by

mammary gland, and mammary blood flow are the fundamental internal factors for milk production (Davis and Collier, 1985). Cooled dairy cattle by misty fan increased in extracellular fluid, blood and plasma volumes in association with a light increase in DMI which partitions the distribution of nutrients to the mammary gland for milk synthesis (Chaiyabutr et al., 2008). Dry cows supplement with high DCAD tend to consume more water, but there were not significant effects on DMI and plasma volume (Delaquis and Block, 1995b). However, lactating cows supplement with high DCAD was higher water intake, DMI and milk yield in early and mid lactation period (Delaquis and Block, 1995a). High water intake may be greater water retention for dairy animals supplement with high DCAD. A greater water conservation would not only provide a reservoir of soluble metabolites for milk synthesis but would be useful in slowing down the elevation in body temperature during high environmental temperature (Chaiyabutr et al., 2000). Although high DCAD supplementation has been reported to increase in milk production, there is a few data on the effects of DCAD on milk production in relation to regulation of body fluid compartments. Thus the third aim of present study is to investigate the effect of high DCAD on milk production in relation to body fluid distribution and ADH concentration in dairy goats (Chapter VI).

Therefore, the objectives of this study were as follow:

1) To investigate the effects of high DCAD conditions on physiological responses, drinking, eating and urination patterns in lactating dairy goats under HTa.

2) To investigate the effects of high DCAD on DMI in relation to ruminal fermentation, microbial protein synthesis, nutrients digestibility and plasma leptin level in lactating dairy goats under HTa.

3) To investigate the effects of high DCAD on milk production in relation to body fluid distribution and ADH concentration in lactating dairy goats under HTa.

The hypotheses of this study were as follow:

1) High DCAD can change physiological responses, drinking, eating and urination patterns in lactating dairy goats.

2) High DCAD can change DMI and plasma leptin level, improve ruminal pH and fermentation, microbial protein synthesis in lactating dairy goats.

3) High DCAD can change body fluid distribution and plasma ADH concentration in lactating dairy goats.

CHAPTER II

REVIEW OF LITERATURE

The literature review would emphasize the information on two possible mechanisms which we intend to investigate in this study as mention above. Firstly, high DCAD can change whole body metabolism via changes in water balance, regulation of feed intake and meal pattern, ruminal function and nutrient digestibility. This experiment was carried out under HTa. Thus, second mechanism may provide some clues which high DCAD can attenuate the effect of HTa condition via changes in rectal temperature, respiration rate, patterns in water intake and urinary excretion and subsequently produce appropriate condition to support the milk synthesis.

2.1 Effects of high environmental temperature in dairy animal

2.1.1 Physiological responses

Thermal physiologists commonly use the term of heat stress to mean the demand made by the environment for heat dissipation (Silanikove, 2000). Heat stress also is characterized by increasing respiration rates and rectal temperature and followed by impairing metabolism (Bandaranayaka and Holmes, 1976). It is more sensitive in high-producing cattle than in low-producing cattle because high-producing cattle produce more milk and require more nutrient intake for milk synthesis and metabolic heat production increase (Kadzere et al., 2002).

Body temperature easily get susceptibility to hot weather, thus it is a sensitive indicator of thermal stress (Araki et al., 1984; Kadzere et al., 2002). According to McDowell et al. (1976), the temperature-humidity index (THI) was used as indicator of thermal conditions. THI values is less than 82 referring to absence of heat stress; 82 – 84 moderate heat stress; 84 – 86 severe heat stress and greater than 86 extreme severe heat stress for sheep and goat (LPHSI, 1990). Heat stress changed physiological responses in dairy cattle and dairy goats such as increasing in respiration rates and rectal temperature as reported by previous studied (Chaiyabutr et al., 2000; Kadzere

et al., 2002; Hamzaoui et al., 2013). Change in respiration rate from influence of environmental temperature may not parallel with the change in body temperature (Bligh, 1984). In addition, respiration rate in animal also depends on the level of carbon dioxide in the blood. High pCO_2 will stimulate an increase in the rate and depth of respiration (Aspinall et al., 2009). Dairy cows supplement with high DCAD from 120.4 to 456 mEq/kg DM increased pCO_2 and respiration rate (West et al., 1992). Thus, in some cases respiration rate is not a good indicator of thermal stress in the animal.

2.1.2 Hormonal and cellular responses

Hormonal responses

High ambient temperature in cattle and buffaloes significantly increased blood cortisol concentration and the cooled animals had low plasma cortisol level than non-cooled animals (Chaiyabutr et al., 2008; Aggarwal and Upadhyay, 2013). Collier et al. (1982) observed that the cortisol concentration elevated in acute heat stress, but it was not in chronic heat stress. Similar findings were found in Friesian calves (Yousef et al., 1997). The reduction of cortisol in heat stress lactating cattle and buffaloes during hot months (prolonged heat stress) may be responsible for reduction of milk components (Aggarwal and Upadhyay, 2013). The increase of cortisol during acute heat stress may be due to the glucocorticoid hormones involve the hyperglycaemic action through the gluconeogenesis process, followed by increasing the blood glucose in acute heat stress animals. The decrease which happens in chronic heat stress is attributed to the fact that cortisol is thermogenic in animals and the decline of adrenocortical activity under thermal stress is a thermoregulatory protective mechanism preventing a rise in metabolic heat production in high environmental temperature.

Thyroid hormones are needed for maximal milk yield. There is declined conversion of thyroxine (T_4) to the active hormone triiodothyronine (T_3) in liver and kidney during lactation, but increased conversion to T_3 in the mammary gland. This could be raised the priority of the mammary gland for metabolites compared to

other body tissues. Animal supplemented with thyroid hormone causes a temporary increase in milk production for several weeks if nutrients were provided sufficiently for supporting the increased metabolism and the increase in production, but milk yield did not change when animal supplemented with thyroid hormones for more than two months (Blaxter KL, 1949). It was known that T_4 and T_3 involved the metabolic homeostasis and susceptible to climate changes (Perera et al., 1985). These hormones decreased during heat stress as compared to thermoneutral conditions (Silanikove, 2000). Season may affect on plasma thyroid hormones and plasma T_3 level was high in winter and low in spring and summer in buffalo and Friesian (Kamal and Ibrahim, 1969). Chaiyabutr et al. (2008) observed that arterial plasma T_4 concentration in cooled dairy cattle was lower than in non-cool dairy cattle at all stages of lactation, but its effect did not occur in arterial plasma T_3 concentration.

Prolactin is needed for the maintenance of milk yield in rats, after bromocriptine administration (bromocriptine blocks the release of prolactin hormone) rat decreases in milk yield of 50% or more. However, prolactin produced less effect on milk yield in ruminant. In addition, milk yield from ruminant has been shown to influence significantly by growth hormone (GH) deficiency. Thus, prolactin seems to play important role on galactopoiesis in rodent and primate. Growth hormone is important hormone in ruminant (Knight and Wilde, 1993). During high environmental temperature, plasma prolactin increased in mammals (Ronchi et al.). An increase in plasma prolactin may be involved in acclimation (Beede and Collier, 1986). Because bromocriptine, a prolactin inhibitor, prevented the function of sweat gland (Kaufman et al., 1988). Plasma prolactin in ruminant decreased due to reduction of nutrient intake as reported by Bocquier et al. (1998).

Growth hormone mediates its function directly via growth hormone receptors (GHR) or indirectly via insulin like growth factor (IGF) system (Bauman and Vernon, 1993; Brooks and Waters, 2010). One of the most important mediators from GH-IGF axis is IGF-1. IGF-1 belongs to a family of insulin-like growth factors (IGFs) that shares close structure homology to the precursor form of insulin (pro-insulin). Although

circulating IGF-1 acts as endocrine fashion appear to come mainly from liver, the local production of IGF-1 as paracrine or autocrine has been known as well (Le Roith et al., 2001). The GH effect on mammary gland function has been proposed to influence by both plasma and local production of IGF system (Flint and Knight, 1997; Brooks and Waters, 2010). It has been know that plasma IGF-1 levels are regulated by GH, insulin and nutritional state. Plasma IGF-1 concentration in dairy cattle reduced during summer time (Butler and Smith, 1989; Hamilton et al., 1999). This may be due to the decline in GH concentration during heat stress animals (Mitra et al., 1972). However, Chaiyabutr et al. (2008) observed that arterial plasma IGF-1 level did not differ between cooled and non-cooled dairy cattle at all stages of lactation, suggesting that the effect of evaporative cooling on increased milk yield was independent on the action of IGF-1. This may be due to slight increase in feed intake in that study. Baumgard and Rhoads (2013) suggested that plasma IGF-1 level decreased during heat stress, follow by reducing the milk synthesis and used these nutrients for maintaining homeothermia. Silanikove et al. (1998) reported that dairy cows supplement with high mineral (Na, K, Cl) increased milk yield, DMI and plasma IGF-1 concentration at week 3 of postpartum. The increase of milk yield during the increase of mineral supplementation for dairy cow may partly relate with increased plasma IGF-1 in that study.

Cellular responses

The cellular response changes in gene expression during high environmental temperature which is an adaptive mechanism of cell (Fujita, 1999). Heat shock proteins help cells to adapt with environmental changes and have important roles in environmental stress tolerant and adaptation (Frydenberg et al., 2003; Hoffmann et al., 2003). HSPs represented between 2 – 15% of total cellular protein expressed by all living organisms (Morimoto et al., 1994). Their functions attribute to cell survival by reducing the accumulation of damaged or abnormal polypeptides within cells (Parsell and Lindquist, 1993) and help the maintenance of proteins in an active form and prevent protein degradation (Neuer et al., 2000), protect the cells against apoptosis (Li and Srivastava, 2001).

HSPs were classified by their molecular weight such as HSP32, HSP40, HSP60, HSP70, HSP90, HSP110 and many others (Kregel, 2002). Family of 70-kDa (HSP70) is one of the most abundant HSPs (Kiang and Tsokos, 1998) and temperature sensitive (Beckham et al., 2004). HSPs become predominant protein synthesis in the cells and reduced expression and synthesis of other proteins during high environmental temperature (Lindquist, 1986; Collier et al., 2008). Min et al. (2015) found that dairy cows expose to high environmental temperature increased in serum HSPs (HSP70, HSP90). Higher expression of HSPs (HSP60, HSP70, HSP90) was also found in tropical goats during summer (Dangi et al., 2012). Moreover, HSP70 was more sensitive and the most abundant in heat stress (Beckham et al., 2004; Min et al., 2015). These results indicated that plasma HSP70 level may be a good indicator of heat stress in animal (Gaughan et al., 2013; Min et al., 2015).

2.1.3 Ruminal fermentation and nutrient digestibility

High environmental temperature may influence the rumen microbes, depressed rumination and reticular motility (Attebery and Johnson, 1969; Aganga et al., 1990). These results lead to change the rumen fermentation. Previous studies observed that heat stress decreased the total production of volatile fatty acids (VFA) (Tajima et al., 2007). Similar findings were also reported by Chanchai (2010). Total VFAs in cooling dairy cattle were higher than in non-cooling dairy cattle during early lactation and tend to be higher during mid and late lactation (Chanchai, 2010). Collier et al. (1982) reported that high producing lactating dairy cattle under heat stress reduced dramatically roughage intake and rumination. Reduction of roughage intake would contribute to reduce VFA production and lead to change in the ratio of acetate and propionate (Kadzere et al., 2002; Nonaka et al., 2008). However, the molar ratio of individual VFAs did not affect by cooling and non-cooling dairy cattle (Chanchai, 2010). The different results of molar of VFAs concentration are possible that animals feed with variety of dietary manipulation such as total mix ration or separating between concentrate and roughage.

Some authors reported that heat stress resulted in improvement of nutrient digestibility in dairy goats (Hamzaoui et al., 2013) and dairy cows (McDowell et al.,

1969), male goats (Hirayama et al., 2004). The higher nutrient digestibility during heat stress may be due to reduction of DMI, a slower rate of digesta passage or a prolonged mean retention time (Robertshaw and Vercoe, 1980; Kadzere et al., 2002). In contrast, there were not significantly different in nutrient digestibility when animal exposed to heat stress (Chanchai, 2010). Bernabucci et al. (1999) reported that Friesian heifers expose to the short term of hot environment increased the nutrient digestibility, but animals during long term of hot environment unchanged the digestibility in compared with thermal comfort conditions. It indicates that there is an adaptation of digestive tract during heat stress.

2.1.4 Dry matter intake and milk production

Dry matter intake

Feed intake in dairy cattle begins to reduce at ambient temperatures of 25 – 26 ^{0}C and decreases more quickly above 30 ^{0}C and reduces up to 40% at 40^0C (NRC, 1989). Reduction of feed intake due to heat stress has been reported in previous studied for dairy goats (Sano et al., 1985; Hamzaoui et al., 2013) and dairy cows (Rhoads et al., 2009; Chanchai, 2010). Partial recovery of DMI in heat stress dairy goats was found by Hamzaoui et al. (2013), reducing with 27% of feed intake during the first of 19 days and then with 14% for days after. However, the recovery of DMI did not happen in dairy cattle under heat stress (Rhoads et al., 2009; Shwartz et al., 2009). This indicates that dairy goats have adapted under heat stress conditions. Collier et al. (1982) reported that high producing lactating dairy cattle under heat stress reduced dramatically in roughage intake and rumination.

Milk production

Lactating saanen goats expose to heat stress conditions from moderate to severe reduced milk yield from 3 to 13% (Sano et al., 1985). Brown et al. (1988) found that dairy goats under heat stress (THI = 79) decreased milk yield in Alpine but not in Nubian goats. In addition, Hamzaoui et al. (2013) reported that dairy goats expose to heat stress condition unchanged the milk yield during late lactation, although heat stress dairy goats significantly decreased the feed intake. Moreover,

during the first of 60 days lactating dairy cattle appeared to have greater effect with climate changes (Sharma et al., 1983). Dairy goats and cattle under heat stress reduced similarly feed intake (22 – 35%), but reduction of milk yield in dairy goats (3 – 13%) was much lower than in dairy cattle (27 – 33%) (Sano et al., 1985; Rhoads et al., 2009; Salama et al., 2014). The different results of milk yield in previous experiments indicate that dairy animals respond to heat stress vary according to species, breed and stage of lactation.

2.2 Effects of DCAD in dairy animal

2.2.1 Definition of DCAD and effect of DCAD on dry matter intake

Dietary cation and anion difference (DCAD) is defined as the proportion of specific fixed ions or the balance between positively charged and negatively charged fixed ions (Beede et al., 1992). The term "fixed ions" refers to bioavailable ions that are not metabolized, namely, Na^+, K^+ and Cl^-. The fixed ion balance plays an important role in determining acid-base balance in biological fluids (Stewart, 1978). Some researchers include sulfur (S) (Dishington, 1975; Block, 1984), although S is not a fixed ion. Because sulfates directly acidify biological fluids and can alter acid-base balance if included at high dietary concentrations. Thus, the DCAD is calculated as either milliequivalent (Na + K – Cl) or (Na + K) – (Cl + S) per 100 g DM or per kg DM. The the milliequivalent (mEq) of an element per 100 g DM is determined by following equation:

mEq/100 g DM = [(grams x Valence) x 1000/ (g atomic weight)]

In addition, the milliequivalent (mEq) of an element per 100 g DM is also calculated by dividing the concentration of the element (% of the diet DM) by the mEq weight of the element. Milliequivalent weights for Na, K, Cl, and S are 0.023, 0.0391, 0.0355, and 0.0160 respectively (Tucker et al., 1991). Na, K, Cl, and S have been chosen to calculate DCAD for ruminants because of their contribution to osmotic potential, acid-base chemistry and pumping mechanisms of cell membranes (Block, 1994).

West et al. (1992) found that high DCAD from +12 to +46 mEq/100g of DM increased the feed intake in heats stress dairy cows. (Delaquis and Block, 1995) observed that DMI increased when dairy cattle fed DCAD from +5.55 to +25.81 mEq/100g of DM during early lactation or +14.02 to +37.27 mEq/100g of DM during mid-lactation. These effects did not occur in late lactation. According to Hu and Murphy (2004) reported a meta-analysis and found that a linear improvement in DMI with high DCAD levels in diet. Similar findings were also reported by previous studies (Oetzel and Barmore, 1993; Hu et al., 2007a). The higher DMI at high DCAD levels in diet could be due to improve the ruminal pH which increased the ruminal microbial activity (Tucker et al., 1988; Sharif M, 2010a). However, Jackson et al. (1992) found that DMI did not differ between treatments when dairy calves fed DCAD from 0 to +52 mEq/100g of DM. Similar findings were also observed by Borucki Castro et al. (2004); Wildman et al. (2007b). The decrease of DMI occurred when animal fed with low or negative DCAD levels in diet (Tucker et al., 1992; Spanghero, 2004). This reduction in DMI may be either poor palatability of anionic salts (Goff et al., 1991) or low DCAD induced slight metabolic acidosis (Block, 1994). The different responds in DMI in the previous experiments depends on level of DCAD in diets, stage of lactation and ingredients of diet, rumen environment.

2.2.2 Water balance and effect of DCAD on water intake and excretion

Water balance

Daily water balance is determined by daily water intake and daily water excretion. Thus, factors regulate water balance which will be gained by regulation of daily water intake and excretion. Daily water intake consists of free water (drinking water), water in feed and metabolism (oxidation) water (Murphy, 1992). Drinking water may involve three separate perspectives: drinking as a homeostatic response (Fitzsimons and Le Magnen, 1969); drinking as an index of circadian rhythms such as temperature and humidity (Johnson and Johnson, 1997) and drinking by eating (Kraly, 1984). Daily water excretion in lactating animals includes skin (sweating), lung (panting), urine, milk and feces. If the water excretion is not replaced by food and drinking water, the total volume of extracellular fluid decreases and the animal

becomes dehydration. In dehydration, osmoregulatory mechanisms will begin to work. The decrease in blood pressure and increase in osmotic pressure result in an increase in the reabsorption of water from collecting ducts, under the control of hormone vasopressin. The osmoreceptors also stimulate the thirst center in the brain and the animal feels the need to drink. Both of these pathways result in a rise in plasma volume and an increase in arterial blood pressure (Aspinall et al., 2009). To maintain the total body water, animals need regulate the balance between water intake and excretion. In addition, total volume and compartmental distribution of body fluids depended on daily variations in dietary salt and water intake (Cowley and Roman, 1989). High DCAD as addition more cationic salts increased water intake and DMI during high environmental temperature as reported in the previous studies (Tucker et al., 1988; Silanikove et al., 1998; Apper-Bossard et al., 2006). As a result, high DCAD is expected that improves the body fluids of dairy goats in the current proposal and it can partly compensate the effects of high environment conditions in regarding to balance of body fluids via regulation of water intake and excretion. Furthermore, an increase of Na and water intake will elevate extracellular fluid and plasma volume follow by increasing of arterial pressure and induce the reduction of vasopressin secretion (Cowley and Roman, 1989). Thus, present study also determined the effects of DCAD on water intake and excretion in relation to vasopressin hormone during time intervals of day. This may provide some information in the mechanism of saving of water as thermoregulation during hot part of day when dairy goats supplement with high DCAD.

