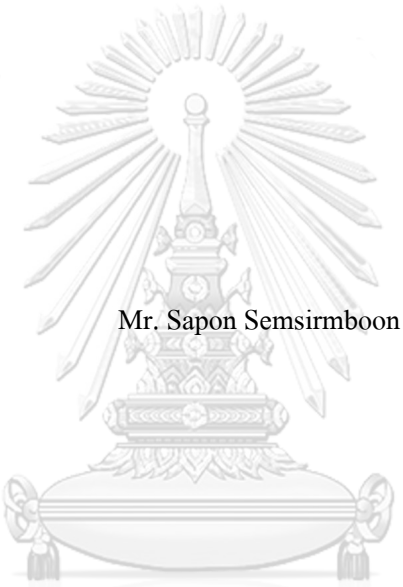
THE EFFECT OF HIGH DOSE ASCORBIC ACID AND HIGH DCAD ADMINISTRATION ON OXIDATIVE STRESS AND MAMMARY GLAND FUNCTION OF DAIRY GOAT FED UNDER TROPICAL CONDITION

Mr. Sapon Semsirmboon



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ผลของการให้ แอสคอร์บิค แอซิด ในระดับสูงและสารอาหารที่มีค่าดีแคดสูงต่อความเครียดออกซิเดชันและการทำหน้าที่เต้านมของแพะนมที่เลี้ยงภายใต้สภาวะเขตร้อน

นายสพล เสมเสริมบุญ

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรดุษฎีบัณฑิต
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สพล เสมเสริมบุญ : ผลของการให้ แอสคอร์บิค แอซิด ในระดับสูงและสารอาหารที่มีค่าดีแคดสูงต่อความเครียดออกซิเดชันและการทำหน้าที่เต้านมของแพะนมที่เลี้ยงภายใต้สภาวะเขตร้อน . (THE EFFECT OF HIGH DOSE ASCORBIC ACID AND HIGH DCAD ADMINISTRATION ON OXIDATIVE STRESS AND MAMMARY GLAND FUNCTION OF DAIRY GOAT FED UNDER TROPICAL CONDITION) อ.ที่ปรึกษาหลัก : รศ. น.สพ.ดร.สัมพันธ์ ธรรมเจริญ, อ.ที่ปรึกษาร่วม : รศ. สพ.ญ.ดร.สุทธาสินี ปุญญโชติ

อุณหภูมิแวดล้อมสูง (High ambient temperature, HTa) เพิ่มอัตราการการหายใจ (respiration rate, RR) และอุณหภูมิทวารหนัก (rectal temperature, Tr) ของแพะนม การตอบสนองดังกล่าวในระดับสูงส่งผลให้เกิดภาวะ การเสียสมดุลกรดด่าง การเสียสมดุลอิเล็กโทรไลต์ และ ความเครียดออกซิเดชัน ซึ่งภาวะดังกล่าวสามารถส่งกระทบทางอ้อมต่อการทำหน้าที่ของเต้านม นอกเหนือจากนั้น HTa ยังสามารถส่งผลกระทบทางตรงต่อการทำงานของเซลล์ซึ่งอาจผ่านทางการเพิ่มขึ้นของความเครียดออกซิเดชัน การเสริมสารอาหารที่มีค่าดีแคดสูง (high dietary cation and anion differences, DCAD) สามารถลดผลกระทบของ HTa ในแพะนมและ การใช้แอสคอร์บิค แอซิด (Ascorbic acid, Asc) ในระดับสูงสามารถลดความเครียดออกซิเดชันในโคนมได้ จากข้อมูลเบื้องต้นดังกล่าวผู้วิจัยตั้งสมมติฐานว่าการทำงานของเต้านมจะเปลี่ยนแปลงหรือไม่ ถ้าผลกระทบของ HTa ลดลงจากเสริมอาหาร DCAD สูง และ Asc ในระดับสูงแผนการศึกษาครั้งนี้ประกอบด้วยการทดลองสองส่วน การทดลองที่หนึ่งมีวัตถุประสงค์เพื่อศึกษาผลของการเสริม Asc ระดับสูงในแพะนม และการทดลองที่สองมีวัตถุประสงค์เพื่อศึกษาสมมติฐานของงานวิจัย การทดลองส่วนที่หนึ่งและสองทำการศึกษาในแพะนมระยะไม่ให้น้ำนมจำนวน 6 ตัวและแพะนมให้นมระยะต้นจำนวน 12 ตัวตามลำดับ ข้อมูลสภาพแวดล้อม RR และ Tr ถูกวัดวันละสามครั้งตอนเวลา 0600, 1300, และ 1800 ตัวอย่างทั้งหมดในการทดลองทั้งสองถูกเก็บในช่วงเวลา 1500 แพะจากการทดลองที่หนึ่งทั้งหมดจะได้รับการเสริมสารสื่อตัวยาและ Asc ระดับสูงในสองวันสุดท้ายของการทดลอง และ ตัวอย่างเลือดจากแพะทดลองเพื่อการตรวจค่าแก๊สในเลือด อิเล็กโทรไลต์และความเครียดออกซิเดชัน แพะจากการทดลองที่สองได้รับการแบ่งอย่างสุ่มเข้ากลุ่มควบคุมและกลุ่ม DCAD โดยที่แพะทั้งสองกลุ่มจะได้รับอาหารควมคุมและอาหาร DCAD สูงเป็นเวลา 8 สัปดาห์ แพะทั้งสองกลุ่มจะได้การเสริม Asc ระดับสูงในสองวันสุดท้ายของสัปดาห์ที่ 4 และ 8 ของการทดลอง การเก็บตัวอย่างเลือดและปัสสาวะของแพะทั้งสองกลุ่มทำในช่วงของการเสริม Asc เพื่อวัดค่าแก๊สในเลือด ค่าอิเล็กโทรไลต์ในเลือด และค่าการทำหน้าที่ของไต นอกเหนือจากนั้นค่าองค์ประกอบน้ำนมและค่าความเครียดออกซิเดชันจะได้รับการตรวจเพิ่มเติมในสัปดาห์ที่ 8 ของการทดลอง จากข้อมูลสภาพแวดล้อมแสดงให้เห็นว่าแพะในการทดลองถูกเลี้ยงภายใต้ HTa และการเพิ่มสูงขึ้นของ RR และ Tr บ่งชี้ถึงการระบายความร้อน การทดลองที่หนึ่งพบว่าการเสริม Asc ในระดับสูงมีแนวโน้มที่จะลดค่าฮีมาโตคริต ซึ่งอาจเป็นผลมาจากการลดลงของภาวะความเครียดออกซิเดชัน ดังนั้นการเสริมสาร Asc ในระดับสูงตามการทดลองที่หนึ่งจะนำไปใช้ต่อในการทดลองที่สอง อัตราการหายใจ ค่า pH ในเลือด และค่าไบคาร์บอเนตในเลือดของแพะกลุ่ม DCAD สูงกว่าแพะกลุ่มควบคุมในช่วงอาทิตย์ที่ 4 ของการทดลองที่สอง การเสริมอาหาร DCAD สูงไม่มีผลกระทบต่ออัตราการหายใจ ค่า pH ในเลือด และค่าไบคาร์บอเนตในเลือดของแพะในช่วงอาทิตย์ที่ 8 ของการทดลองที่สองซึ่งเป็นช่วงเวลาที่แพะกลุ่ม DCAD มีอัตราการขับทิ้งอิเล็กโทรไลต์ที่สูงขึ้น นอกเหนือจากนั้น การเสริมสารทั้งสองอย่างมีผลร่วมในการลด ค่า creatinine และ malondialdehyde ในพลาสมา การลดลงดังกล่าวตรวจพบพร้อมกับการเปลี่ยนแปลงขององค์ประกอบน้ำนม ผลการทดลองนี้ชี้ให้เห็นการเสริมอาหาร DCAD สูง และ Asc ในระดับสูงมีผลร่วมในการเพิ่มน้ำในร่างกายและลดความเครียดออกซิเดชัน การลดลงของความเครียดออกซิเดชันดังกล่าวเปลี่ยนแปลงการทำหน้าที่ของเต้านมแพะ ดังนั้นจากผลการทดลองสามารถสรุปได้ว่าการเสริมสารอาหาร DCAD สูง และ Asc ในระดับสูงมีผลร่วมในการลดผลกระทบของภาวะอุณหภูมิแวดล้อมสูงต่อการทำหน้าที่ของเต้านมของแพะนมที่เลี้ยงภายใต้สภาวะอากาศร้อน

สาขาวิชา	สรีรวิทยาการสัตว์	ลายมือชื่อนิสิต .....
ปีการศึกษา	2565	ลายมือชื่อ อ.ที่ปรึกษาหลัก .....
		ลายมือชื่อ อ.ที่ปรึกษาร่วม .....

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KEYWORD: acid-base disturbance, dairy goat, evaporation, oxidative stress, vitamin c

Sapon Semsirmboon : THE EFFECT OF HIGH DOSE ASCORBIC ACID AND HIGH DCAD ADMINISTRATION ON OXIDATIVE STRESS AND MAMMARY GLAND FUNCTION OF DAIRY GOAT FED UNDER TROPICAL CONDITION. Advisor: Assoc. Prof. Dr. Sumpun Thammacharoen, D.V.M., Ph.D. Co-advisor: Assoc. Prof. Dr. SUTTHASINEE POONYACHOTI, D.V.M., Ph.D.