Effect of DCAD on water intake and excretion

Previous studies found that high DCAD increased water intake and DMI during high environmental temperature (Tucker et al., 1988; Silanikove et al., 1998; Apper-Bossard et al., 2006). The amounts of water intake correlated positively with the quantity of food ingest (Murphy, 1992). In addition, about 80% of water intake happens around meals in monogastric and ruminants (Bigelow and Houpt, 1988; Forbes and Barrio, 1992; Rossi and Scharrer, 1992). Theoretically, high DCAD may relate to the increase in Na or K intake followed by increasing plasma Na and K. This

causes an increase of osmotic pressure of plasma. These conditions will stimulate the thirst center in the brain and the animal feel the need to drink (Blair-West et al., 1992; Aspinall et al., 2009). However some studies found that high DCAD did not effect on water intake due to unchanged DMI (Delaquis and Block, 1995b). Dairy animals fed with high DCAD were higher water excretion in urine and increased the blood pH and HCO_3^- concentration (Jackson et al., 1992; Delaquis and Block, 1995a). Thus, higher water intake by high DCAD diet may result the increased excretion of HCO_3^- in the urine (Delaquis and Block, 1995a; Delaquis and Block, 1995b). It has been known that animals under high environmental temperature will increase respiration and the expiration of CO_2 exceeds the rate of its formation in the body. The partial pressure of CO_2 of blood reduces, creating a deficit of blood carbonic acid and resulting in respiratory alkalosis. An increase in blood and urine pH and reduced net acid excretion rate result. Thus, animals compensate for high blood pH by excreting HCO_3^- ions into urine. As a result, animal can maintain normal blood pH, although blood HCO_3^- concentration was lower normal (Sanchez et al., 1994). Dairy animals supplement with high DCAD will compensate the loss of HCO_3^- ions into urine during high environmental temperature. This result may change the pattern of urinary excretion and save the water for thermoregulation and also maintain the balance of body fluid compartments.

2.2.3 Effect of DCAD on ruminal fermentation and nutrients digestibility

A part of DCAD effect on dairy animals may result from altered rumen fermentation. Ruminal pH is reduced by lactic acid production when high grain concentrations are fed (Russell and Hino, 1985), which decreases fiber digestibility (Snyder et al., 1983). According to Mertens and Ely (1979) ruminal pH from 6.4 to 6.8 is optimal for fiber digestion. Tucker et al. (1988) reported that increased DCAD improved ruminal pH and unchanged fermentation patterns in dairy cows. Similarly, increased DCAD in dairy cows increased ruminal pH and could not affect on ruminal acetate and propionate (Roche et al., 2005). Wilkens et al. (2015) found that male sheep fed either low or high DCAD were not statistically significant in total volatile fatty acids and ruminal osmolality. However, Sharif et al. (2010b) observed that

ruminal pH, ruminal ammonia nitrogen, acetate and acetate: propionate ratio were greater in buffalo bulls fed with medium and high DCAD (220 and 330 mEq/kg DM, respectively) than those fed anion diets or low DCAD (110 mEq/kg DM). In addition, dairy cows during early lactation fed with high DCAD in low crude protein (CP) diets (15%) were higher the microbial protein synthesis and butyrate concentration than low DCAD and was similar for low and high DCAD in high CP (17%) diets (Wildman et al., 2007a). Similarly, Eriksson and Rustas (2014) reported that dairy cows in mid-lactation fed with high potassium bicarbonate (cationic salt) were higher allantoin excretion than those fed with low potassium bicarbonate. Moreover, Hu et al. (2007a) found that plasma branched chain amino acids (AA) and essential AA: total AA ratio were higher in dairy cows during early lactation fed with DCAD of 47 mEq/100g DM than those fed with DCAD of 22 mEq/100g DM and indicated that more microbial protein flowing to the small intestinal. These results suggest that high DCAD improves ruminal characteristics and microbial protein synthesis, but the DCAD supplement in response to rumen function varies to species and stage of lactation.

The effects of varying DCAD on nutrient digestibility were reported by Delaquis and Block (1995b). They found that high DCAD did not differ on ADF and NDF digestibility in dry cows, but DM digestibility was slightly higher and unchanged for ADF and NDF digestibility in lactating cows. Some studies supplement with sodium bicarbonate as cationic salt for high DCAD have improved DM, OM, ADF digestibility when dairy cows fed corn silage as based diet and not for alfalfa hay (Stokes and Bull, 1986). Similar findings were reported by Eriksson and Rustas (2014).

2.2.4 Effect of DCAD on milk production and composition

Hu and Murphy (2004) in the meta-analysis reported that dairy cows fed with high DCAD had a positive on milk production and 4% fat corrected milk yield (4% FCM yield). Tucker et al. (1988) found that lactating dairy cattle increased milk yield up to 8.6% when DCAD level increased from -10 to +20 mEq/ (Na + K –Cl)/ 100g of DM in diet. Similarly, improvement of milk yield and 4% FCM yield found from 16.4 to 19.7 kg/day when dairy cattle fed with DCAD from -11.66 to +31.24 mEq/100g of DM (West et al., 1991). Similar findings were also reported by Borucki Castro et al.

(2004); Wildman et al. (2007a). The increase of milk production at higher DCAD level could be due to improved DMI. However, some studies found that high DCAD did not improve milk production such as DCAD level from +12.04 to +45.6 mEq/100g of DM (West et al., 1992) or +21 to +127 mEq/100g of DM (Roche et al., 2003) or -15 to +15 mEq/100g of DM (Wu et al., 2008). According to Delaquis and Block (1995a), high DCAD level improved milk yield in early (from 25 to 50 post partum) and mid lactation. These effects were not obtained in late lactation. However, high DCAD supplemented immediately for dairy cows after calving (from day 0 to day 42 postpartum) did not improve milk production (Chan et al., 2005). Similar findings were also reported by Hu et al. (2007a); Hu et al. (2007b) when dairy cows fed with DCAD of 22 to 47 mEq/100g of DM. Some studies reported in meta-analysis that optimal level of DCAD for lactation rations ranged at +25 and +30 mEq/100g of DM and the milk yield begin reducing when DCAD exceeds about 30 mEq/100g of DM (Sanchez et al., 1994; Hu and Murphy, 2004). These different results can be attributed to the range of DCAD and the stage of lactation used in the different experiments.

Milk protein and fat percentage unchanged by high DCAD level in previous studies (Tucker et al., 1991; West et al., 1991). However, some studies have demonstrated that increased milk fat content when dairy animals fed high DCAD levels (Roche et al., 2005; Razzaghi et al., 2012). High DCAD levels have changed the ruminal pH and fermentation which enhance the de novo fatty acid synthesis and followed by increasing milk fat (Wildman et al., 2007a; Sharif M, 2010a).

2.3 Regulation of feed intake and effect of leptin on feed intake

2.3.1 Regulation of feed intake

Ruminant ate their feed in the discrete meals. There are several theories which have been developed in the search for factors affecting feed intake. Generally, theories could be divided into two groups: physical and physiological regulation. Theories focusing on physical regulation suggest that the capacity of the digestive tract is an important limiting factor in feeding. Physiological regulation (metabolic regulation) could be determined as feedback signals arising from receptors in the

periphery which inform the central nervous system about the metabolic status of the individual. In the brain, these signals are integrated and decisions are made whether or not to eat.

The feed intake is measured by the filling of the rumen as the physical mechanical control mechanisms. The filling of the rumen depended on the ruminal fermentation and passage rate. The rate of passage depends on the particle size and structure of feed and the ruminal fermentation is influenced by the quality of feed (Azizi, 2008). Beauchemin et al. (1994) reported that with short chopped alfalfa silage in diets DMI reduced less than 0.5 kg/d, whereas DMI decreased up to 3kg/d when the animal feed with long chopped alfalfa silage in diets. The decrease in forage particle size could result in increased DMI when distention in the reticulo-rumen limited DMI (Azizi, 2008).

Physical regulation of feed intake happens when DMI was limited by the time required for chewing or by distension within the gastrointestinal tract (Allen, 2000). Dietary factors that increase eating time could result in decreased ruminating time, which raises the filling effect of diet. Distension stimulates the stretch receptors in the muscle layer in the wall of the reticulo-rumen (Harding and Leek, 1972). Brain satiety centers integrate these and other stimuli to signal the end of a meal (Forbes, 1996). However, the response to distention regarding to meal end might not be the same between animals and physiological status (Allen, 2000). The threshold of stimulation by reticulo-rumen fill in each animal may depend on nutrient absorption and possible hormone (Mbanya et al., 1993). It has been reported that dairy animals during high environmental temperature decreased feed intake and increased water intake (Sano et al., 1985; Rhoads et al., 2009; Salama et al., 2014). The reduction of feed intake may be due to the animal attempt to reduce metabolic heat production (Kadzere et al., 2002) or gut filled by water (Salama et al., 2014). However, dairy animals supplement with high DCAD in hot weather increased both water intake and DMI as reported by previous studies (Tucker et al., 1988; Silanikove et al., 1998; Apper-Bossard et al., 2006). The responses of animals between high environment

temperature and high DCAD diet may involve the physical regulation of feed intake and it may change their meal pattern.

Ruminal osmolality depends on the content of mineral salts and fermentation of organic matters in diets. Increased osmolality is associated with various physiological responses that may affect satiety (Azizi, 2008). Baile and Mayer (1969) found that infusion of Na acetate into the reticulo-rumen may increase osmolality and it follow to reduce feed intake, whereas injection with the same amount of Na acetate into the jugular vein had no effect on DMI. In contrast, Choi and Allen (1999) found that infusion of NaCl into the reticulo-rumen DMI unchanged over the 12h infusion period, but decreased meal size and inter-meal interval. However, Rossi et al. (1998) found that Pygmy goats supplement with 3% NaCl in diet reduced feed intake and unchanged water intake. They suggested that an increase in ruminal osmolality could control the feeding, but it seems to be less important for control of drinking. The meal size in ruminant depends on the ruminal osmolality (Carter and Grovum, 1990) and animal fed with energy rich feeds increased ruminal osmolality faster than low energy feeds (Bennink et al., 1978). There is evidence that absorption of propionate induces satiety. Anil and Forbes (1980) reported that infusion of propionate into the portal vein of sheep decreased feed intake over 80% compared to control. Additionally, Choi and Allen (1999) reported that propionate infusion into reticulo-rumen of lactating reduced the meal length and meal size compared with infusion NaCl or acetate. The effect of propionate infusion on the reduction of DMI may be caused by an increased insulin secretion (Grovum, 1995). Insulin has been reported to reduce DMI in sheep (Foster et al., 1991).

2.3.2 Effect of leptin on feed intake

Leptin is a protein hormone (16 kDa) produced by white adipose tissue and is the product of the obese gene. When there is a lot of adipose tissue, production of leptin increases to activate the satiety centers in the hypothalamus and reduces food intake. In contrast, when adipose tissue reserves reduce due to limited availability of food, leptin levels decrease and appetite increases. Thus, leptin is an important regulation of appetite, whole-body energy balance and body composition

(Squires, 2003). Leptin administration in ewes reduced feed intake approximately one third after 3 days (Henry et al., 1999). Similarly, high leptin content in blood followed by the reduction of feed intake in dairy goats (Thammacharoen et al., 2014) and dairy cows (Chanchai, 2010). However, the anorectic effect of leptin was lost when growing and adult sheep were underfed (Morrison et al., 2001). The increase of leptin expression in adipocytes from heat shock indicates that leptin help to limit the body hyperthermia by reducing feed intake, energy metabolism and body fat (Houseknecht et al., 1998). This may be a possible adaptive mechanism during heat shock of the cell (Collier et al., 2006). Thus, high DCAD for dairy goats during HTa may increase feed intake and it may partly relate the alteration of plasma leptin level which is expected in current study.

CHAPTER III

MATERIALS AND METHODS

The experiment was carried out at Veterinary student training center, Faculty of Veterinary Science, Chulalongkorn University.

3.1 Experimental design and animals care

The procedures of this experiment were performed in according to the guidelines and approved by the Animals Care and Use Committee, Faculty of Veterinary Science, Chulalongkorn University (#1531074).

Ten multiparous crossbred Saanen lactating goats, average body weight (BW) 34.48 ± 1.42 kg, were used in this experiment. One month before parturition, all animals were kept in individual metabolic cages in 2 x 1 m shaped pens with plastic floors for adaptation. One week after parturition (PP-1), the animals were divided into two groups based on body weight, with five animals in each group. They were offered two experimental diets either control DCAD or high DCAD (control: 22.81 or DCAD: 39.08 mEq/100g DM respectively). Two diets contained 44% corn stover by-product silage and 56% concentrate. The corn stover by-product silage, used in the diets, was chopped on a 2 cm. The diets were formulated as a total mixed ration (TMR) according to national research council recommendation (NRC, 1981). The levels of $NaHCO_3$ and K_2CO_3 were added to diets to obtain the diets with DCAD of 22.81 or 39.08 mEq/100g DM. The ingredients of the experimental diets were presented in Table 3.1 These diets were offered for goats twice daily at 07:00 h and 14:00 h (refusals always exceeded 10% of experimental diets) with free access to water and feed.

Table 3.1 The ingredients of experimental diets on the DM basis

Item	Control	High DCAD
Corn stover by-product silage	44	44
Cassava	3.26	3.26
Soybean meal	19.62	19.62
Molasses	3.69	3.69
Corn meal	25.86	24.42
Rice bran	2.25	2.25
Limestone	0.9	0.9
$NaHCO_3$	0.14	0.62
K_2CO_3	0.28	1.24

3.2 Data collection and measurements

Feed samples were collected every day throughout the experiment and divided into two parts; one half was immediately dried in the oven at 105 ^{o}C over-night to determine dry matter and the remaining samples were kept frozen at -20 ^{o}C until chemical analysis. At the end of experiment, all feed samples were thawed and mixed thoroughly, and subsamples were dried at 65 ^{o}C overnight (approximately 12 h) and for nitrogen and ash analysis according to AOAC (1990), neutral detergent fiber (NDF) and acid detergent fiber (ADF) using the procedure developed by of Van Soest et al. (1991). Milk yield was daily recorded twice at 06:00 h and 13:00 h from parturition to day 56 postpartum. After parturition all goats were weighed before morning feeding, once per week throughout the experiment. The experiment was done in 49 days, from 2nd to 8th weeks of postpartum (PP-2 to PP-8, Figure 3.1). The digestibility measurement was in the last seven days (PP-8) of experiment by total fecal collection method.

The ambient temperature and relative humidity at goat barns were recorded by using a thermohygrometer (Thermohygrometer, Sato, Taiwan). The ambient temperature and relative humidity were measured every two hours of day time, one per week throughout the experiment (Figure 1). The temperature and humidity index (THI) was determined in according to NRC (1971) as follow:

$$\text{THI} = (1.8 \times Tdb + 32) - ((0.55 - 0.0055) \times RH \times (1.8 \times Tdb - 26.8))$$

Where: Tdb = dry bulb temperature and is expressed as ^0C; RH = relative humidity

Rectal temperature was determined by a digital clinical thermometer (digital clinical thermometer C202, Terumo, Tokyo, Japan). Respiration rate was measured by counting the movement of the flank. The rectal temperature and respiration rate were measured every two hours of day time, one per week throughout the experiment (Figure 3.1).
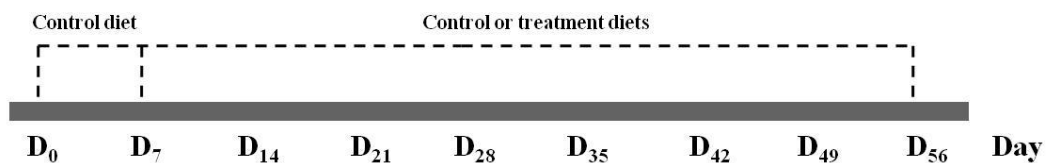
Figure 3.1 Experimental procedure, from day 0 to day 7 postpartum ten lactating dairy goats received control diet. After that, five goats were received with control diet and the others were fed with DCAD diet throughout the experiment.

- Blood collection for hormones was measured on D_{27} and D_{55} (4th and 8th postpartum, PP-4 and PP-8)

- Body weight was measured on D_0, D_7, D_{14}, D_{21}, D_{28} D_{35}, D_{42}, D_{49} and D_{56}

- Meal pattern was measured on D_{26} and D_{54} (PP-4 and PP-8)

- Body fluid was measured on D_{28} and D_{56} (PP-4 and PP-8)

- Rumen fluid was collected on D_{28} and D_{56} (PP-4 and PP-8)

- Nutrient digestibility and milk sample was measured on D_{49-56}

- Temperature, relative humidity, water intake, urinary volume, respiration rate and rectal temperature was measured at 09:00 h, 11:00 h, 13:00 h, 15:00 h, 17:00 h and 19:00 h.

3.3 Determination of dry matter intake, water intake and balance, nutrient digestibility

Feed offered and feed refusals were daily recorded from parturition to the end of experiment. Daily dry matter intake is calculated by following formula:

Daily feed intake = feed offered - feed refusals (Dry matter basis)

Water intake (WI) was daily measured from parturition to the end of experiment. In addition, water intake was also determined by day time (every two hours from 07:00 h to 19:00 h) and night time through the experiment (Figure 1). The measurement of water intake was performed by subtracting the weight of water offered with the weight of water refusal.

Apparent water balance was measured by following formula:

Apparent water balance = water ingest (water intake + water in feed) – water losses (milk, urine, feces), without taking account the metabolic (Chaiyabutr et al., 1980).

Total fecal collections were daily performed in the last seven days of experiment. Subsamples (about 10% of total amount) of feces were collected and dried in oven until constant weight to determine dry matter. Another subsample of feces was performed and kept at -20 ^{0}C for later analysis. The fecal samples were analyzed for DM, N, Ash, NDF and ADF levels.

Nutrient digestibility was determined by:

$$\text{Digestibility of nutrients (\%)} = \frac{\text{Nutrients in feed intake – Nutrients in feces}}{\text{Nutrients in feed intake}} \times 100$$

Meal patterns were determined on day 26 and day 54 postpartum (PP-4 and PP-8). Meal patterns were recorded continuously for 24h using digital balance equipped to data processing software (PBA 665 & Weigh Term 231G, Mettler Toledo, Zürich, Switzerland). Digital balances were fixed under the feed containers of goats. Balance with feed containers was protected by wood boxes. The actual weight of feed containers was checked and recorded automatically by a personal computer in each minute. Meals were defined as feed removals exceeding 5g that were separated by at least 15 minutes of non-feeding (Rossi et al., 1998). Parameters recorded were meal size, meal duration, meal frequency and inter-meal interval. Meal size is total amount of feed ingest during each meal. Meal duration is calculated as the time

intervals between the beginning and ending of each meal. Meal frequency is calculated by counting the number of meal per day. Inter-meal interval is time intervals between meals (Nielsen, 1999).