High ambient temperature (HTa) increased the respiration rate (RR) and rectal temperature (Tr) of dairy goats. The excess response resulted in acid-base imbalance, electrolyte imbalance, and systemic oxidative which could indirectly affect the mammary gland. Besides, HTa did directly alter cell function, and this has been proposed to be mediated by oxidative stress. We previously showed that high dietary cation and anion differences (DCAD) could decrease the HTa effect. In dairy cows, a high dose ascorbic acid (Asc) has been used to decrease oxidative stress. Then, It was hypothesized whether mammary gland function could be altered when the HTa was alleviated by DCAD and Asc supplements. This current study consisted of two main experiments. The first aimed to investigate the Asc supplement protocol in dairy goats while the second was planned to investigate the hypothesis. The first and second experiments were carried out on six non-lactating goats and twelve lactating goats, respectively. During these experiments, the ambient condition, RR, and Tr were measured three times a day, at 0600, 1300, and 1800. All samples were collected at 1500 of both experiments. In the first experiment, goats were intravenously supplemented with vehicle and Asc on the last two days of the experiment and only blood samples were used to analyze blood gas, electrolytes, and oxidative stress. In the second experiment, goats were randomly assigned to control and DCAD groups which were fed with the control diet and a high DCAD diet for 8 weeks. The protocol of Asc supplement was done on the last two days of the 4^{th} and 8^{th} weeks of the experimental period. On these supplemented days, blood and urine were collected to measure blood gas, electrolytes, and renal function, while both milk composition and plasma oxidative stress were measured only on the 8^{th} of the experimental period. The ambient condition showed that all goats were fed under HTa, while an increased RR and Tr indicated heat dissipation. In the first experiment, Asc tended to decrease hematocrits which might be mediated by depleted oxidative stress. Then, this protocol was then used in the second experiment. On the 4^{th} week of the second experiment, RR, blood pH and bicarbonate were higher in the DCAD group compared with the control group. These DCAD effects were no longer observed on the 8^{th} week of this study when fraction excretion of electrolyte was increased in the DCAD group. The presence of both supplements synergistically depleted plasma creatinine and malondialdehyde. The depletion of plasma malondialdehyde was observed with the alteration of milk composition. Based on these data, the high DCAD and high dose Asc synergistically increased body water but decreased oxidative stress. The depletion of oxidative stress altered the mammary gland function of dairy goats. Therefore, the presence of high DCAD and high dose Asc supplement did synergistically alleviate the HTa effect on mammary gland function of lactating goat fed under tropical condition.

Field of Study:	Animal Physiology	Student's Signature .....
Academic Year:	2022	Advisor's Signature .....
		Co-advisor's Signature .....

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Sapon Semsirmboon

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Ascorbic acid	Asc
Ambient temperature	Ta
Ammonium excretion	NH_4
Bicarbonate	HCO_3
Blood urea nitrogen	BUN
Body weight	BW
Chloride	Cl
Dietary cation and anion difference	DCAD
Dry matter	DM
Dry matter intake	DMI
Fractional excretion	FE
Free fatty acids	FFA
Glutathione peroxidase	GPx
Hematocrit	Hct
High ambient temperature	HTa
Hydrochloric acid	HCL
Hydrogen peroxide	H_2O_2
Malondialdehyde	MDA
Milk yield	MY
Milliequivalents	mEq
Net acid excretion	NAE
Partial pressure of Carbon dioxide	PCO_2
Pentose phosphate pathway	PPP
Potassium	K
Potassium carbonate	K_2CO_3
Reactive oxygen species	ROS
Rectal temperature	Tr
Relative humidity	RH
Respiration rate	RR

Sodium	Na
Sodium bicarbonate	$NaHCO_3$
Sodium hydroxide	NaOH
Temperature humidity index	THI
Titratable acid	TA
Water intake	WI

CHAPTER I

INTRODUCTION

Milk synthesis is a main function of the mammary gland, and this function is compromised by high ambient temperature (HTa). In tropical conditions, milk production of goats and cows in the summer was lower than production in the winter (Saipin et al., 2020a; Thammacharoen et al., 2021). It is known that the effect of HTa on milk production was partially mediated by a decreased food intake. This indirect effect was responsible for a 35 – 50 % reduction in milk production (Rhoads et al., 2009; Xiao et al., 2020) while others could be mediated by other indirect effects including acid-base imbalance, electrolyte imbalance, and systemic oxidative stress (Schneider et al., 1988a; Schneider et al., 1988b; Tanaka et al., 2007). However, the study in mammalian cells showed that HTa could directly affect cell metabolism, and oxidative stress has been proposed to mediate this effect (Collie et al., 2006; Kobayashi et al., 2018; Saipin et al., 2020b).