3.4 Determination of milk composition

A thirty milliliters of milk sample was daily collected from each animal at the last seven days of experiment and kept at − 20 ^{o}C until analysis. Milk compositions were analyzed for total solids, solid no fat, fat, lactose and protein by using Milkoscan (FT2; Foss, Hilleroed, Denmark).

3.5 Ruminal function: analysis of volatile fatty acids

Ruminal fluid collection

Rumen fluid samples were collected from dairy goats on day 28 and day 56 of postpartum (PP-4 and PP-8) by using a stomach tube connected to a syringe. Approximately 20 ml of fluid samples were taken at 09:30 h after morning feeding. The pH was immediately determined by using pH meter (pH221, Lutron, Taipei, Taiwan). After that, the ruminal fluid samples were filtered through two layers of cheese-cloth and added with 1ml 6N HCl for preservation. Then, samples were frozen at -20 ^{o}C for later analysis of volatile fatty acids and ammonium nitrogen.

Sample preparation and analysis

Volatile fatty acids were prepared and analyzed as described by Thammacharoen et al. (2001). Briefly, 5 ml of rumen fluids were thawed, added 1ml into a centrifuge tube and then centrifuged at 4000 r.p.m for 20 minutes and the clear supernatants were ready to inject in the gas chromatograph. Ammonium nitrogen was determined by a Salicylate-hypochlorite.

VFAs are determined by Hewlett Packard GC system HP6890A, equipped with a 30m x 0.32mm x 0.15 μm film fused silica capillary columns (HP Innowax, Agient, USA). Injector and detector temperatures are 250^{0}C and 300^{0}C respectively. The temperature program (column temperature) is used from 60^{0}C to 200^{0}C (20 ^{0}C/min,

10 min). Carrier gas-nitrogen flow 1ml/min. The sample is used 1 µl for injection and the analysis time is taken about 15 minutes.

3.6 Urine collection and analysis

Total urine was collected and measured on the same days as feces by using plastic containers with amount 10% sulfuric acid solution (15 ml H_2SO_4 10% in 90 ml urine) added to prevent nitrogen loss (final pH of urine was kept below 3). The total urine from each day was then collected at 10% of total volume, were kept in a refrigerator and pooled at the end of each period to be analyzed for nitrogen by the Kjeldahl method (AOAC, 1990) and for allantoin excretion by colorimetric method according to (Chen and Gomes, 1992).

Additionally, urine was also records by separating the day time and night time. On the day time urine was determined 2 hour-intervals from 07:00 h to 19:00 h, one per week.

To determine the nitrogen retention the following formula was performed:

Nitrogen retention = Total nitrogen in feed intake − (total nitrogen in feces + total nitrogen in urine + total nitrogen in milk)

3.7 Determination of plasma hormones

On day 27 and day 55, blood samples were collected at 09:00 h and 16:00 h for analysis of minerals and at 16:00 h for analysis of plasma leptin and ADH concentrations. The samples were obtained from the jugular vein, placed in lithium heparin tube, kept in crushed ice and then centrifuged at 3,000 rpm for 10 minutes. The plasma samples were stored at -20 ^{0}C until analysis. Sodium and potassium were measured by flame photometer (Flame photometer 410C, Ciba Corning Inc., USA). Chloride was determined by chlorimeter (Chloride 925, Ciba Corning Inc., USA). Osmolality was measured by osmometer (Osmometer 3D3, Advance Instrument Inc., USA). Plasma leptin concentration was determined using an Enzyme-Linked ImmunoSorbent Assay (ELISA) kit specific for multi-species hormone (MBS018743, MyBioSource, San Diego, USA). Plasma ADH is determined by ELISA kit specific for

goat and employs the competitive enzyme immunoassay technique (MBS006109, MyBioSource, San Diego, USA).

3.8 Determination of body fluid compartments

On PP-4 and PP-8 (day 28 and day 56 PP), dairy goats were inserted with catheters (18G, NIPRO Corporation, Osaka, Japan) into the jugular vein for dye solution injection at 16:00 h. The catheters were stopped with three-way stopcocks (NIPRO Corporation, Osaka, Japan). After insertion with catheters, dairy goats were prevented with eating and drinking, and then blood was sampled. Plasma volume (PV) was measured by intravenous injection of 3 ml of 0.5% Evans blue dye (Sigma-Aldrich, Steinheim, Germany) via the jugular vein. Determination of extracellular fluid (ECF) was taken by intravenous injection of 5 ml of 10% sodium thiocyanate solution (Sigma-Aldrich, Steinheim, Germany) and total body water (TBW) was performed by intravenous injection of 5 ml of 20% antipyrine solution (Sigma-Aldrich, Steinheim, Germany) via the jugular vein. Three milliliter blood samples from the jugular vein into the EDTA tube were collected before and after injection of dye solution at 20, 30, 40 and 50 minutes for PV, ECF and TBW was taken the blood at the same time points of PV and ECF and continuously collected at 120, 180 and 240 minutes, kept in crushed ice. An aliquot of the blood samples at 0 minutes (before dye injection) was taken to determine for packed cell volume (PCV) by microhaematocrit centrifuge and then all blood samples were centrifuged at 3,000 rpm for 10 minutes for harvesting the plasma. Every withdrawn the blood, each animal was infused with normal saline that was equivalent to the volume of blood withdrawn. Plasma samples were analyzed the concentration of Evans blue dye and NaSCN according to Medway and Kare (1959) and antipyrine as described by Weiss (1958) by using spectrophotometer. The blood volume (BV) was calculated from plasma volume and packed cell volume (Chaiyabutr et al., 1980).

Plasma volume (PV) was calculated from equation:

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

Percentage of plasma volume (%PV) is calculated from the plasma volume and body weight (BW) as equation:

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

Extracellular fluid (ECF) is calculated as equation:

$$ECF = \frac{\text{Amount of sodium thiocyanate injected}}{\text{Concentration of sodium thiocyanate at zero time concentration}}$$

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

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

Total body water (TBW) is calculated as equation:

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

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

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

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

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

Percentage of blood volume (BV) is calculated from the blood volume (BV) and body weight (BW) as equation:

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

3.9 Statistical analysis

All data were reported as the mean value ± SE. The experimental results from different periods were compared by analysis of variance with repeated measurements and post-hoc analysis was performed by Bonferroni t-test; the data between groups were compared by using unpaired t-test. Differences were considered significant when $P<0.05$.
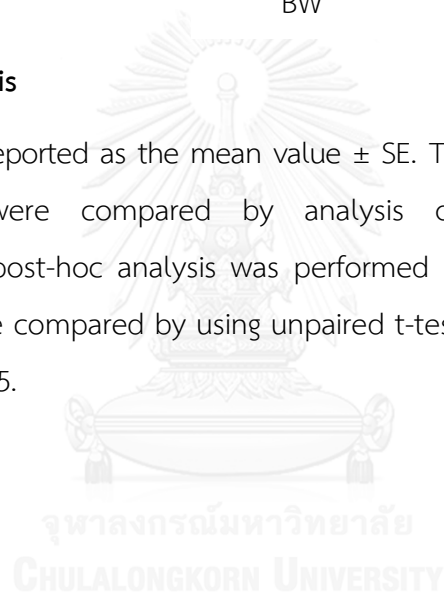
CHAPTER IV

THE EFFECT OF HIGH DIETARY CATION AND ANION DIFFERENCE ON DRINKING, EATING AND URINATION PATTERNS IN DAIRY GOATS UNDER HIGH AMBIENT TEMPERATURE

INTRODUCTION

In tropical countries, dairy animals are confronted with prolonged HTa during prolonged summers. Decreased feed intake and increased water intake during HTa have been report in both dairy cattle and goat (Sano et al., 1985; Rhoads et al., 2009; Salama et al., 2014). Dairy animals exposed to heat stress changed their meal patterns by increasing meal frequency, consuming smaller meals and eating for longer periods (Alam et al., 2011; Shiao et al., 2011). The reduction of feed intake during HTa may be in part due to the animal's attempt to reduce metabolic heat production or a gut filled by water (Kadzere et al., 2002; Salama et al., 2014). Supplementation with high dietary cation and anion in both species increased both water intake and dry matter intake as reported by previous studies (Tucker et al., 1988; Apper-Bossard et al., 2006). Further, comparable responses have also been demonstrated in dairy cattle fed under mild degree HTa (Silanikove et al., 1998). An increase in DMI effect by DCAD has been described partly via the improvement of rumen function such as ruminal pH, ruminal microbial activities and fermentation (Tucker et al., 1988; Sharif et al., 2010b).

It was well accepted that goats fed under HTa have different mechanisms and tolerance compared with cattle. Specifically, small ruminants tend to use panting rather than sweating, as in large ruminants (Robertshaw, 2006). Dairy cattle responded to acute heat stress by increasing respiration rate and body temperature (Chaiyabutr et al., 2008; Kanjanapruthipong et al., 2015) and altering the rumen function (Nonaka et al., 2008). Because dairy goats are different from dairy cattle in terms of their responses to HTa and because there was no behavioral information about the effect of DCAD on eating and drinking patterns, the present study aimed to

investigate the potential effect of higher DCAD to alleviate the HTa effect on diurnal variations in feed intake, water intake and urinary patterns.

MATERIALS AND METHODS

4.1.1 Animals and management

The details of experimental design and management of animal were described in Chapter III. Briefly, ten crossbred Saanen goats that were in late pregnancy, and with an average body weight (BW) 34.48 ± 1.42 kg were selected and used in this experiment. One week after parturition (PP-1), animals were divided into two groups based on body weight, with five animals in each group. They were offered two experimental diets of either control DCAD or high DCAD (control: 22.8 or DCAD: 39.1 mEq/ 100 g DM respectively). The experiment was started and lasted for 7 weeks (2nd–8th week postpartum, PP-2 to PP-8). The experimental diets contained 44% corn stover by-product silage and 56% concentrate. The diets were formulated as a total mixed ration according to national research council recommendation (NRC, 1981). The ingredients of the experimental diets are presented in Table 3.1 (Chapter III). These diets were offered for goat twice daily at 07:00 h and 14:00 h (refusals always exceeded 10% of experimental diets) with free access to water and feed.

4.1.2 Data collection and measurements

Feed and refusal samples were collected every day throughout the experiment and divided into two parts; one half was immediately dried in the oven at 105 °C overnight to determine dry matter, and the remaining samples were kept frozen at – 20 °C until chemical analysis. The details of analysis methods in chemical composition of experimental diets were described in Chapter III. Sodium and potassium were measured by atomic absorption spectrophotometer (Thermo iCE 3000 series, Thermo Fisher Scientific, China), chloride (Cl) was determined by colorimetric titration and sulfate (SO_4^{2-}) was measured by spectrophotometer (UV-VIS 1800 Shimadzu, Japan).

Body weight was measured from all of the goats once per week from PP-1 to PP-8. Rectal temperature (Tr), respiration rate (RR), water intake (WI), urine volume,

ambient temperature (Ta) and relative humidity (RH) were determined once per week throughout the experimental period, and the measurements were done every 2 h during daytime. Tr was determined by a digital clinical thermometer (digital clinical thermometer C202, Terumo, Tokyo, Japan). RR was measured by counting the movement of the flank within one minute. The measurement of WI was performed by subtracting the weight of water offered with the weight of water refusal. Urine volume was collected using a plastic container and weighted at specific time points. Ambient temperature and relative humidity at goat barns were recorded using a thermohygrometer (Thermohygrometer, Sato, Taiwan). The temperature and humidity index (THI) was determined according to NRC (1971).

On PP-4 and PP-8, eating and meal patterns were recorded continuously for 24 h using digital balance equipped to data processing software (PBA 665 & Weigh Term 231G, Mettler Toledo, Zürich, Switzerland). For each goat, the digital balance was fixed under the feed container. The balance with feed container was protected by a wooden box. The actual weight of feed containers was checked and recorded automatically once per minute using a personal computer. Meals were defined as feed removals exceeding 5 g that were separated by at least 15 minutes of non-feeding (Rossi et al., 1998; Thammacharoen et al., 2017). Parameters recorded were meal size, meal duration, meal frequency and inter-meal interval.

4.1.3 Statistical analyses

All data were presented as the mean and standard error of measurement and analyzed with the repeated two-way analysis of variance (ANOVA). Significance of main effects was performed by the Bonferroni t-test. The data of two diets were compared using an unpaired t-test. Statistical significance was declared at $P < 0.05$.

RESULTS

4.2.1 Chemical composition of experimental diets

The chemical composition of experimental diets is presented in Table 4.1. The control and DCAD diets were similar in DM, Ash, OM, CP, NDF and ADF contents, but contents of Na, K, Cl and S ion in the DCAD diet were greater than in the control diet and consequently DCAD in miliequivalents of (Na+K) - (S+Cl)/ 100g of DM was greater in the DCAD diet than in the control diet.

4.2.2 Environmental condition and the effects of high DCAD on rectal temperature and respiration rate

The average Ta, RH and THI at 07:00 h and 13:00 h were 27.6±0.4 and 35.3±0.5 $^{\circ}$C, 50.6±1.06 and 71.3±0.78%, 78.1±0.57 and 85.5±0.47, respectively. During the experimental period, there were no difference of Ta, RH and THI at 13:00 h between control and DCAD groups (P>0.05, Table 4.2). Similarly, there was no interaction effect between week and high DCAD supplementation on Tr and RR in this study (P>0.05). The average Tr at 13:00 h for the control and DCAD groups were not significantly different throughout the experimental period (39.6±0.13 and 39.6±0.10 $^{\circ}$C, P>0.05, Table 4.2). However, the average of RR at 13:00 h from DCAD (148±10 time per min) was higher than in the control (124±10 time per min, P<0.05, Table 4.2).

4.2.3 Effects of high DCAD on dry matter intake and meal patterns

There was no interaction effect between week and high DCAD supplementation on total daily, day time and night time DMI (P>0.05, Fig. 4.1a; 4.1b; 4.1c). Under HTa condition, the average daily DMI from both groups was 34.7±0.2 g/kg (1.2±0.1 kg). Dry matter intake of day time and night time from both groups was 32±0.2 and 2.8±0.1 g/kg (1.1±0.1 and 0.1±0.0 kg). The average total daily, day- and night-times DMI from 8 weeks during experimental period did not differ between groups (P>0.05, Fig. 4.1a, 4.1b, 4.1c). In this current study, eating and meal pattern were also measured on PP-4 and PP-8. The 24 h DMI at PP-8 (P<0.05), but not at PP-4 (P>0.05), from the DCAD group was significantly higher than from the control group (Table 4.3). This result corresponded to the larger 24 h and day time meal sizes of DCAD group than those

of the control group (P<0.05, Table 4.3). In addition, there were also significantly longer meal duration and less meal frequency during day time in the DCAD group than in the control group (P<0.05, Table 4.3).

4.2.4 Effects of high DCAD on water intake and urinary excretion

There was no interaction effect between week and high DCAD supplementation on daily, day time and night time water intake (P>0.05, Fig 4.2a; 4.2b; 4.2c). Under HTa conditions, the average daily WI from both groups was 169±18 ml/kg (5.7±0.08 L). The day- and night-time WI from both groups were 149±15 and 19±5 ml/kg BW (5.1±0.07 and 0.6±0.02 L, respectively). The average daily total and day-time WI during the experimental period from both groups were not significantly different (P>0.05, Fig. 4.2a and 4.2b). However, night-time WI was higher in the DCAD than in the control when the DCAD diet provided from PP-2 to PP-8 (P<0.05, Fig. 4.2c).

Daily, day time and night time urine volume did not affect by week and high DCAD supplementation (P>0.05, Fig 4.3a; 4.3b; 4.3c). The average daily UV from both groups was 91±14 ml/kg (3.1±0.4 L). The day- and night-time UV from both groups were 45±8 and 40±6 ml/kg (1.5±0.2 and 1.4±0.2 L, respectively). The average daily UV during experimental period was not significantly different (P>0.05, Fig. 4.3a). There was no difference in UV when day- and night-time UV were analyzed as well (P>0.05, Fig. 4.3b and 4.3c).

Table 4.1 The chemical composition of experimental diets on a DM basis

Item	Control	High DCAD
Dry matter, %	35.69	35.49
Crude protein, %	16.68	16.84
Ash, %	6.36	7.24
Organic matter, %	93.64	92.76
Acid detergent fiber, %	26.12	25.16
Neutral detergent fiber, %	51.61	50.03
Net energy[1], Mcal/kg DM	1.66	1.67
Ca,[1] %	0.52	0.54
P,[1] %	0.36	0.49
Na, %	0.10	0.16
K, %	1.46	1.87
Cl, %	0.37	0.20
S, %	0.13	0.15
DCAD[2], mEq/100g DM	23.52	39.89

[1] calculated according to NRC (1981)

[2] DCAD in miliequivalents of (Na + K) – (S + Cl)/100 g DM

Table 4.2 The environmental conditions and animal responses from the present experiment

Item[1]		Weeks of postpartum							
		1	2	3	4	5	6	7	8
Ta (°C)	Control[2]	34.10±0.93	35.10±0.78	35.50±1.00	35.80±0.58	35.25±1.09	34.50±0.91	34.60±1.04	34.30±1.03
	DCAD[2]	35.80±0.64	36.05±0.66	36.20±0.60	35.35±0.98	35.60±1.11	35.45±0.90	35.35±0.89	35.45±1.09
RH (%)	Control[2]	52.00±1.60	49.20±2.22	48.70±2.54	49.50±1.16	52.80±2.68	52.90±1.50	51.40±0.97	53.20±1.93
	DCAD[2]	48.00±2.12	47.90±1.55	48.10±1.38	51.20±2.32	51.20±1.76	51.60±1.34	51.00±1.29	50.90±2.77
THI	Control[2]	84.21±1.01	84.95±0.66	85.34±0.85	85.97±0.67	85.83±1.01	84.91±0.97	84.76±1.21	84.67±1.01
	DCAD[2]	85.64±0.64	85.95±0.69	86.18±0.55	85.66±0.87	86.01±1.19	85.92±0.97	85.67±0.87	85.70±0.97
Tr (°C)	Control[2]	39.6±0.19	39.7±0.19	39.6±0.21	39.7±0.20	39.5±0.12	39.4±0.13	39.6±0.19	39.7±0.14
	DCAD[2]	39.5±0.12	39.6±0.16	39.8±0.12	39.6±0.11	39.7±0.18	39.7±0.10	39.6±0.06	39.5±0.17
RR *	Control[2]	121.3±11.77	117.9±14.03	120.5±18.10	123.7±9.47	122.0±14.05	121.3±13.84	128.2±9.46	127.4±17.12
(breath/min)	DCAD[2]	119.9±15.64	136.9±11.23	158.9±17.09	145.6±13.29	160.2±15.87	163.0±13.67	156.4±10.76	143.6±13.74

1 Ta, Ambient temperature; RH, relative humidity; THI, temperature and humidity index; Tr, rectal temperature; RR, respiratory rate.