The evaporative heat dissipation was the main heat loss mechanism of dairy ruminants fed under HTa (Robertshaw, 2006; Saipin et al., 2020a). The respiration rate was increased during the daytime HTa to dissipate the heat load and this response resulted in respiratory hypocapnia (Do Nguyen et al., 2022). To compensate for this imbalance, the excretion of blood bicarbonate was increased through the kidney (Barker et al., 1957; Arbus et al., 1969). Indeed, the rate of metabolism was increased during the early lactation while heat dissipation also increased this rate (Maia et al., 2016; Gärtner et al., 2019). The high metabolic rate could result in metabolic acidosis and this imbalance might be increased during HTa exposure according to the decreased blood buffer as bicarbonate. Besides, the change in plasma electrolytes could affect the acid-base balance (Torrenta Artero, 2017). With HTa, the sweating rate of ruminants was increased to dissipate heat on the skin (Jenkinson and Mabon, 1973; Maia et al., 2016) and the excess excretion could deplete body water and electrolytes (Schneider et al., 1988a; Schneider et al., 1988b; Beatty et al., 2006). Moreover, an increased metabolic rate did increase oxidative stress and the antioxidant was depleted in the presence of HTa (Sie et al., 2017; Guo et al., 2018). The alteration of this antioxidant was included in the adaptive mechanism to HTa and this was

proposed to be involved in milk synthesis under HTa (Saipin et al., 2020a). These previous observations suggested the mammary gland function under HTa might be compromised by acid-base imbalance, electrolyte imbalance, and oxidative stress.

We previously demonstrated that high dietary cation and anion difference (DCAD) could increase heat dissipation and alleviate the HTa effect (Nguyen et al., 2018; Nguyen et al., 2019). The previous study in ruminants showed that high DCAD could increase blood buffer and maintain plasma electrolytes, these effects were observed with the alteration of kidney function (Wildman et al., 2007a,b,c). Regarding oxidative stress, ascorbic acid (Asc) had been used to decrease oxidative stress in dairy cows and a high dose Asc supplement could alter mammary gland oxidative stress (Chaiyowittayakun et al., 2002; Matsui, 2012). Therefore, we hypothesized whether the mammary gland function under HTa could be altered when both imbalance and oxidative stress were compromised by high DCAD and Asc supplements.

Keywords (Thai): การรบกวนสมดุลกรดด่าง, แพะนม, การระเหย, ความเครียดออกซิเดชัน, วิตามิน ซี

Keywords (English): Acid-base disturbance, dairy goat, evaporation, oxidative stress, vitamin c

Objectives of study

1. To investigate the effect of high DCAD and high dose Asc supplement on the acid-base balance of dairy goats fed under HTa.

2. To investigate the effect of high DCAD and high dose Asc supplement on the electrolyte balance of dairy goats fed under HTa.