2 DCAD level: Control = 22.8 mEq/ 100g DM; DCAD = 39.1 mEq/ 100g DM

*Significance DCAD effect: P<0.05
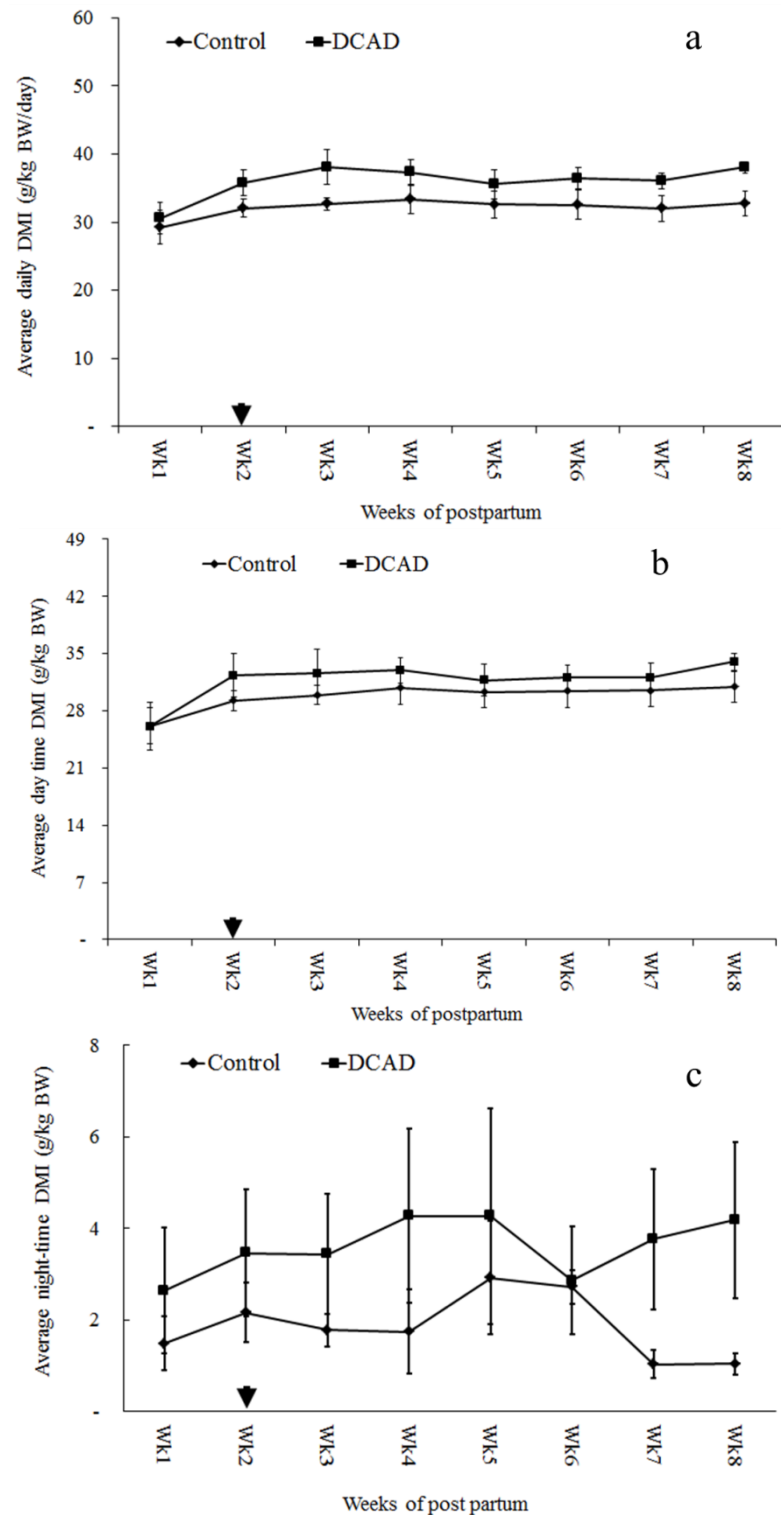
Figure 4.1 Effect of high dietary cation and anion difference on dry matter intake (DMI). Control or DCAD rations were provided from the 2nd week postpartum (PP-2, arrow) and DCAD effect was observed until the 8th postpartum (PP-8). There were no effects of DCAD on the average daily DMI (a), day-time DMI (b) and night-time DMI (c).

Table 4.3 Effect of high DCAD on eating and meal patterns at the 4th and 8th weeks postpartum

Eating and meal patterns[1]		PP-4		PP-8	
		Control[2]	DCAD[2]	Control[2]	DCAD[2]
DMI (g DM/kg BW)	24 h	34.73±2.26	38.17±3.04	33.51±2.29	41.16±1.94*
	Day time	31.62±1.99	33.69±3.35	32.36±2.06	36.98±1.80
	Night time	1.74±0.92	4.28±1.90	1.04±0.24	4.18±1.71
Meal size (g DM/kg BW)	24 h	2.23±0.24	2.35±0.25	2.35±0.29	3.48±0.27*
	Day time	3.05±0.48	2.89±0.40	2.93±0.26	3.98±0.14*
	Night time	0.30±0.12	0.80±0.31	0.35±0.11	1.64±0.71
Meal duration (min.)	24 h	34.60±3.61	35.40±2.29	35.00±2.70	46.40±4.48
	Day time	42.80±6.01	41.40±3.57	40.40±2.98	53.20±4.53*
	Night time	17.60±1.25	19.20±2.78	16.80±1.80	19.80±3.88
Meal frequency	24 h	16.00±0.80	16.00±1.20	14.00±0.90	12.00±1.40
	Day time	11.00±1.00	12.00±0.70	1100.±0.30	9.00±0.40*
	Night time	5.00±0.70	4.00±0.70	3.00±0.70	3.00±0.70
Inter-meal interval (min.)	24 h	49.00±5.21	42.20±5.91	56.40±8.83	58.20±7.79
	Day time	22.40±2.50	17.40±1.29	23.20±2.48	24.60±2.91
	Night time	100.00±8.56	103.60±20.10	208.60±93.30	210.80±46.62

DMI: dry matter intake; PP-4: the 4th week postpartum; PP-8: the 8th week postpartum.

*Significance DCAD effect: P<0.05 compare between control and DCAD at PP-8.

[1]: 24 h from 07:00 h to 06:00 h; day time from 07:00 h to 19:00 h; night time from 19:00 h to 06:00 h.

[2]: DCAD level :Control =22.8 mEq/ 100g DM; DCAD =39.1 mEq /100g DM
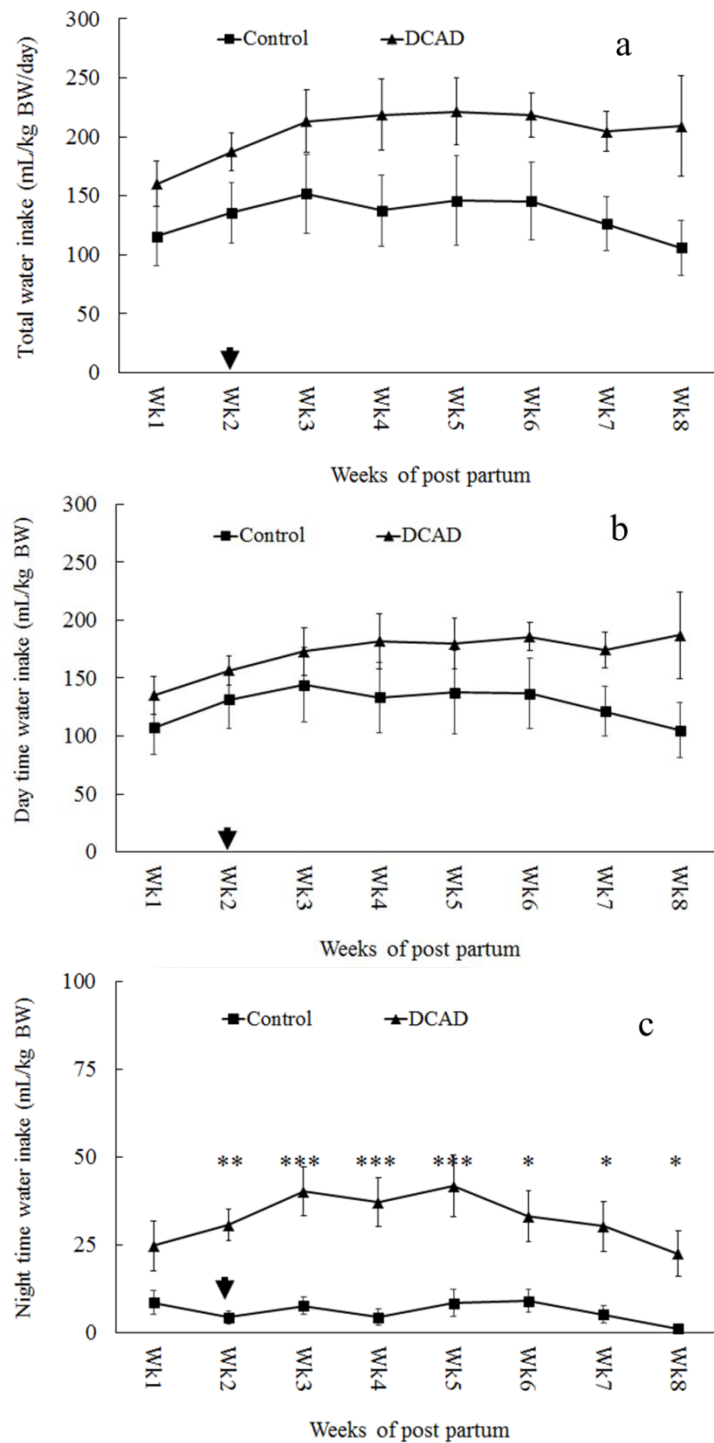
Figure 4.2 Effect of high dietary cation and anion difference on total water intake (WI). Control or DCAD rations were provided from the 2nd week postpartum (Wk2, arrow) and DCAD effect was observed until the 8th postpartum (Wk8). High DCAD had no effects on the average daily WI (a) and day-time WI (b). However, the night-time WI (c) from DCAD group was higher than from the control group. Asterisks at the specific time point indicate a significant difference between treatments.*: P<0.05; **: P<0.01; ***: P<0.001.
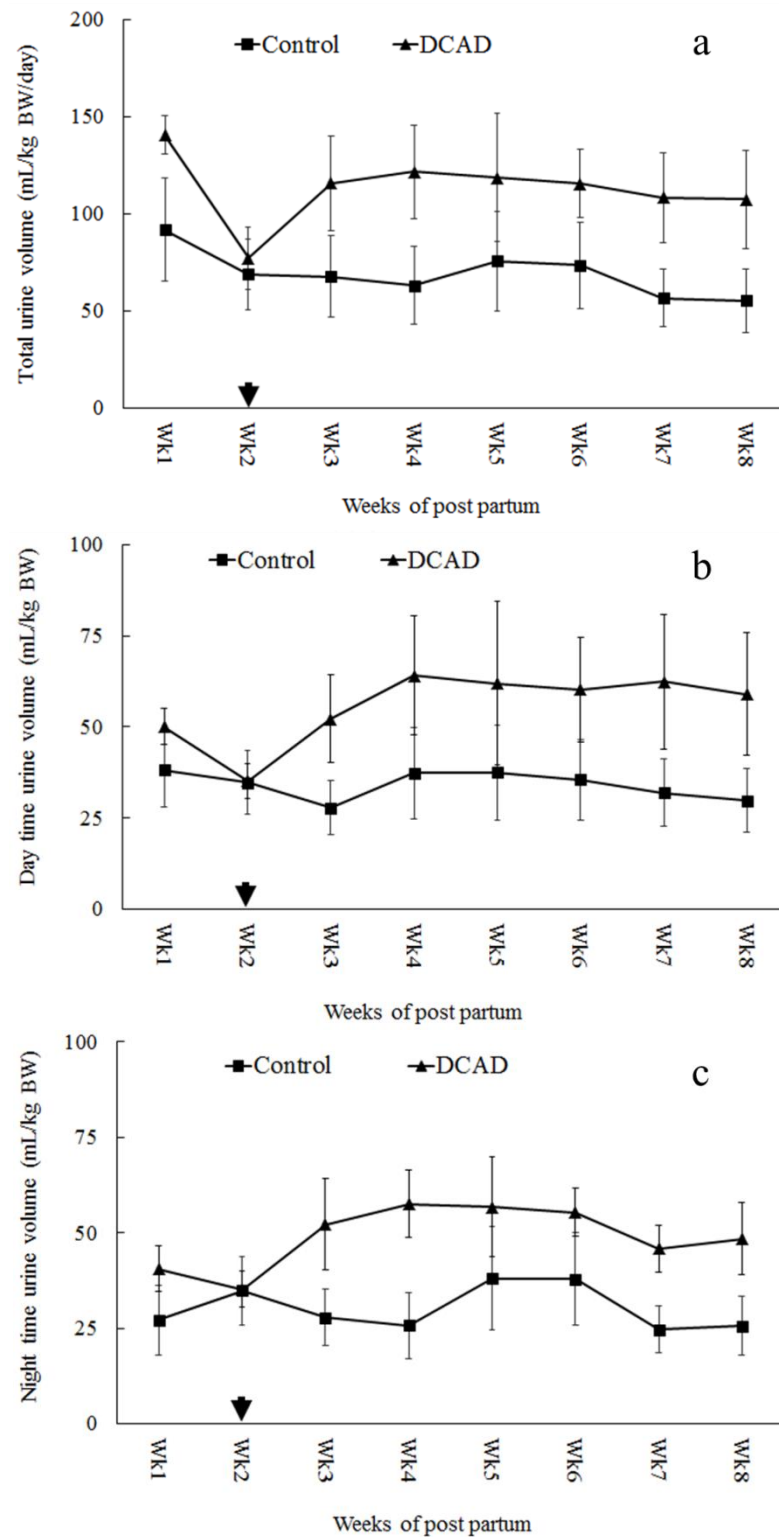
Figure 4.3 Effect of high dietary cation and anion difference on urine volume (UV). Control or DCAD rations were provided from the 2nd week postpartum (WK2, arrow) and DCAD effect was observed until the 8th postpartum (Wk8). There were no effects of DCAD on the average daily UV (a), day-time UV (b) and night-time UV (c).

DISCUSSION

The data reported here revealed the effect of high DCAD on eating, drinking, and urination behaviors in crossbred dairy goats fed under HTa conditions. Dietary high DCAD produced a positive effect on meal and eating patterns. In addition, the higher night-time WI and the non-significant difference of urination pattern apparently contributed to evaporative heat dissipation during the day time.

It was clear from the environmental conditions and physiological responses (Tr and RR) reported in the present experiment that all goats fed under HTa conditions were at the stage of heat stress (Kadzere et al., 2002; Hamzaoui et al., 2013). The RR reported here suggested that goats were at the early phase of panting which was thermal tachypnea (Hales and Webster, 1967; Robertshaw, 2006). Moreover, the significantly higher RR from the DCAD over control groups in this study suggests the effect of high DCAD on acid base homeostasis. In principle, high DCAD ration via supplementation of fix cation (Na or K) with HCO_3^- tends to induce alkalosis (Block, 1994). In dairy cattle, the typical acid base balance after high DCAD have been demonstrated under normal Ta and HTa conditions. Although high DCAD increased blood pH, HCO_3^-, PCO_2 and RR (Tucker et al., 1988; West et al., 1991; West et al., 1992; Kadzere et al., 2002), the indices of blood gas were within the normal range (Stevens et al., 1994). The significantly higher RR in the DCAD over control groups was in line with previous results and suggested that under HTa high DCAD ration apparently produced a minor effect on the acid-base balance.

Dairy goats fed high DCAD diets tend to increase daily DMI under HTa. High DCAD ration increased week 8-PP DMI by enhancing meal size and duration. However, it is difficult at this point to conclude that high DCAD under the HTa condition increases DMI in crossbred dairy goats as in previous reports in dairy cattle (Tucker et al., 1988; West et al., 1991; West et al., 1992; Delaquis and Block, 1995a). This is because there was no significant difference in daily DMI throughout the 8 weeks of the experimental period. In addition, the effect of high DCAD on eating and meal patterns from the present study extended the behavioral phenomenon of the DCAD effect on

DMI. Similar to other mammals, ruminants use their basic taste perceptions for selecting suitable diet (Ginane et al., 2011). Behavioral responses of a tasty diet, as pre-absorptive signals, have been demonstrated in laboratory rodents as well as in humans (Berridge, 2003). The analysis of eating behavior across the dietary shift revealed that rats fed a high fat and carbohydrate diet increased their eating and meal patterns within the first week (Treesukosol, 2014). An increase in meal patterns has been demonstrated within the similar period as well in crossbred dairy goats fed with fat supplemented ration (Thammacharoen et al., 2017). Although the present results could not determine whether the pre-absorptive or the post-absorptive signals was the crucial mechanisms behind the effect of high DCAD on eating patterns (Smith, 1996; Ginane et al., 2011), the effect of high DCAD on eating pattern from the current study likely came from the post-absorptive signals (satiation hormones and plasma metabolites) rather than pre-absorptive signals or palatability effect. This is because the basic taste perception effect of pre-absorptive signals could be demonstrated at the early phase of dietary shift (Treesukosol, 2014; Thammacharoen et al., 2017) and when a DCAD diet was provided from 2nd to 8th weeks of postpartum the significant increase in meal pattern was detected at PP-8, but not at PP-4.

In the current study, the patterns of WI and UV from both groups were different depending on the time of the day: day (high THI) and night (moderate THI). Animals fed high DCAD drank more water throughout the night-time, but the urine volume from high DCAD group was not greater than the control group. The results suggested that water retention in the DCAD group was higher than the control group during night-time. The total water consumption values from the control and DCAD groups reported for this study were 4.6 and 6.8 L/day (average 5.7 L/day or 16% BW). This finding was in accordance with previous reports of goats fed under HTa conditions (9.7 L/day or 22% BW, Hamzaoui et al. (2013) and was much greater than the level reported for goats fed under temperate zone, approximately 1-2.5 L/day, 3.0-5.5% BW (Rossi et al., 1999; Kokkonen et al., 2001). Further, analysis of the day- and night-time WI ratios from both groups revealed that the ratio was approximately 9:1, however, it was approximately 2:1 when goats were fed under temperate zone conditions (Rossi et al.,

1999) Greater water conservation would be useful for heat dissipation mechanisms during HTa (Chaiyabutr et al., 2008). The higher total WI in response to HTa and the shift in day- and night-time WI ratio suggested that goats fed with high DCAD could improve water retention by modifying night time WI to support heat dissipation capacity.

In conclusion, the results from the study of Chapter IV suggest that dairy goats fed high DCAD rations used addition behavioral strategies to ameliorate heat stress. This adjustment is considerable as an adaptive reaction to support evaporative heat dissipation under HTa conditions. Reduction of the negative effects of heat stress may in part contribute to an increase in eating and meal pattern. Taken together, the results suggested the potential positive effect of a high DCAD formulation, which may be selected as a choice of dietary strategy for ruminants fed under prolonged HTa condition.

CHAPTER V

DIETARY CATION AND ANION DIFFERENCE: EFFECTS ON FEED INTAKE, RUMINAL

FUNCTION AND PLASMA LEPTIN IN LACTATING DAIRY GOATS

UNDER TROPICAL CONDITION

INTRODUCTION

Heat production is directly regulated by the endocrine and nervous systems via modifications of appetite, digestive process and indirectly controlled by changes of the activity of respiratory enzymes and protein synthesis (Yousef, 1985). Dairy animals under HTa condition decreased feed intake (Sano et al., 1985; Rhoads et al., 2009; Salama et al., 2014) and altered the ruminal function such as lower ruminal pH, microbial activities and fermentation characteristics (Nonaka et al., 2008). In addition, previous studies reported that plasma leptin concentration increased in heat stress dairy cows (Bernabucci et al., 2006) and heat stress dairy goats (Al-Dawood, 2017). In ruminant, leptin as adiposity hormone has been shown to involve in satiation (Thammacharoen et al., 2014). Hence, heat stress dairy animal reduced feed intake as protective mechanism against hyperthermia which alters either ruminal function or plasma leptin content.

It has been known that K and Na requirement increased during hot weather and dry matter intake increased when these minerals were supplemented for lactating cows (Schneider et al., 1986; West et al., 1987). In addition, West et al. (1992) found that heat stress cows increased DMI when dietary cation and anion difference (DCAD) level increased from 12 to 46 mEq/100 g DM. Moreover, Delaquis and Block (1995a) observed that DMI increased when dairy cattle fed DCAD from +5.55 to +25.81 mEq/100 g of DM during early lactation or +14.02 to +37.27 mEq/100 g of DM during mid-lactation, but these effects did not occur in late lactation. However, other studies found that DMI did not differ among treatments when DCAD increased in dairy calves (Jackson et al., 1992) and dairy cows (Wildman et al., 2007b). In general, the different responses in DMI in the previous experiments depend on levels of DCAD in diets, stage of lactation and ingredients of diet (Sharif et

al., 2010a). In addition, Hu and Murphy (2004) suggested that DMI peaked at DCAD of 34 to 40 mEq/100 g DM. In ruminant, the effects of DCAD diet on DMI apparently came in part from the changes in ruminal pH, the ruminal microbial activities and the fermentation products (Tucker et al., 1988; Wildman et al., 2007b; Eriksson and Rustas, 2014). However, the mechanisms underlying the effects of high DCAD on DMI in lactating dairy goats under tropical conditions did not fully understand. It is possible that the mechanisms of high DCAD diet on DMI in lactating goats may mediate through either the alterations in ruminal function or plasma leptin level. Therefore, the aims of present study are to investigate the effect of high DCAD on DMI, ruminal function and plasma leptin in lactating dairy goats under tropical conditions.

MATERIALS AND METHODS

5.1.1 Animals and management

The details of the experimental design and animal management were described in Chapter III. Briefly, ten crossbred Saanen goats that were in late stages of pregnancy (3-4 years old) and with an average body weight (BW) 34.48 ± 1.42 kg were selected and used in this experiment. A week after parturition (PP-1), animals were divided into two groups based on body weight, with five animals in each group and lasted for 7 weeks (2nd – 8th week postpartum, PP-2 to PP-8). They were offered two experimental rations of either control DCAD (control, 22.81 mEq/100 g DM) or high DCAD (DCAD, 39.08 mEq/100 g DM). The ingredients and chemical compositions of the experimental diets are presented in Table 3.1 and Table 4.1 (Chapter III and IV). Dairy goats received TMR, twice daily at 07:00 h and 14:00 h with free access to water and feed. After parturition, all of the goats were weighed before morning feeding, once per week throughout the experiment. The average temperatures during the experiment at 07:00 h, 13:00 h and 19:00 h were 27.64±1.45 $^{\circ}$C, 35.28±0.50 $^{\circ}$C and 30.35±0.26 $^{\circ}$C, respectively. The percentages of relative humidity at 07:00 h, 13:00 h and 19:00 h were 71.29±2.92 %, 50.60±1.06 % and 65.37±0.41 %, respectively. The temperature and humidity index (THI) was calculated according to

NRC (1971)'s formula from above information as 78.12±2.13, 85.48±0.47 and 81.33±0.39, respectively.

5.1.2 Data collection and analysis

Feed intake, water intake were recorded daily and then calculated for morning (from 07:00 h to 13:00 h), afternoon (from 13:00 h to 19:00 h), night-time (from 19:00 h to 06:00 h) and whole day (23 h). In addition, feed intake and water intake were also measured once per week throughout the experiment and the measurements were recorded two-hour intervals from 07:00 h to 19:00 h. Chemical composition of experimental diets was analyzed for DM, OM, Ash, CP, NDF, ADF, Na, K, Cl and SO_4^{2-} contents. The details of protocol were described in Chapter III.

5.1.3 Rumen collection and volatile fatty acids analysis

Rumen fluid samples were collected from dairy goats at PP-4 and PP-8 by using a stomach tube connected to a syringe. Approximately 20 ml of fluid samples were taken at 2.5 hours (09:30 h) after morning feeding. The pH was immediately determined by using pH meter (pH221, Lutron, Taipei, Taiwan). After that, the ruminal fluid samples were filtered through two layers of cheese-cloth and added with 1 ml 6N HCl for preservation. Then, samples were frozen at -20^{0}C until analysis of volatile fatty acids (VFAs) and NH_3-N. VFAs were prepared and analyzed as described by Thammacharoen et al. (2001). NH_3-N was determined by a Salicylate-hypochlorite method (Bower and Holm-Hansen, 1980).

5.1.4 Apparent digestibility, urine collection and analysis

The digestibility was measured using total fecal collection technique. Each goat was daily collected and mixed the feces at PP-8. The subsamples from each animal was kept under -20 ^{0}C until chemical analysis. Chemical composition of fecal samples was analyzed for DM, OM, CP, NDF, ADF contents. The details of protocol were described in Chapter III.

Total urine was collected and measured in the same week as fecal collection by using plastic containers with amount 10% sulfuric acid solution added to prevent nitrogen loss (15 ml H_2SO_4 10% in 90 ml urine and final pH of urine was kept below

3). The total urine from each day was then collected at 30 ml, kept under -20 ^{0}C and pooled at the end of experiment to be analyzed for nitrogen by the Kjeldahl method (AOAC, 1990) and for allantoin excretion by colorimetric method according to Chen and Gomes (1992). Nitrogen retention was calculated from difference between nitrogen input (feed intake) and nitrogen output (total nitrogen in feces, urine, and milk).

5.1.5 Determination of plasma leptin concentration and blood glucose

On day 27 and day 55 postpartum, blood samples were collected at 16:00 h. The blood glucose was measured immediately using glucometer (Accu-Chek Adv II, Roche diagnostic GmbH, Manheim, Germany). The samples are obtained from the jugular vein, placed in lithium heparin tube, kept in crushed ice and then centrifuged at 3,000 rpm for 10 minutes. The plasma samples were stored at -20 ^{0}C until analysis. Plasma leptin concentration was determined using an Enzyme-Linked ImmunoSorbent Assay (ELISA) kit specific for goat leptin hormone (MBS018743, MyBioSource, San Diego, USA).

5.1.6 Statistical analysis

The data were presented as the mean ± SEM. The data for plasma leptin were analyzed with the repeated measures two-way analysis of variance (ANOVA). Significance of main effects was performed by Bonferroni t-test. The data for feed intake, water intake, ruminal parameters, nutrient digestibility, allantoin excretion and nitrogen balance were averaged and compared using an unpaired t-test. Statistical significance was declared at $P<0.05$.

RESULTS

5.2.1 Dry matter intake and water intake

There was no significant difference in dry matter intake per body weight (DMI/BW) between groups during morning and afternoon feeding, night time throughout experiment (P>0.05, Fig. 5.1). However, dairy goats in DCAD group tended to consume more total daily DMI/BW throughout experiment (P=0.08, Fig. 5.1) and significant higher at PP-8 (P<0.05; Table 5.1). Significantly higher in DMI/BW at PP-8 from high DCAD group was found during afternoon feeding (P<0.05, Table 5.1) and tended to increase in DMI/BW during night-time (P=0.08, Table 5.1). Two-hour intervals of DMI/BW were unaffected by DCAD during day time (P>0.05, Fig. 5.2). Similarly, body weight and blood glucose did not differ between groups (P>0.05, Table 5.1).

There was an effect of DCAD supplementation on 24 h and night time water intake, animals in DCAD group drank more water than in control group (P<0.05, Fig. 5.3). But water intake during morning and afternoon feeding was similar between groups (P>0.05, Fig. 5.3). In contrast, two-hour intervals of water intake were affected by DCAD during day time (from 07:00 h to 19:00 h). Significantly higher water intake in animals from DCAD group than those from control was found from 09:00 h to 11:00 h (P<0.01, Fig. 5.4) and tended to increase from 15:00 h to 17:00 h (P=0.06) and from 17:00 h to 19:00 h (P=0.07). Other time intervals were similar between groups (P>0.05, Fig. 5.4)

5.2.2 Ruminal fermentation characteristics and total apparent digestibility

Ruminal fermentation characteristics

Daily average NH_3-N concentration was not affected by DCAD (P>0.05, Table 5.2). The dairy goats in the DCAD group had higher ruminal pH than those in the control group (P<0.05). Total VFA concentration, propionate molar proportion and average ratio of acetate to propionate were not affected by DCAD supplementation (P>0.05, Table 5.2). In contrast, acetate molar proportion was greater and butyrate molar proportion was lower than in DCAD group (P<0.05).

Total apparent digestibility

The apparent nutrient digestibility had higher from the DCAD group than from the control group (P<0.05, Table 5.1). The mean values of apparent digestibility (%) for control versus (vs.) DCAD groups were 74.40 ± 0.90 vs. 78.75 ± 0.83 for DM, 68.05 ± 1.48 vs. 74.86 ± 1.26 for OM, 73.03 ± 1.24 vs. 78.08 ± 1.47 for CP, 59.15 ± 2.23 vs. 67.85 ± 1.44 for NDF and 48.62 ± 4.05 vs. 59.24 ± 1.79 for ADF, respectively.

5.2.3 Urinary allantoin excretion and nitrogen balance

Urine output and urinary allantoin excretion were greater from DCAD group than from control group (P<0.05, Table 5.3). However, there were no significant differences in nitrogen intake and excretion in urine, feces and milk (P>0.05). As a result, nitrogen balance was similar between groups (P>0.05, Table 5.3).

5.2.4 Plasma leptin concentration

There was no interaction effect between week and high DCAD supplementation on plasma leptin concentration (P>0.05). However, there was an effect of DCAD on plasma leptin level throughout the experiment. Dairy goats in DCAD group had significantly higher plasma leptin concentration than those in control group (P<0.05, Fig. 5.5). In addition, when plasma leptin content was analyzed by weeks the concentration of plasma leptin in DCAD group was significantly greater than in control at PP-4 (P<0.05), but not for PP-8.

Table 5.1 Effect of dietary cation and anion difference on dry matter intake and apparent digestibility at PP-8 in dairy goats

Item	Treatment		P
	Control	DCAD	
Initial BW, kg	34.60±1.69	34.36±2.48	0.94
Final BW, kg	35.18±1.44	34.14±2.26	0.71
Blood glucose, mg/dL	77.00±4.00	79.00±2.25	0.68
Dry matter intake[1], g/kg BW			
24 h	32.76±1.85	38.02±0.8	0.03
Morning	15.53±0.91	14.52±1.19	0.52
Afternoon	15.41±1.31	19.48±0.66	0.02
Night-time	1.79±0.36	4.22±1.17	0.08
Apparent digestibility, %			
Dry matter	74.40±0.90	78.75±0.83	0.001
Organic matter	68.05±1.48	74.86±1.26	0.001
Crude protein	73.03±1.24	78.08±1.47	0.02
Neutral detergent fiber	59.15±2.23	67.85±1.44	0.01
Acid detergent fiber	48.62±4.05	59.24±1.79	0.02

Treatment: Control = 22.81 mEq/ 100g DM; DCAD = 39.08 mEq/ 100g DM

[1]: 24 h from 07:00 h to 06:00 h; morning from 07:00 h to 13:00 h; afternoon from 13:00 h to 19:00 h; night time from 19:00 h to 06:00 h.

Table 5.2 Effect of dietary cation and anion difference on ruminal fermentation characteristics in dairy goats

Item	Treatment		P
	Control	DCAD	
Ruminal fluid pH	6.54±0.05	6.68±0.01	0.01
NH$_3$-N, mg/dL	18.10±2.35	17.03±1.84	0.71
Total VFA, mmol/L	64.25±4.85	63.97±2.33	0.96
VFA, mol/100 mol			
Acetate	54.81±1.05	59.25±1.54	0.04
Propionate	34.85±1.25	33.00±1.34	0.40
Butyrate	10.34±0.55	7.75±0.45	0.01
Acetate: propionate ratio	1.58±0.08	1.82±0.15	0.22
Osmolality (mOsm/kg)	241.20±25.89	223.20±11.80	0.48

Treatment: Control = 22.81 mEq/ 100g DM; DCAD = 39.08 mEq/ 100g DM

Table 5.3 Effect of dietary cation and anion difference on urinary allantoin excretion and nitrogen balance at PP-8 in dairy goats

Items	Treatment		P
	Control	DCAD	
Urine, kg/d	2.04±0.52	3.95±0.30	0.013
Allantoin excretion, mg/BW/day	13.41±2.65	22.75±3.01	0.05
Nitrogen intake, g/d	30.86±2.60	34.89±1.92	0.25
Nitrogen excretion			
Urine, g/d	8.73±0.76	9.78±0.76	0.34
Feces N, g/d	6.25±0.51	5.92±0.47	0.65
Milk, g/d	6.48±0.98	6.78±0.85	0.82
Nitrogen balance, g/d	9.40±1.72	12.39±1.24	0.20