3. To investigate the effect of high DCAD and high dose Asc supplement on oxidative stress of dairy goats fed under HTa.

4. To investigate the effect of high DCAD and high dose Asc supplement on mammary gland function under HTa.

Hypothesis of study

1. The high DCAD and high dose Asc supplement change the acid-base balance of dairy goats fed under HTa.

2. The high DCAD and high dose Asc supplement change the electrolyte balance of dairy goats fed under HTa.

3. The high DCAD and high dose Asc supplement change the oxidative stress of dairy goats fed under HTa.

4. The high DCAD and high dose Asc supplement increased the milk yield of dairy goats fed under HTa.

CHAPTER II

LITERATURE REVIEWS

High ambient temperature and physiological response

The regulation of body temperature is essential physiology, and this is maintained by the balance between heat load and heat loss (Hall, 2015). The metabolism and high ambient temperature (HTa) were responsible for the internal and external heat load of the body (Mercer, 2001). In dairy ruminant, the animal first moved out of HTa and decreased dry matter intake (DMI) to reduce both external and internal heat load (Ominski et al., 2002; Kendall et al., 2006; Schütz et al., 2009). If these responses were not enough to maintain body temperature, four main heat loss mechanisms were activated. These mechanisms consisted of radiation, convection, conduction, and evaporation (Hall, 2015). The efficiency of the first three heat loss mechanisms depended on a temperature gradient between the animal and the environment (Campos Maia et al., 2005; Maia et al., 2008; Hall, 2015; Maia et al., 2015). However, the daytime HTa of tropical was close to the body temperature of dairy ruminant (Saipin et al., 2020a; Thammacharoen et al., 2021) and this limited the efficiency of radiation, convection, and conduction. These observations suggested that evaporation was the main heat loss mechanism of dairy ruminants. This mechanism exists in both the respiratory system and the sweat gland and the water is required to evaporate the heat out of the body (Campos Maia et al., 2005; Robertshaw, 2006; Maia et al., 2008; Maia et al., 2015). In dairy goats, the response of the respiratory system was mainly responsible for the evaporative heat loss mechanism (Robertshaw, 2006). With HTa of Thailand, rectal temperature (Tr) was increased by 1°C and respiration rate (RR) was doubled during the daytime (Saipin et al., 2020a). Besides, the dairy goat fed under HTa had higher water intake (WI) when compared to the goats fed under control ambient temperature (Ta) (Escobosa et al, 1984; Hamzaoui et al. 2013; Khelil-Arfa et al., 2014). These indicated an activated heat loss mechanism during daytime HTa. These responses were included as the adaptive mechanism of dairy goats fed under HTa (Saipin et al., 2020a). Even though the effect of HTa on dairy production was proposed to involve in stress response (Alvarez and Johnson, 1973; Guerrini and Bertchinger, 1982; Burhans et al., 2022), the effect of season on milk yield was not observed with

an increased plasma cortisol level of the dairy goats (Saipin et al., 2020a). With this observation, the effect of daytime HTa on dairy goats was not involved in the response of the hypothalamic-pituitary-adrenal gland or stress axis. However, the previous publication showed that an increased HTa could affect blood gas parameters in both lactating cows and goats (Sivakumar et al., 2010; Thammacharoen et al., 2013; Mehaba et al., 2019; Façanha et al., 2020). Moreover, the plasma antioxidant was decreased in the presence of HTa (Sivakumar et al., 2010; Chaiyabutr et al., 2011). Therefore, the negative effect of HTa could be mediated through both acid-base balance and oxidative stress.

High ambient temperature and milk production

The dairy ruminant is domesticated to serve the needs of milk consumption, and both high yield and high consistency of milk production are contributing to the food security of humans. With the HTa, the milk production of dairy cow and goat were compromised especially in the tropical region (Ominski et al., 2002; Saipin et al., 2020a; Thammacharoen et al., 2021) and this caused an economic loss in the dairy industry (St-Peirre et al., 2003; Key and Sneeringer, 2014; Guun et al., 2019). HTa of tropical condition decreased milk production of cows and goats by 16% and 20% when compared to control Ta (Saipin et al., 2020a; Thammacharoen et al., 2021). The tropical climate was a year-round HTa and high relative humidity (RH) which caused a high temperature humidity index (THI) (Saipin et al., 2020a; Thammacharoen et al., 2021). This climate limited the evaporative heat loss and exacerbated the effect of HTa (Campos Maia et al., 2005; Maia et al., 2016), then, the milk production of dairy ruminants fed under tropical is susceptible to the negative effect of HTa. The heat-induced hypophagia was previously proposed to limit the nutrient supply which could indirectly affect the mammary gland function (Lu, 1989; Rhoads et al., 2009; Xiao et al., 2020). Indeed, this indirect effect of HTa was responsible for 35-50% of milk production while other parts might be affected by either other indirect effects or a direct effect of HTa (Rhoads et al., 2009; Xiao et al., 2020). The previous study in dairy goats suggested that acid-base imbalance and oxidative stress might be included as indirect effects of HTa (Sivakumar et al., 2010; Façanha et al., 2020; Saipin et al., 2020a). Besides, the dairy ruminant was susceptible to both acid-base imbalance and oxidative stress during early lactation

(Garnsworthy et al., 2006; Putman et al., 2018; Gärtner et al., 2019; Saipin et al., 2020a). Regardless of the indirect effect, the function of cultured cells could be altered by HTa treatment (Collie et al. 2006; Kobayashi et al., 2018; Saipin et al., 2020b). This direct effect was proposed to be mediated through oxidative stress (Baumgard and Rhoads, 2013; Saipin et al., 2020a). Interestingly, the alteration of oxidative stress within the mammary gland across the lactation stage was related to the milk composition (Garnsworthy et al., 2006; Zachut et al., 2013) and the acute oxidative stress within the mammary gland also decreased both milk yield (MY) and composition (Oshima and Fuse, 1981; Silanikove et al., 2016). These observations suggested that acid-base imbalance, systemic oxidative stress, and mammary gland oxidative stress might mediate the negative effect of HTa on milk synthesis.