Treatment: Control = 22.81 mEq/ 100g DM; DCAD = 39.08 mEq/ 100g DM
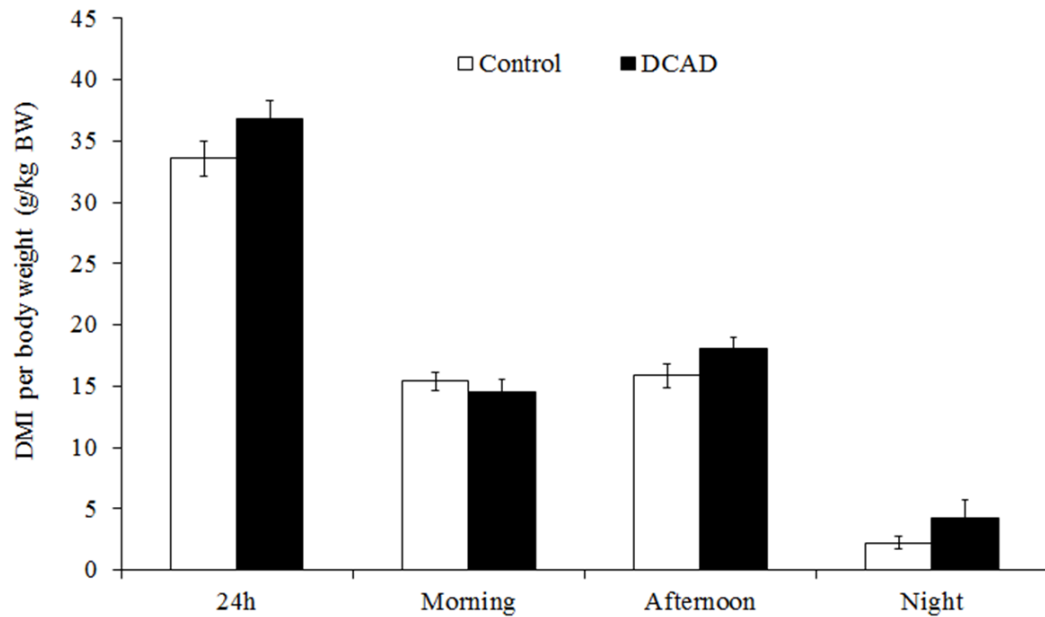
Figure 5.1 Effect of dietary cation and anion difference on total dry matter intake per body weight; morning from 07:00 h to 13:00 h; afternoon from 13:00 h to 19:00 h; night from 19:00 to 07:00 h. Treatment: Control = 22.81 mEq/ 100g DM; DCAD = 39.08 mEq/ 100g DM.
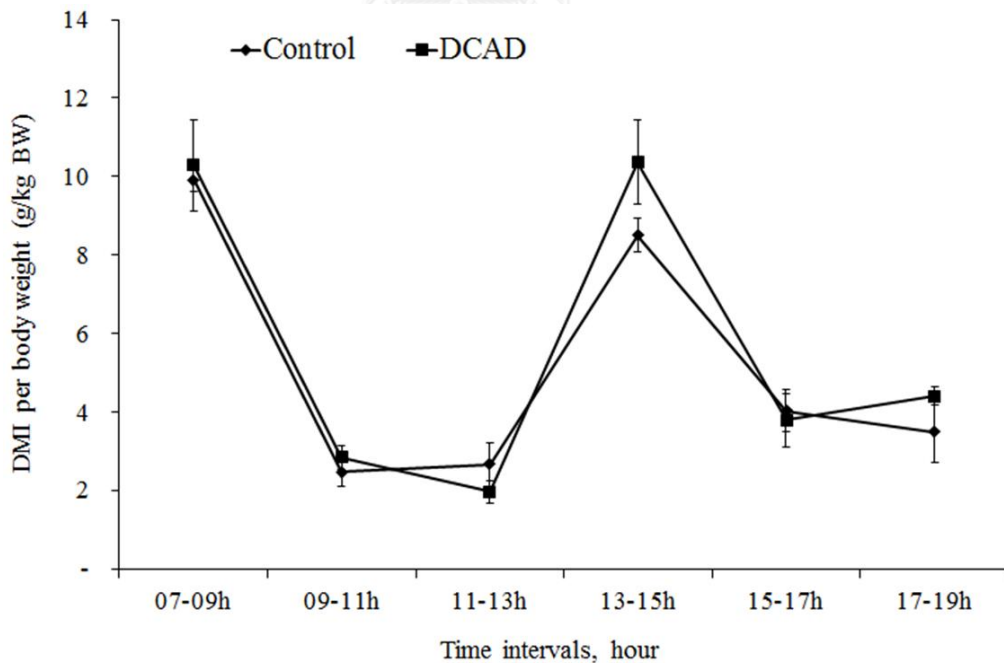
Figure 5.2 Effect of dietary cation and anion difference on dry matter intake per body weight at two hour - intervals from 07:00 h to 19:00 h. Treatment: Control = 22.81 mEq/ 100g DM; DCAD = 39.08 mEq/ 100g DM.
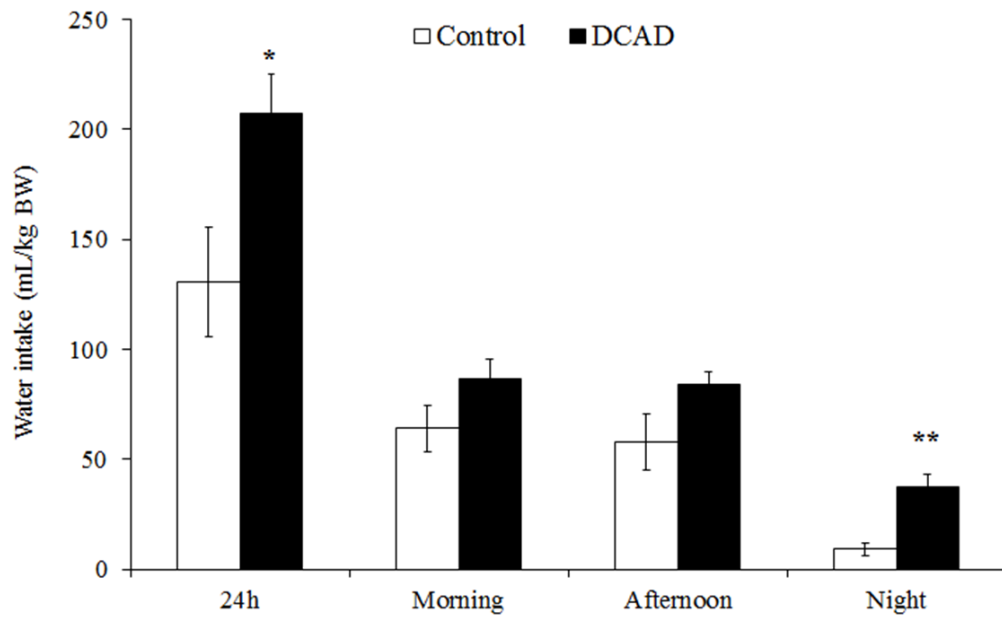
Figure 5.3 Effect of dietary cation and anion difference on daily water intake; morning from 07:00 to 13:00 h; afternoon from 13:00 to 19:00 h; night from 19:00 to 07:00 h. Treatment: Control = 22.81 mEq/ 100g DM; DCAD = 39.08 mEq/ 100g DM. *: $P<0.05$; **: $P<0.01$.
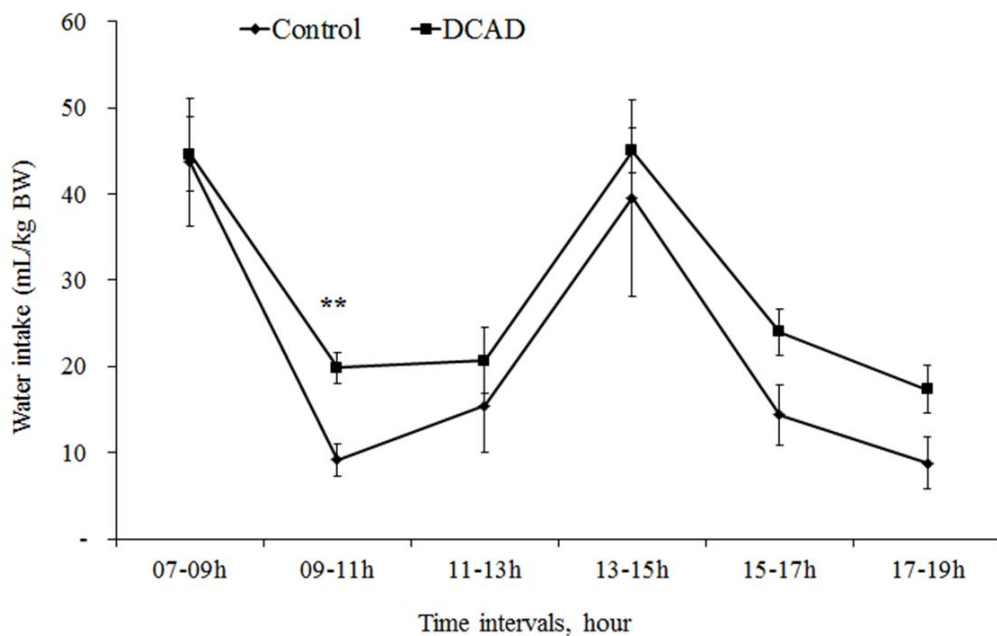
Figure 5.4 Effect of dietary cation and anion difference on water intake at two hour - intervals from 07:00 h to 19:00 h. Treatment: Control = 22.81 mEq/ 100g DM; DCAD = 39.08 mEq/ 100g DM. **: $P<0.01$.
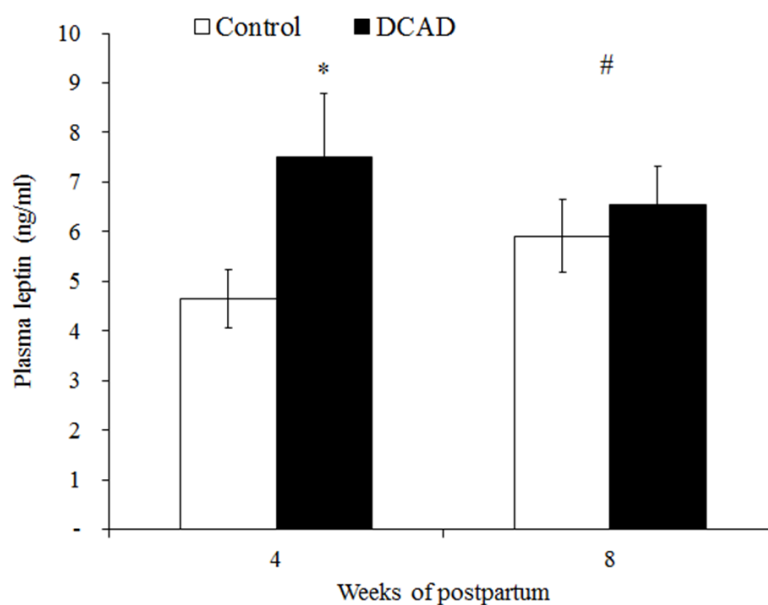
Figure 5.5 Effect of dietary cation and anion difference on plasma leptin concentration in lactating dairy goats at week 4th and 8th of postpartum. Treatment: Control = 22.81 mEq/ 100g DM; DCAD = 39.08 mEq/ 100g DM. *: Significant effect between treatments at 4th week (unpaired t-test). #: Significance of main effects by DCAD diets (repeated measures two-ways ANOVA).

DISCUSSION

The data in this study revealed that high DCAD were positive effects on water intake, ruminal function, microbial protein synthesis and nutrient digestibility. However, dairy goats fed with high DCAD tended to increase DMI/BW. The effects of DCAD on DMI/BW may be independent from the action of leptin in the current study.

In this study, there was no effect of DCAD on DMI/BW during morning, afternoon and night time or two-hour intervals during day time throughout the experiment. However, total daily DMI/BW tended to increase in DCAD group and significantly increased at PP-8 when DCAD increased. The increase in DMI/BW could be due to the improvement of ruminal pH, higher in microorganism activities via increasing of urinary allantoin excretion and nutrient digestibility, rather than by changing in plasma leptin concentration. Significantly higher total daily DMI/BW in DCAD group at PP-8 mainly contributed from afternoon feeding (P<0.05, Table 5.1) and partly at night-time (P=0.08, Table 5.1), but not for morning feeding in the present study. Previous study suggested that animal could maintain an osmotic pressure balance of body fluids either increasing the water consumption and the rumen influx of water

from plasma or decreasing feed intake (Langhans and Scharrer, 1995). In addition, low ruminal pH may also be associated with increased osmolality of the ruminal contents, which in turn inhibits feed intake (Carter and Grovum, 1990). Under present experimental condition, dairy goats in control group drank less water and consumed less bicarbonate than those in DCAD at night-time (P<0.05), thus the animals from control group had to maintain osmolality by reducing feed intake. In contrast, ruminal osmolality at 2.5 hour after morning feeding (09:30 h) were similar between groups because of higher water intake from DCAD group at 09:00 h to 11:00 h in this study. As a result DMI/BW was not affected by DCAD during this time. Several experiments suggested that DCAD supplementation for ruminant increased DMI as increasing DCAD levels (West et al., 1992; Delaquis and Block, 1995a; Hu and Murphy, 2004). However, Jackson et al. (1992) found that DMI did not differ between groups when dairy calves fed DCAD from 0 to +52 mEq/100 g of DM. Similar findings were also observed by (Wildman et al., 2007b). The decrease of DMI occurred when animal fed with low or negative DCAD levels in diet (Tucker et al., 1992; Spanghero, 2004). This reduction in DMI may be either poor palatability of anionic salts (Goff et al., 1991) or low DCAD induced slight metabolic acidosis (Block, 1994).

Water consumption was affected by DCAD supplementation. Dairy goats in DCAD group increased water intake compared with those in control group. This finding was similar with previous studies (Silanikove et al., 1998; Apper-Bossard et al., 2006). In contrast, some studies found that high DCAD did not affect on water intake due to unchanged DMI (Delaquis and Block, 1995b). The present study observed that the effect of DCAD on increasing water intake came from night time, during 09:00 h to 11:00 h and from 15:00 h to 19:00 h. But other time intervals did not differ between groups. According to Murphy (1992) indicated that the amounts of water intake positively correlated with the quantity of feed ingest. In addition, about 80% of water intake happens around meals in monogastric and ruminants (Forbes and Barrio, 1992; Rossi and Scharrer, 1992). However the data from current study revealed that animal in both groups drank from 44 to 61% of water during two-hour of main

meals (from 07:00 h to 09:00 h for morning feeding and 13:00 h to 15:00 h for afternoon feeding).

A part of DCAD effect on dairy animals may result from alteration of ruminal fermentation. Under present experiment, ruminal pH at 2.5 h after morning feeding increased with regard to DCAD increase. Higher ruminal pH could cause either from increasing water intake and solid passage rates (Rogers, 1982) or from neutralizing hydrogen ion from rumen (Kohn, 1998). In this study, water intake from DCAD group was greater than from control during 09:00 h to 11:00 h (P<0.01, Fig. 5.4). Moreover in order to increase DCAD in this study, sodium bicarbonate and potassium carbonate were added more in high DCAD than in control. Thus they can contribute to bicarbonate for the rumen buffer, and consequently increased ruminal pH. The concentrations of acetate and butyrate were affected by DCAD supplementation. Dairy goats fed with high DCAD had higher acetate level in rumen than those fed with control. This result happened in response to higher allantoin excretion as indicator of microorganism activities, greater fiber digestion in the current study. However butyrate molar proportion from DCAD was lower than in control though the reason was unclear. The levels of NH_3-N, total VFA, propionate molar proportion and average ratio of acetate to propionate were similar between groups. The effect of DCAD on concentrations of VFA, acetate, propionate and butyrate in this study may relate to the changes in water intake (dilution), rates of liquid passage from the rumen. Thus if we consider only on ruminal concentrations without accounting for those factors, these results could be in different and incorrect interpretations. The results from ruminal function in this study were consistent with Fraley et al. (2015), who reported that dairy cow fed with DCAD of 16 to 53.5 mEq/ 100 g DM increased ruminal pH, acetate concentration and tended to decrease butyrate level. In addition, Tucker et al. (1988) observed that high DCAD improved ruminal pH and unchanged fermentation patterns in dairy cows. Similarly, high DCAD in dairy cows increased ruminal pH and could not affect on ruminal acetate and propionate (Roche et al., 2005). In contrast, Sharif et al. (2010b) observed that ruminal pH, ruminal ammonia nitrogen, acetate and acetate: propionate ratio were greater in

buffalo bulls fed with medium and high DCAD (220 and 330 mEq/kg DM, respectively) than those fed anion diets or low DCAD (110 mEq/kg DM). The present results suggested that high DCAD improved ruminal characteristics and increased microbial protein synthesis in lactating goats.

Total apparent digestibility of DM, OM, CP, NDF and ADF increased in response to DCAD increase in the current experiment. Higher fiber digestibility in DCAD group could cause from higher pH, followed by higher cellulolytic bacteria activity in ruminal fluid, which can be indicated by an increase in acetate molar proportion and allantoin excretion in this study. The effects of varying DCAD on nutrient digestibility were reported by (Delaquis and Block, 1995a; Delaquis and Block, 1995b). They found that high DCAD did not differ on ADF and NDF digestibility in dry and lactating cows, but DM digestibility was slightly higher in lactating cows. Stokes and Bull (1986) indicated that supplement with sodium bicarbonate as cationic salt for high DCAD have improved DM, OM, ADF digestibility when dairy cows fed corn silage as based diet and not for alfalfa hay. Similarly, Eriksson and Rustas (2014) reported that DM, OM, NDF digestibility increased as $KHCO_3$ addition increase in dairy cows. These findings were similar the present experiment when dairy goats fed with corn silage as roughage of diets and increased nutrient digestibility as DCAD increase.

Dairy goats in high DCAD group were higher urinary allantoin excretion than those in control group. Higher allantoin excretion could be from increase of ruminal pH in this study or from increase of water consumption, consequently, an increase in rumen liquid fractional passage rate and in microbial growth rate as reported by Dijkstra et al. (1998). Dairy cows fed with high DCAD in low crude protein (CP) diets (15%) were higher the microbial protein synthesis than low DCAD (Wildman et al., 2007a). Similarly, Eriksson and Rustas (2014) reported that dairy cows in mid-lactation fed with high potassium bicarbonate (cationic salt) were higher allantoin excretion than those fed with low potassium bicarbonate. Moreover, (Hu et al., 2007a) found that plasma branched chain amino acids (AA) and essential AA: total AA ratio were higher in dairy cows fed with DCAD of 47 mEq/100g DM during early lactation than those fed with DCAD of 22 mEq/100g DM and indicated that more microbial protein

flowing to the small intestine. These results suggest that high DCAD improves microbial protein synthesis, but the effect of DCAD supplementation on microbial protein synthesis varies to levels of DCAD.

Nitrogen intake and excretion in urine, milk and feces did not differ between treatments. As a result, nitrogen balance was not affected by DCAD. Similar findings were found by (Delaquis and Block, 1995a) when dairy cows fed with high DCAD during early and late lactation, but significantly higher during mid-lactation. However, some studies reported that nitrogen intake and retention were higher in buffalo fed with medium and high DCAD than in those fed with low or negative DCAD (Sharif et al., 2010b). The positive N balance in this study is in line with the N balance reported by Ho (2015) and Nguyen (2011) in dairy goats during early lactating period.

Throughout experiment plasma leptin concentration significantly increased in DCAD group. In principle, leptin acts on hypothalamic neurons to inhibit food intake and increase energy expenditure, leading to decrease body weight (Campfield LA, 1995; Halaas JL, 1995). However, the current result is not explained by confounding factors. Firstly, DMI/BW tended to increase in DCAD group. Secondly, animal in both groups were similar in body weight throughout the study. Thirdly, plasma glucose did not differ between groups (P>0.05, Table 5.1). Under HTa animal can maintain normal blood pH, but blood HCO_3^- concentration was lower normal. This can represent compensated metabolic acidosis in heat stress animals (Sanchez et al., 1994). Dairy animals fed with high DCAD increased blood pH and HCO_3^- concentration (Tucker et al., 1988; West et al., 1991) and may partly compensate the effect of HCO_3^- loss via urine under HTa. The DCAD effect on hyperleptinemia may cause either from acid base status or alkaline diets. Previous studies have demonstrated that leptin secretion was affected by acidosis in animal and human. Uraemic rats supplementation with sodium bicarbonate tended to increase serum leptin level and unchanged food intake (Teta et al., 1999). However, acidosis decreased leptin secretion in cultured adipocytes (Teta et al., 2003). In human, fish rich diets induce acidosis was lower serum leptin concentration than in vegetarian diets (Winnicki et al., 2002). Moreover, the effect of DCAD on plasma leptin level may relate to the

glucose utilization in muscle due to increase of panting (movement of muscle). Previous studies observed that injection of leptin into medial hypothalamus stimulated glucose uptake by skeletal muscle, brown adipose tissue and heart but not in white adipose tissue (Kamohara et al., 1997; Minokoshi et al., 2012) and leptin plays important roles on glucose metabolism, independent of actions on food intake and body weight (Berglund et al., 2012). Similarly, Chanchai (2010) reported that plasma leptin, respiration rate and rectal temperature from non-cooling dairy cattle were higher than in cooling dairy cattle during late lactation, but DMI was similar in both groups. In contrast, non-cooling dairy cattle during mid lactation decreased DMI due to greater plasma leptin level, respiration rate and rectal temperature. These results suggested that effect of high DCAD on leptin secretion in the current study may relate to acid-base chemistry and glucose utilization during panting. An increase in plasma leptin concentration is normally related to weight loss. Thus, lower plasma leptin content from control group may adaptively ameliorate the effects of compensated metabolic acidosis under HTa.

Here, we provide the evidence that dairy goats fed high DCAD under HTa increase plasma leptin concentration and are independent the action from feed intake. In addition, the results from present study indicated that dairy goats fed high DCAD tended to increase DMI. Higher DMI may be in part from effects of high DCAD on increasing ruminal pH, microbial activity via urinary allantoin excretion, and nutrition digestibility. The improvement of rumen function from dairy goats fed high DCAD would help to decrease negative effects on rumen health under HTa conditions.

CHAPTER VI

DIETARY CATION AND ANION DIFFERENCE: EFFECTS ON MILK PRODUCTION AND BODY FLUID DISTRIBUTION IN LACTATING DAIRY GOATS UNDER TROPICAL CONDITION

INTRODUCTION

In Thailand, dairy animals are confronted with prolonged HTa. Goats are considered more tolerant to HTa conditions compared with dairy cows (Silanikove, 2000). Dairy goats and cattle under HTa similarly reduced feed intake (22 – 35%), but reduction of milk yield in dairy goats (3 – 13%) was much lower than (27 – 33%) in dairy cattle (Sano et al., 1985; Rhoads et al., 2009; Salama et al., 2014). Heat accumulation and production in dairy animals are one of the major causes that decrease the production of milk. The reduction of milk yield under HTa conditions may be in part due to lower in feed intake and increase the demand for maintenance of body temperature (Rhoads et al., 2009). Because of the reduction of feed intake during HTa conditions, intake of mineral elements is also decreased. Therefore, the increasing elemental levels in diet were strategy to meet mineral element requirements during HTa (Sanchez et al., 1994).

Increasing dietary cation and anion difference (DCAD) by addition of cations such as sodium (Na^+) and potassium (K^+) has positive effect on milk production when lactating dairy cows fed with high DCAD from 34 to 40 mEq/100 g DM (Hu and Murphy, 2004). Some studies reported that dairy cows fed with high DCAD increased (Tucker, et al., 1988; Apper-Bossard, et al., 2006) or unchanged milk production (Roche et al., 2003; Wu et al., 2008). However, the mechanisms underlying the effects of high DCAD on milk production do not fully understand when dairy animals supplement with high DCAD during HTa conditions. It has been known that milk production is affected by both internal and external factors e.g. substrates utilization by mammary gland, mammary blood flow, food and water intake, and environmental temperature. Body fluid homeostasis was the fundamental internal factor for milk production (Davies and Collier, 1985). Cowley and Roman (1989)

reported that total volume and compartmental distribution of body fluids depended on daily variations in dietary salt and water intake. Normally, goats under HTa increased water intake and minimized water loss in urine and feces (Silanikove, 2000). Alamer (2011) reported that goat and sheep during summer season consumed more water and higher total body water than those during winter season. Cooled dairy cattle by misty fan increased in extracellular fluid, blood and plasma volume in association with a light increase in DMI which partitions the distribution of nutrients to the mammary gland for milk synthesis (Chaiyabutr et al., 2008). High DCAD as addition more cationic salts increased water intake and urinary excretion in lactating cows as reported by previous studies (Delaquis et al., 1995; Khelil-Arfa, et al., 2014). As a result, high DCAD supplementation is expected that alters body fluid compartments via changes in water balance. Thus the current study aimed to investigate the effect of high DCAD on milk production in relation to water balance, body fluid distribution and plasma ADH concentration in lactating dairy goats under tropical conditions.

MATERIALS AND METHODS

6.1.1 Experimental design and animal care

The details of experimental design and animal care were described in Chapter III. Briefly, ten crossbred Saanen goats that were in the late stages of pregnancy (3-4 years old) and with an average body weight (BW) of 34.48 ± 1.42 kg were selected and used in this experiment. A week after parturition (PP-1), animals were divided into two groups based on body weight, with five animals in each group. They were offered two experimental rations of either control DCAD (control, 22.81 mEq/100 g DM) or high DCAD (DCAD, 39.08 mEq/100 g DM). The ingredients and chemical compositions of the experimental diets are presented in Table 3.1 and 4.1 (Chapter III and IV). Dairy goats received TMR, twice daily at 07:00 h and 14:00 h with free access to water and feed. Animals were milked twice a day at 06:00 h and 13:00 h by hand milking.

The average temperatures during the experiment at 07:00 h, 13:00 h and 19:00 h were 27.64±1.45 $^{\circ}$C, 35.28±0.50 $^{\circ}$C and 30.35±0.26 $^{\circ}$C, respectively. The percentages of relative humidity at 07:00 h, 13:00 h and 19:00 h were 71.29±2.92 %, 50.60±1.06 % and 65.37±0.41 %, respectively. The temperature and humidity index (THI) was calculated according to (NRC, 1971)'s formula from above information as 78.12±2.13, 85.48±0.47 and 81.33±0.39, respectively.

6.1.2 Data collection and analysis

Feed intake, water intake and milk yield were recorded daily after parturition until PP-8. In addition, water intake and urinary excretion were also measured once per week throughout the experiment and the measurements were recorded every 2 h during day time and 24 h; on PP-8 the feces from each goat was separately collected from urine by using the metabolic cage. Following on, apparent water balance was calculated using the difference between water input (ingested water, feed water) and water output (milk, urinary excretion, feces) throughout the experiment, without taking account of the water in metabolism (Chaiyabutr et al., 1980). In addition, the difference between water intake and urinary excretion (Diff WI-Uex) from specific time points was calculated using the following formula: Diff WI-Uex = Water intake – Urinary excretion. Urinary pH was determined by using pH meter (pH221, Lutron, Taipei, Taiwan).

Rectal temperature (Tr) and respiration rate (RR) were determined one per week and measurements were collected at two-hour intervals during the day time (from 09:00 to 19:00 h). Then the percentage changes in either rectal temperature or respiration rate were calculated using the difference between 09:00 h and other time points of day time (11:00 h, 13:00 h, 15:00 h, 17:00 h, 19:00 h). Rectal temperature was determined by a digital clinical thermometer (digital clinical thermometer C202, Terumo, Tokyo, Japan). Respiration rate was measured by counting the movement of the flank within one minute.

Chemical composition of experimental diets was analyzed for DM, OM, Ash, CP, NDF, ADF, Na, K, Cl and SO_4^{2-} contents. The details of protocol were described in Chapter III.

6.1.3 Determination of milk composition, plasma electrolytes, osmolality and plasma ADH

Milk samples from each goat were collected daily at PP-8 and kept at – 20 $^{\circ}$C until analysis. Milk compositions were analyzed for total solids, solid no fat, fat, lactose and protein by using Milkoscan (FT2; Foss, Hilleroed, Denmark).

4% fat corrected milk yield (4% FCM) for goat and sheep was calculated according to Mavrogenis and Papachristoforou (1988) as following formula:

4% FCM = M (0.411 + 0.147 f).

Where: M: milk yield (kg/day) and f: milk fat composition (%)

On PP-4 and PP-8, four milliliter blood samples were collected at 09:00 h and 16:00 h from the jugular vein, placed in a lithium heparin tube, kept in crushed ice and then centrifuged at 3,000 rpm for 10 minutes. The plasma samples were stored at -20 $^{\circ}$C until analysis. Sodium and potassium were measured by flame photometer (Flame photometer 410C, Ciba Corning Inc., USA). Chloride was determined by chlorimeter (Chloride 925, Ciba Corning Inc., USA). Plasma cation and anion difference (CAD) was calculated using the equation mEq (Na^+ + K^+ - Cl^-)/L. Osmolality was measured by osmometer (Osmometer 3D3, Advance Instrument Inc., USA). Plasma ADH concentration at 16:00 h was determined by ELISA kit specific for goat and employed the quantitative sandwich enzyme immunoassay technique (MBS006109, MyBioSource, San Diego, USA). The sensitivity of this kit was 0.1 pg/ml. The intra-assay variation was 0.41%.

6.1.4 Determination of body fluid compartments

On PP-4 and PP-8, dairy goats were inserted with catheters (18G, NIPRO Corporation, Osaka, Japan) into the jugular vein for dye solution injection at 16:00 h. The catheters were stopped with three-way stopcocks (NIPRO Corporation, Osaka,

Japan). After insertion with catheters, dairy goats were prevented from eating and drinking, and then blood samples were taken. Plasma volume (PV) was measured by intravenous injection of 3 ml of 0.5% Evans blue dye (Sigma-Aldrich, Steinheim, Germany) via the jugular vein. Determination of extracellular fluid (ECF) was taken by intravenous injection of 5 ml of 10% sodium thiocyanate solution (Sigma-Aldrich, Steinheim, Germany) and total body water (TBW) was performed by intravenous injection of 5 ml of 20% antipyrine solution (Sigma-Aldrich, Steinheim, Germany) via the jugular vein. Three milliliter blood samples from the jugular vein into the EDTA tube were collected before and after injection of dye solution at 20, 30, 40 and 50 minutes for PV and ECF and TBW was taken the blood at the same time points of PV and ECF and continuously collected at 120, 180 and 240 minutes, kept in crushed ice. An aliquot of the blood samples at 0 minutes (before dye injection) was taken to determine for packed cell volume (PCV) by microhaematocrit centrifuge and then all blood samples were centrifuged at 3,000 rpm for 10 minutes for harvesting the plasma. Once blood was withdrawn, each animal was infused with normal saline that was equivalent to the volume of blood withdrawn. Plasma samples were analyzed for the concentration of Evans blue dye and NaSCN according to Medway and Kare (1959) and antipyrine as described by Weiss (1958) by using a spectrophotometer. The blood volume (BV) was calculated using plasma volume and packed cell volume (Chaiyabutr et al., 1980).

6.1.5 Statistical analysis

The data for Tr, RR, dry matter intake/ body weight (DMI/BW), water intake, urinary excretion and pH, apparent water balance, Diff WI-Uex, milk yield and 4% FCM were averaged from PP-2 to PP-8. Data for plasma electrolytes, plasma osmolality, body fluid compartments and plasma ADH level were averaged at PP-4 and PP-8. Subsequently, the data from the two diets were compared using an unpaired t-test. Statistical significance was declared at $P < 0.05$.

RESULTS

6.2.1 Effects of dietary cation and anion difference on rectal temperature and respiration rate

The average Tr was not affected by DCAD throughout the day time (P>0.05, Table 6.1). There was no difference in RR at 11:00 h, 15:00 h and 19:00 h, but RR from the DCAD group tended to be higher than from the control group at 09:00 h (P=0.06) and 13:00 h (P=0.09). The percentage change of RR did not differ between groups. But there was a significantly lower percentage change of Tr from the DCAD group compared with the control group at 09:00 h to 13:00 h (P<0.01, Table 6.1). The percentage change of Tr was similar between DCAD and control groups at other time points.

6.2.2 Effects of dietary cation and anion difference on dry matter intake, milk yield and compositions

The average total DMI/BW tended to be higher in the high DCAD than the control (P=0.08, Table 6.2) throughout the experiment. There was an effect of DCAD on total daily water intake (P<0.05). Animals in the high DCAD groups drank more than those in the control group. Urine volume and pH, milk yield, 4% FCM and milk compositions were similar in both groups (P>0.05, Table 6.2).

6.2.3 Effects of dietary cation and anion difference on plasma electrolytes and osmolality

There was no effect of DCAD supplementation on plasma concentrations of K^+, Cl^- and plasma osmolality at 09:00 h and 16:00 h (P>0.05, Table 6.3). Similarly, there was no difference of plasma Na^+ level and CAD between groups at 09:00 h. However, the plasma Na^+ concentration and CAD from DCAD was higher than in the control at 16:00 h (P<0.05, Table 6.3).

6.2.4 Effects of dietary cation and anion difference on water balance

There was an effect of DCAD on apparent water balance during the post-treatment period (from PP-2 to PP-8). Animals in the DCAD group were higher apparent water balance over 24 h than those in the control (P<0.01, Fig. 6.1). Higher Diff WI-Uex from DCAD group came from 09:00 h to 11:00 h (P<0.01, Table 6.4) and partly from 15:00 h to 17:00 h (P = 0.10). However, Diff WI-Uex at two-hour intervals such as 07:00 h to 09:00 h, 09:00 h to 11:00 h, 11:00 h to 13:00 h, 13:00 h to 15:00 h, 15:00 h to 17:00 h, 17:00 h to 19:00 h did not differ between groups (P>0.05).

6.2.5 Effects of dietary cation and anion difference on body fluid compartments

The absolute values and relative values as a percentage of body weight of TBW increased as levels of DCAD increased (P<0.05, Table 6.5). In contrast, DCAD did not influence on absolute values and relative values as a percentage of body weight of ECF, PV and BV. The PCV tended to increase in DCAD group (P=0.07). There was no effect of DCAD on plasma ADH concentration between groups (P>0.05, Table 6.5).
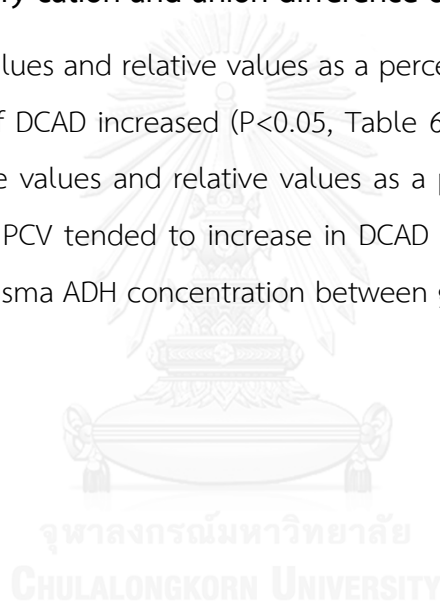
Table 6.1 Effect of dietary cation and anion difference on average values and percentage changes of rectal temperature and respiration rate in dairy goats under high ambient temperature (means ± S.E.M)

Hours	Treatment[†]		P
	Control	DCAD	
Rectal temperature ($^{\circ}$C)			
09:00 h	39.30±0.10	39.44±0.06	0.29
11:00 h	39.47±0.12	39.49±0.08	0.91
13:00 h	39.60±0.11	39.63±0.07	0.86
15:00 h	39.72±0.13	39.81±0.09	0.58
17:00 h	39.82±0.13	39.81±0.09	0.99
19:00 h	39.65±0.14	39.70±0.12	0.80
Respiration rate (breaths/min.)			
09:00 h	90.92±6.30	112.40±7.20	0.06
11:00 h	106.81±7.49	128.24±9.00	0.11
13:00 h	123.77±8.06	151.73±11.50	0.09
15:00 h	121.79±7.34	142.58±10.31	0.14
17:00 h	111.67±8.12	129.23±7.77	0.16
19:00 h	97.19±6.17	109.92±4.85	0.15
Percentage change of rectal temperature (%)			
09:00 to 11:00 h	0.66±0.26	0.11±0.11	0.10
09:00 to 13:00 h	0.76±0.06	0.47±0.05	0.01
09:00 to 15:00 h	1.04±0.16	0.91±0.11	0.53
09:00 to 17:00 h	1.28±0.17	0.93±0.15	0.17
09:00 to 19:00 h	0.87±0.21	0.64±0.17	0.42
Percentage change of respiration rate (%)			
09:00 to 11:00 h	18.42±3.97	12.50±1.91	0.23
09:00 to 13:00 h	25.96±2.46	25.42±2.59	0.88
09:00 to 15:00 h	25.05±2.33	20.77±2.49	0.24
09:00 to 17:00 h	17.07±3.35	12.40±2.11	0.28
09:00 to 19:00 h	4.09±2.18	2.40±3.21	0.14

[†]DCAD level: Control = 22.81 mEq/ 100g DM; DCAD = 39.08 mEq/ 100g DM

Table 6.2 Effect of dietary cation and anion difference on dry matter intake, water intake, urine volume, milk yield and composition in dairy goats under high ambient temperature (means ± S.E.M)

Item	Treatment[†]		P
	Control	DCAD	
DMI, g/kg BW/day	31.56±1.62	36.76±2.01	0.08
Water intake, ml/kg BW/day	130.81±24.74	207.29±17.68	0.04
Urine volume, ml/kg BW/day	65.82±18.48	109.25±22.26	0.17
Urine pH	8.79±0.06	8.81±0.04	0.80
Milk yield, kg/day	1.47±0.22	1.54±0.19	0.82
4% FCM[††], kg/day	1.48±0.19	1.62±0.23	0.64
Milk composition, %			
Fat	4.10±0.39	4.24±0.58	0.81
Protein	2.75±0.27	2.82±0.17	0.84
Lactose	4.29±0.11	4.41±0.05	0.34
Total solid	12.14±0.74	12.48±0.68	0.75

[†]DCAD level: Control = 22.81 mEq/ 100g DM; DCAD = 39.08 mEq/ 100g DM

4% FCM[††] = Milk yield (0.411 + 0.147 (% fat)).

Table 6.3 Effect of dietary cation and anion difference on plasma electrolytes and plasma osmolality in dairy goats under high ambient temperature (means ± S.E.M)

Item	09:00 h		16:00 h	
	Control[†]	DCAD[†]	Control[†]	DCAD[†]
Na^+ (mEq/L)	144.54±1.57	142.42±1.26	150.14±1.85	158.89±2.32*
K^+ (mEq/L)	3.74±0.18	3.70±0.07	3.82±0.17	4.10±0.16
Cl^- (mEq/L)	99.45±1.98	98.40±2.03	93.65±2.83	91.15±0.67
CAD[‡] (mEq/L)	49.13±1.53	47.72±2.38	60.31±4.26	71.84±2.28*
Osmolality (mOsm/kg)	290.20±5.80	291.40±8.98	296.60±8.35	293.60±4.41

[†]DCAD level: Control = 22.81 mEq/ 100g DM; DCAD = 39.08 mEq/ 100g DM

[‡]CAD = Cation and anion difference (Na + K – Cl) in mEq/L

*P<0.05, Control vs. DCAD at specific time points

Table 6.4 Effect of dietary cation and anion difference on difference between water intake and water output at different time intervals in dairy goats under high ambient temperature (means ± S.E.M)

Item	Water intake (l/animal)		Urine volume (l/animal)		Diff WI-Uex[†] (l/animal)	
	Control[‡]	DCAD[‡]	Control[‡]	DCAD[‡]	Control[‡]	DCAD[‡]
07:00 to 09:00 h	1.48±0.26	1.53±0.13	0.12±0.02	0.22±0.04*	1.37±0.24	1.30±0.11
09:00 to 11:00 h	0.31±0.07	0.67±0.03**	0.17±0.05	0.21±0.08	0.14±0.05	0.46±0.07**
11:00 to 13:00 h	0.53±0.18	0.69±0.10	0.22±0.06	0.43±0.09	0.31±0.15	0.26±0.05
13:00 to 15:00 h	1.36±0.43	1.54±0.06	0.16±0.06	0.22±0.05	1.20±0.37	1.32±0.06
15:00 to 17:00 h	0.49±0.14	0.82±0.09	0.27±0.10	0.38±0.08	0.22±0.07	0.44±0.10
17:00 to 19:00 h	0.30±0.11	0.60±0.10	0.27±0.09	0.39±0.07	0.04±0.14	0.21±0.13

[†]Diff WI-Uex: difference between water intake and urinary excretion

[‡]DCAD level: Control = 20 mEq/ 100g DM; DCAD = 40 mEq/ 100g DM

*P<0.05; **P<0.01, Control vs. DCAD

Table 6.5 Effect of dietary cation and anion difference on body fluid compartments, packed cell volume and plasma ADH level in dairy goats under high ambient temperature (means ± S.E.M)

Item	Treatment[†]		P
	Control	DCAD	
Total body water (l)	22.39±0.71	25.63±1.10	0.04
Total body water (%BW)	65.62±2.19	76.29±2.87	0.02
Extracellular fluid (l)	10.09±0.42	11.71±0.69	0.08
Extracellular fluid (%BW)	29.48±1.18	34.94±2.13	0.06
Plasma volume (l)	2.09±0.22	2.27±0.31	0.60
Plasma volume (%BW)	6.21±0.77	6.65±0.80	0.68
Blood volume (l)	2.80±0.30	3.11±0.40	0.50
Blood volume (%BW)	8.25±1.05	9.31±1.04	0.47
Packed cell volume (%)	25.53±0.52	27.15±0.87	0.07
ADH (pg/ml)	4.92±0.43	4.02±0.48	0.20

[†]DCAD level: Control = 22.81 mEq/ 100g DM; DCAD = 39.08 mEq/ 100g DM
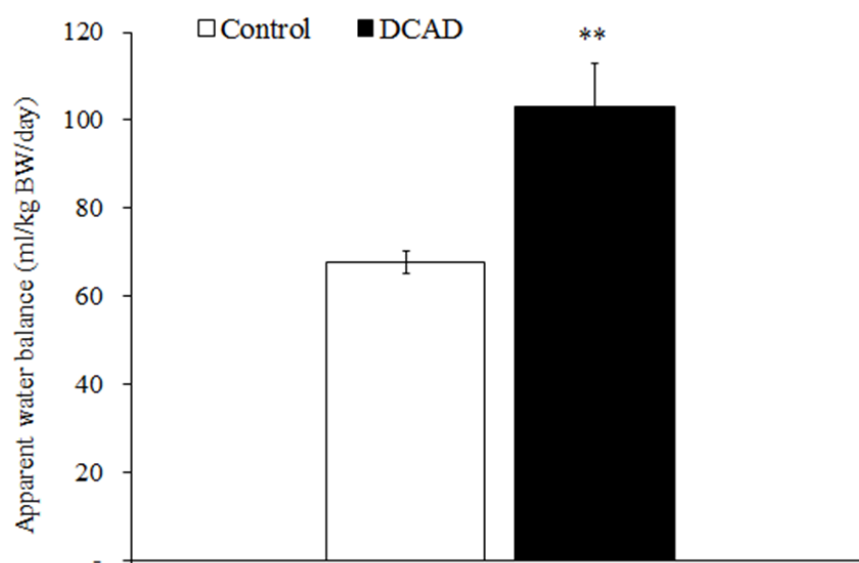
Figure 6.1 Effect of dietary cation and anion difference on apparent water balance during 24 h (means ± S.E.M). DCAD level: Control = 22.81 mEq/ 100g DM; DCAD = 39.08 mEq/ 100g DM. **P<0.01.