High ambient temperature and acid-base balance

The blood pH represents the concentration of proton, and the altered pH could affect normal cell function (Hall, 2015). The major acid and base substances in the blood are carbon dioxide (CO_2) and bicarbonate (HCO_3), respectively (Hall, 2015). Even though the amount of both substances is needed to be balanced, a change in both blood cation and anion could affect blood pH according to electroneutrality (Constable, 2014; Torrenta Artero, 2017). The main cation and anion that could contribute to the blood pH are sodium (Na), potassium (K), and chloride (Cl). The evaporative heat dissipation was a main heat loss mechanism in ruminants fed under HTa and the excess response could interfere with acid-base balance (Schneider et al., 1988a; Schneider et al., 1988b; Robertshaw, 2006). In ruminants, evaporative heat dissipation exists in the respiratory tract and skin (Maia et al., 2015; Maia et al., 2016). The high RR or panting during heat dissipation increased carbon dioxide exhalation and decreased blood partial pressure CO_2 (PCO_2) (Robertshaw, 2006; Mehaba et al., 2019; Façanha et al., 2020). In humans, this acid-base imbalance was compensated by an increased HCO_3 excretion, and the kidney was mainly responsible for HCO_3 excretion (Barker et al., 1957; Arbus et al., 1969). Besides, sweat is secreted from the gland, and heat is then evaporated out of the skin (Jenkinson and Robertshaw, 1971; Jenkinson and Mabon, 1973). Sweat consists of water and the main blood electrolyte, and the high level of sweating rate could eventually affect blood pH (Beatty et al., 2006). The dairy

ruminants fed under HTa require high energy to serve heat dissipation mechanisms (NRC, 1981; Maia et al., 2016) while this requirement is also increased acid production during early lactation (Gärtner et al., 2019). High level of acid from both dissipation and lactation could overwhelm the buffer and exacerbate acid-base imbalance in early lactating ruminants. Without HTa, the buffer supplement could decrease acid-base imbalance and alter milk composition of the dairy cow (Hu and Murphy, 2004; Hu et al., 2007; Wildman et al., 2007a). These observations indicated that the milk synthesis under HTa might be altered when the acid-base imbalance was decreased.

High ambient temperature and electrolyte balance

The extracellular electrolytes are important for normal cell function and the kidney is mainly responsible for balance (Hall, 2015; Hamm et al., 2015). The alteration of electrolytes could be mediated by the change in dietary intake, urinary excretion, and water balance (Chapman et al., 2020). Both HTa and lactation could affect these factors and interfere with electrolyte balance (Ulutas et al., 2003; Beatty et al., 2006). With lactation and HTa, an increased WI and decreased electrolyte excretion indicated a high requirement of both electrolyte and water (Ulutas et al., 2003; Beatty et al., 2006; Hamzaoui et al., 2013; Saipin et al., 2020a). The dairy ruminant increased both RR and sweating rate to dissipate the heat load (Jenkinson and Mabon, 1973; Saipin et al., 2020a). The excess of these responses depleted extracellular fluid, and this resulted in an increased hematocrit (Hct) and electrolytes in goats (El-Nouty et al., 1980). Then, WI was increased to serve heat dissipation while the electrolyte excretion was decreased to conserve the extracellular fluid (Beatty et al., 2006; Chapman et al., 2020; Saipin et al., 2020a). These physiological responses were proposed to be mediated by two hormones, aldosterone, and vasopressin (Chapman et al., 2020). An increased level of these hormones in goats was observed when Tr was increased by 3°C but not at Tr was increased by 1°C (El-Nouty et al., 1980). In natural HTa, Tr of the dairy goat was increased by only 1°C and the HTa effect was alleviated without the change in vasopressin (Nguyen et al., 2018; Nguyen et al., 2019). Besides, the high salt diet increased body fluid and promoted heat acclimation in humans and the high salt diet in goats also alleviate the negative effect of HTa (Allsopp et al., 1998; Nguyen et al., 2018; Nguyen

et al., 2019). Therefore, this study aimed to use this dietary supplement to alleviate the negative effect of HTa on electrolyte and acid-base and it might indirectly alter mammary gland function.

High ambient temperature and antioxidant depletion

Reactive oxygen species (ROS) are a part of cell metabolism, and the amount of ROS is balanced by antioxidants (Sie et al., 2017). There are many types of ROS but important ROS are superoxide anion radical, hydroxyl radical, and hydrogen peroxide (H_2O_2). Even though the first two molecules have higher reactivity, their half-life is shorter than H_2O_2. Besides, this latter molecule plays a role in cell signaling (Slimen et al., 2014) but excess H_2O_2 could overwhelm antioxidants which causes oxidative stress. Increased oxidative stress damages intracellular molecules and alters cell function (Sie et al., 2017). Among the cell components, lipid compounds are susceptible to oxidative stress and the product of this reaction is malondialdehyde (MDA). In terms of antioxidants, there were two groups which are enzymatic and non-enzymatic systems. The first antioxidant lowers the ROS activity through enzymatic reactions and this group consists of three mains enzyme including glutathione peroxidase (GPx), superoxide dismutase, and catalase. However, the produced H_2O_2 is mainly neutralized by GPx and this enzyme is also the main extracellular antioxidant (Chang et al., 2020). The activity of this enzyme is maintained by the presence of nicotinamide adenine dinucleotide phosphate (NADPH) and Asc (Puskas et al., 2000; Fico et al., 2004). In acute oxidative stress, a glucose metabolic partway was altered to serve the existing antioxidants (Kuehne et al., 2015) while intense and prolonged oxidative stress activated the production of antioxidants (Sthijns et al., 2016). An increase in ROS, MDA, and antioxidants was observed during lactation (Piccione et al., 2007; Radin et al., 2015). This indicated that dairy ruminants during early lactating were susceptible to oxidative stress. In addition, an ability to defend against oxidative stress (antioxidant capacity) was proposed to be responsible for the persistence of milk synthesis of dairy cows fed under HTa (Chaiyabutr et al., 2011; Chaiyabutr, 2012). The presence of HTa decreased plasma Asc, plasma and cellular (red blood cell) GPx activity, while increased plasma MDA of dairy cows and rats (Padilla et al., 2006; Tanaka et al., 2007; Bhat et al., 2008; Tanaka et al., 2011; Guo et al., 2018). These observations indicated that HTa decreased both systemic and cellular antioxidant capacity.

In tropical condition, the milk production of dairy ruminants was reduced by 16-20% during the summer seasons and the activation of GPx activity was included as the adaptive mechanism to HTa (Saipin et al., 2020a; Thammacharoen et al., 2021). Indeed, Tr of dairy goats and cows fed under tropical condition was increased by 1°C (38 to 39°C) during the daytime HTa and this range promoted the heat acclimation of the mammalian cells (Lord-Fontaine and Averill, 1999; Saipin et al., 2020a; Thammacharoen et al., 2021). An increased GPx activity and altered glucose metabolism within the cell were a phenotype of thermotolerant mammalian cells (Moon et al., 2010; Glory and Averill-Bates, 2016; Tchouague et al., 2019). In general, GPx activity requires glutathione for its reaction, and the absence of glutathione affected the survival of mammalian cells cultured at HTa (Lord-Fontaine and Averill, 1999). To maintain both GPx and glutathione, the intracellular glucose was diverted to produce an electron donor, NADPH (Lord-Fontaine and Averill-Bates, 2002; Kuehne et al., 2015). This molecule is produced by two glucose pathways including the pentose phosphate pathway (PPP) and the citric acid cycle. The first pathway is mainly responsible for the antioxidant defense while the second is used for energy production. In the mammary gland, oxidative stress could affect major milk composition including lactose, milk fat, milk protein, and free fatty acids (FFA) and these were observed with the change of systemic antioxidant (Silanikove et al., 2016; Nedic et al., 2019). During the antioxidant defense, milk citrate was decreased while glucose was proposed to be diverted toward PPP. The activation of PPP increased glucose and glucose -6-phosephate (G6P) in milk (Garnsworthy et al., 2006; Silanikove et al., 2016). These observations suggested that there might be the link between systemic, mammary gland oxidative stress, milk synthesis in the presence of HTa.

High ambient temperature and dietary cation and anion difference

High dietary cation and anion difference (DCAD) has been used to manage the acid-base and electrolyte imbalance in lactating dairy cows (Riond, 2001; Hu and Murphy, 2004). The level of DCAD is defined as the milliequivalents (mEq) of Na + K -Cl per 100 gram dry matter (DM) and the previous study demonstrated that high DCAD (+39 mEq/ 100 gDM) could alleviate the HTa effect and increased DMI of lactating goat (Nguyen et al., 2018; Nguyen et al., 2019). With HTa, an increased RR of goats depleted PCO_2 and the excretion of HCO_3 was increased to