DISCUSSION

The lactating dairy goats in this study were exposed to HTa from 33 to 35 ^{0}C from 11:00 h to 17:00 h with THI from 80 to 85, whereas animals stayed at low Ta and THI during early morning (27.64 and 78.12 respectively). This experimental condition indicated that animals were exposed to modest heat stress conditions from 11:00 h to 17:00 h and there was an absence of heat stress conditions during early morning (Silanikove and Koluman, 2015). Heat stress in dairy animals changes physiological responses such as increasing in RR and Tr as reported by previous studies (Chaiyabutr et al., 2000; Kadzere et al., 2002; Hamzaoui et al., 2013). The current study shows that dairy goats in both groups increased Tr and RR in relation to increments of ambient temperature and THI throughout the day. However, the animal's responses under HTa were not similar between groups. Animals in the high DCAD group demonstrated a lower percentage change in Tr than those in the control between 09:00 h and 13:00 h. This may be due to higher heat dissipation from both higher in RR and greater in water intake in the high DCAD group. Similarly, goats under HTa limited water consumption which was lower in RR and higher in Tr than those in control (Kaliber et al., 2016). In this study, animals fed high DCAD tended to increase

RR, even when there was an absence of heat stress (THI at 07:00 h was 78.12±2.13). The effect of DCAD on RR was similar to that shown in previous studies. When dairy cows were supplemented with high DCAD (from 120.4 to 456 mEq/kg DM) pCO_2 and RR increased (West et al., 1992). The present results suggest that dairy goats fed low DCAD under HTa may be more sensitive than those fed high DCAD due to the higher percentage change in the level of Tr.

The present study found that in these climate conditions there was no effect of DCAD on milk yield and 4% FCM. This result agrees with previous studies where high DCAD did not improve milk production in dairy cows (West et al., 1987; Sanchez et al., 1997). Hadjipanayiotou (1988) showed that addition of $NaHCO_3$ to the diet did not increase the milk production in dairy goats during early lactation. Similarly, high DCAD immediately supplemented in dairy cows after calving (from day 0 to day 42 postpartum) found milk yield unchanged (Chan et al., 2005). According to Delaquis and Block (1995a), high DCAD level improved milk yield in early and mid lactation, but were not obtained in late lactation. In dairy goats, the impact of HTa conditions varies in regard to stage of lactation. Lactating dairy goats decreased milk yield up to 9% during early lactation period, whereas they decreased milk yield up to 3% during late lactation (Hamzaoui et al., 2012). Although 4% FCM was similar to both groups, dairy goats in the high DCAD group increase 4% FCM up to 8.84%. Similarly, Tucker et al. (1988) observed that lactating dairy cattle increased milk yield up to 8.6% when DCAD levels were increased. These different results can be attributed to the range of DCAD, the stage of lactation that was used in the different experiments. In addition, the lack of DCAD effect on milk yield in this study could be due to the different responses between dairy goats and dairy cattle under HTa. Because goats are considered more tolerant to HTa conditions compared with dairy cows (Silanikove, 2000) and the reduction in milk yield in dairy goats was much lower than in dairy cattle (Sano et al., 1985; Rhoads et al., 2009; Salama et al., 2014). The current study found that milk composition was not affected by DCAD. These findings agree with other studies in dairy cows (Tucker et al., 1991; West et al., 1991; Apper-Bossard et al., 2010). However, some studies have demonstrated that there is an increase in

milk fat content when dairy animals are fed high DCAD levels (Hu et al., 2007a; Razzaghi et al., 2012). A high DCAD level increases the ruminal pH and subsequently increases acetate and butyrate concentrations which enhance the de novo fatty acid synthesis and there follows an increase in milk fat (Wildman et al., 2007a; Sharif et al., 2010a).

Dairy goats fed with high DCAD tended to increase DMI/BW under HTa. Higher DMI/BW could be due to higher ruminal pH and fermentation which increased the nutrient digestibility in the current study (Chapter V). Earlier studies have demonstrated that there is a positive relationship between the effect of DCAD and DMI (West et al., 1991; Hu et al., 2007a). In addition, Delaquis and Block (1995a) reported that DCAD increased DMI during early and mid lactation, but there was no difference for cows in late lactation. Similarly, West et al. (1992) found that high DCAD increased DMI in heat stress cows. They indicated that the increase in blood buffering capacity with high DCAD may be partly responsible for increase in DMI (Block, 1994). In dairy cattle, high DCAD have been demonstrated to increase blood pH and HCO_3^-, plasma CAD and urinary pH (Tucker et al., 1988; West et al., 1991; Hu and Murphy, 2004). The results from present study reveal that high DCAD increased plasma CAD at 16.00 hour, but it did not affect on urinary pH. Thus, it is difficult at this point to conclude that effects of high DCAD under HTa condition increase DMI in crossbred dairy goats in relation to blood buffering capacity as previous reports (West et al., 1992; Block, 1994). However, Wildman et al. (2007b) reported that high DCAD did not improve DMI in late lactating dairy cows under hot weather. The different responses among studies may be due to a different stage of lactation, levels of DCAD in diets and climate conditions.

Plasma concentrations of Na^+ and K^+ were not altered by DCAD at 09:00 h although dietary levels of Na and K were much greater for dairy goats fed with high DCAD compared with those fed with control. The non-significant difference of high DCAD on plasma Na^+ and K^+ concentrations could be due to the excessive excretion via kidney, because the urine volume in the DCAD group was significantly higher than the control group from 07:00 h to 09:00 h throughout the study (P<0.05, Table 6.4).

These findings are consistent with previous reports (Tucker et al., 1991; Hu and Murphy, 2004; Hu et al., 2007b), which found that plasma Na^+ and K^+ levels were not affected by DCAD in lactating cows. However, plasma concentration of Na^+ from DCAD at 16:00 h, but not plasma K^+, was higher than the control. Greater plasma Na^+ level could be due to higher Na intake and a similar urine volume from 13:00 to 19:00 h in the current study (P>0.05, Table 6.4). This agrees with study by Roche et al. (2005), who reported that the plasma Na^+ level increased as DCAD increase. Plasma concentration of Cl^- was unaffected by DCAD at 09:00 h and 16:00 h in this study. However, Hu et al. (2007b) reported that blood Cl^- concentration tended to be lower when DCAD increased from 22 to 47 mEq/100 g DM. There was no effect of DCAD on plasma CAD at 09:00 h, but plasma CAD increased at 16:00 h in animal fed high DCAD diets. Higher CAD from high DCAD group is primarily caused by the increase in plasma Na^+ concentration. Certain studies showed that serum or plasma CAD was significantly higher for cows fed diets with high DCAD (Tucker et al., 1991; Wildman et al., 2007a). In contrast, there was no effect of DCAD on serum or plasma CAD as reported by previous studies (West et al., 1991; Chan et al., 2005). Although DCAD increased plasma Na^+ concentration at 16:00 h, but plasma osmolality was not affected by DCAD at 09:00 h and 16:00 h. The average of plasma osmolality, Na^+ and K^+ in this study was within the normal range (Stevens et al., 1994). However, plasma Cl^- level was lower compared with those reports. Plasma Cl^- from the current study offered similar findings to those observed by Hu and Murphy (2004), who reported that plasma Cl^- concentration in dairy cows fed with different levels of DCAD varied from 87.37 to 107.80 mEq/L.

Although animals in both groups were exposed to HTa in the same conditions, their capacities for water balance were different. Animals in the DCAD group drank more water than those in the control group. In contrast, animals in both groups similarly excreted the water in milk, feces and urinary volume. As a result, animals in the DCAD group had significantly greater water balance during 24 h than those in the control group. Higher water balance would not only provide a reservoir of soluble metabolites for milk synthesis but would be useful in slowing down the elevation in

body temperature during HTa in dairy cows (Chaiyabutr et al., 2000) and in dairy goats when the percentage change in Tr between 09:00 h and 13:00 h was significantly lower in DCAD group than in control group (P<0.01, Table 6.1). The present results are similar to previous studies when high DCAD as supplemented by potassium bicarbonate also increased the water balance in dairy cow (Eriksson and Rustas, 2014). However, other studies demonstrated that there was no effect of increase in sodium bicarbonate supplementation on water balance in dry and lactating dairy cows (Khelil-Arfa et al., 2014). It may be due to higher water intake and urinary excretion in animals fed high DCAD in those experiments. The results from this study indicate that dairy goats in the DCAD group have improved their water balance by increasing water intake and unchanging urinary excretion. It would contribute to maintaining normal body temperature under HTa in this study.

There were no effects of DCAD on absolute values and relative values as percentage of body weight of PV and BV and tended to be higher in PCV and ECF in the current study. However, both absolute values and relative values of TBW in this study in DCAD group were greater than those in control group. An increase in TBW under HTa may be an adaptive mechanism for heat tolerance (El-Nouty et al., 1988), because it acts as a heat sink and therefore heat is transferred from body to ingested water during the hot hours of the day and dissipates it during the cooling hours of night. The increase in TBW in DCAD group may come from either the ECF or transcellular fluids (mainly digestive tract). In ruminant water firstly retains in the rumen and gastrointestinal tract and then passes to the vascular compartments. Fraley et al. (2015) reported that fractional liquid passage rate was a linear increase when dairy cows fed with DCAD from 16 to 53.5 mEq/100 g DM. Thus an increase in TBW under HTa of this study may also come from the increasing liquid passage rate from digestive tract to the vascular compartments. It is noted that the kidney is an important organ in the regulation of body fluid compartments in part through the plasma ADH concentration. The effects of TBW and water balance from this study may have mediated the effects of DCAD on urinary volume in relation to plasma ADH level. Theoretically, high DCAD may relate to the increase in Na or K intake

followed by increasing plasma concentrations of Na^+ and K^+. This causes an increase of osmotic pressure of plasma. These conditions would stimulate the thirst center in the brain and increased water intake by the animal (Blair-West et al., 1992; Aspinall et al., 2009) and followed by increasing BV, reducing ADH secretion and renal water reabsorption. Consequently, urinary excretion would increase. However, the present results indicated that animals in DCAD group under HTa significantly increased water intake, similar plasma ADH concentration and unchanged urinary volume during 24 h compared with control group (P>0.05, Table 6.2 and 6.4). As a result, high DCAD would improve TBW and water balance. The results from body fluid compartments in control group agree with previous studies in dairy goats (Saipin et al., 2014) and growing goats during summer season (Alamer, 2011), but relative values as percentage of body weight of ECF and TBW from DCAD group were higher than those studies.

In conclusion, the results from the current study of Chapter VI indicate that animals fed high DCAD diets increased water intake and a similar volume of urine. These results are attributed to an increase in the TBW and water balance. These have contributed to a thermoregulatory advantage and would be useful in slowing down the elevation in body temperature under HTa. However, dairy goats fed high DCAD diets tended to increase DMI/BW while milk yield and composition remained unchanged.

CHAPTER VII

SUMMARY

7.1 Summary

This study emphasized the information on two possible mechanisms by which DCAD supplementation affected on milk production and DMI in relation to physiological responses, diurnal variations in eating and meal patterns, ruminal function and nutrition digestibility, water balance and body fluid compartments in dairy goats fed under HTa condition (Fig. 7.1). Firstly, high DCAD can change whole body metabolism via water balance, regulation of feed intake and meal pattern, ruminal function and nutrient digestibility. This experiment was carried out under HTa. Thus, second mechanism may provide some clues which high DCAD can attenuate the effect of HTa condition via changing in rectal temperature, respiration rate and subsequently produce appropriate condition to support the mammary function. The main findings of current study are an increase in water intake and water balance, ruminal pH and allantoin excretion, nutrient digestibility, plasma leptin concentration and alterations of meal pattern when dairy goats consume with high DCAD diets under HTa conditions.

THI in this study varied on the time of day. Dairy goats exposed to heat stress from 11:00 to 17:00 h. This environmental condition has affected on the respiration rate and rectal temperature. Dairy goats in both groups increased rectal temperatures and respiration rates in regarding with the increment of ambient temperature and THI throughout the day. However, the responses with DCAD supplementation were not similar between groups. Animal fed high DCAD diets were greater than respiration rates, even when animal were an absence of heat stress (Chapter IV). These results suggest that DCAD supplementation may change the acid-base status, follow by alteration of respiration in dairy goats.
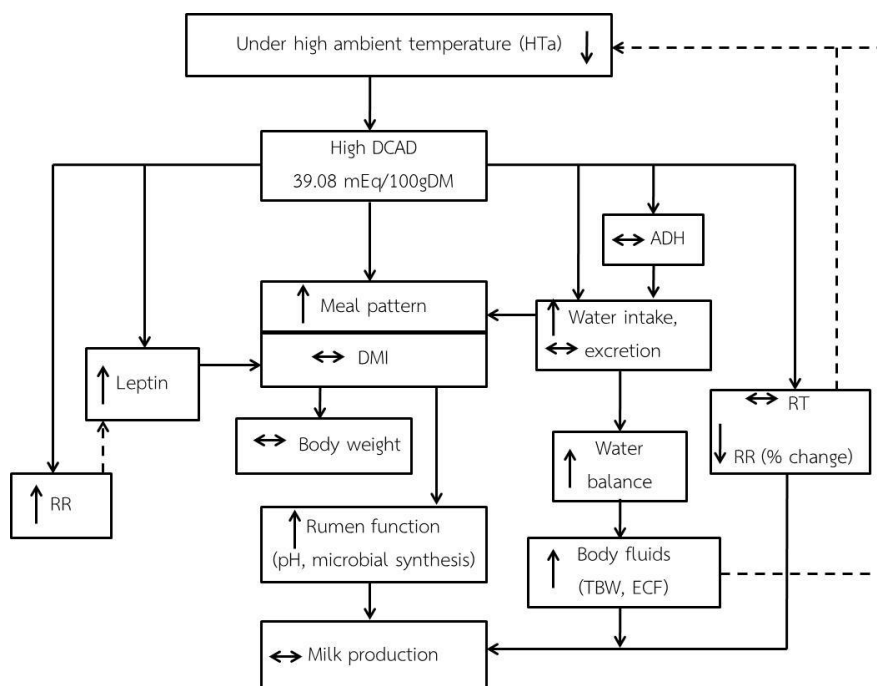
Figure 7.1 Diagram represents two possible mechanisms by which DCAD supplementation affected on milk production and DMI in relation to physiological responses, diurnal variations in eating and meal patterns, ruminal function, water balance and body fluid compartments in dairy goats fed under HTa conditions. Solid arrows represent demonstrated pathways whereas dashed arrows represent speculative pathways. ↑: increase; ↓ decrease; ⟷ : unchange

The results of this study revealed that dairy goats fed with high DCAD have altered meal pattern by increasing meal size, meal duration, and unchanged meal frequency and inter-meal interval (Chapter IV). This could contribute to improve DMI in dairy goats fed high DCAD diet under HTa at PP-8 and overall tended to increase DMI thorough experiment (Chapter IV, V, VI). Higher DMI caused either from increasing ruminal pH, fermentation characteristics, microbial activities via urinary allantoin excretion and nutrition digestibility (Chapter V) or from the improvement of water balance (Chapter VI) for heat dissipation mechanisms during HTa, rather than changing in plasma leptin concentration in the current study (Chapter V). Moreover, dairy goats fed with high DCAD under HTa have changed the patterns of water intake and urinary excretion throughout the day (Chapter IV, V, VI). An increase in water intake has been found in high DCAD group (Chapter V, VI). In addition, plasma ADH concentration was similar between groups and consequently urine volume unchanged. These results were attributed to increase both total body water and

water balance (Chapter VI). Although dairy goats fed with high DCAD under HTa increased ruminal pH, fermentation characteristics, microbial activities, nutrition digestibility and water balance. However, DCAD supplementation did not improve milk production and quality in this study (Chapter VI).

7.2 Benefits and limitations of study

The study has provided some physiological mechanisms by which high DCAD may attenuate HTa via increasing water intake and water balance. In addition, high DCAD also decreases negative effects from HTa on ruminal function via increasing ruminal pH, microbial protein synthesis and nutrient digestibility. These results suggest that high DCAD may produce appropriate conditions to increase the feed intake for dairy goats under HTa. However, the results from present study also reveal that dairy goats fed high DCAD increase RR under HTa condition. Thus, it is difficulty to conclude that dairy goats fed high DCAD during summer time in Thailand whether get the benefits or not from the diets with DCAD of 40 mEq/ 100g DM. In contrast, the results from eating pattern indicate that controlling of the time of feeding is also beneficial, during early morning and late evening feeding help to push the peak heat of fermentation to cooler parts of the day.

The present study reveals that high DCAD increases the respiration rate and plasma leptin secretion in dairy goats. However, we do not know whether the effects of high DCAD on respiration rate, acid-base status in relation to plasma leptin secretion in lactating goats. Thus, further study need to determine the effects of high DCAD on respiration rate, acid-base status in relation to plasma leptin secretion in lactating goats under HTa. In addition, the results from this study suggest that high DCAD increases water intake, total body water and water balance. This could be explained by unchanging urine volume between groups. However, another mechanism may involve on water balance and body fluid that is water transfer from rumen. So, further study need to determine the effects of high DCAD on water transfer from rumen in relation to water balance and body fluid distribution in dairy goats under HTa.

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