compensate for this acid-base imbalance (Hamzaoui et al. 2013; Mehaba et al., 2019; Façanha et al., 2020). The effect of DCAD on DMI was observed with an increase in blood pH and HCO_3 (Schneider et al., 1986; Hu and Murphy, 2004) and these responses were mediated through the kidney function (Wildman et al., 2007a,b). Even though the degree of these responses depends on DCAD level, the duration of the supplement also affected the result of acid-base balance (Wildman et al., 2007b,c; Hersom et al., 2010). In short-term supplement, high DCAD did increase plasma electrolytes and increased WI (Tucker et al., 1988). An increased WI by short-term supplement was observed with an increased blood pH and HCO_3 (Escobosa et al, 1984; Delaquis and Block, 1995a,b). Interestingly, the short-term supplement did not affect fractional excretion of electrolytes (Delaquis and Block, 1995a,b) while these parameters were increased as the compensation mechanism (Wildman et al., 2007a,b,c). The effect of DCAD on blood gas parameters was not prominently observed in the long-term supplement (Hu et al., 2007; Wildman et al., 2007a,b,c) and the difference in kidney function could be responsible for this response. With HTa, the effect of high DCAD on WI and DMI of lactating goats were prominently observed on the 4^{th} and 8^{th} week of supplement, respectively (Nguyen et al., 2018; Nguyen et al., 2019). Besides, this effect of high DCAD was related to an increased WI and body water (Nguyen et al., 2018; Nguyen et al., 2019). These observations suggested the effect of DCAD was related to both HCO_3 and body water which are mainly controlled by kidney function. An increased blood HCO_3 could be mediated by two mechanisms including the reabsorption of filtered HCO_3 and the production of new HCO_3 (Hamm et al., 2015). Most of the filtered HCO_3 was reabsorbed with Na at the tubular cell of the proximal tubule while the rest was reabsorbed at the distal tubule and collecting duct (Hamm et al., 2015). Regarding HCO_3 production, an increased acid excretion by ammonium excretion is responsible for HCO_3 production in the kidney (Hamm et al., 2015). With a high level of blood HCO_3, the excreted HCO_3 overwhelmed the reabsorption mechanism and increased the concentration of urine HCO_3 (Delaquis and Block, 1995a,b; Wildman et al., 2007a). These observations inspired us to study both electrolyte balance and kidney function in dairy goats fed under HTa. Therefore, we hypothesized whether the

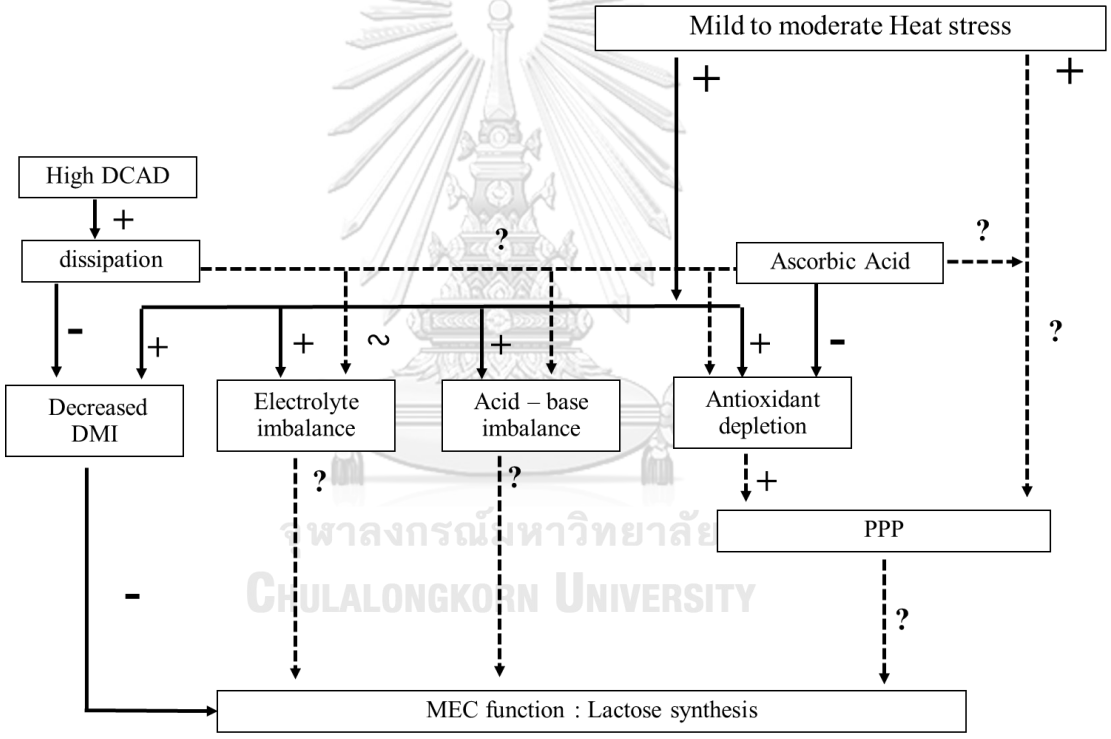
mammary gland function under HTa was altered when the effect of HTa on electrolyte balance and renal function were modified by high DCAD.

High ambient temperature and ascorbic acid

Asc is known as vitamin C and it is a natural antioxidant that is important for normal physiology (Gegotek and Skrzydlewska, 2022). The depleted Asc caused oxidative stress and caused disease in both humans and animals. In humans, food is a main source of Asc while the ruminant could produce it by themselves. Even though Asc could be produced, the depletion of Asc was observed in dairy ruminants fed under HTa (Tanaka et al., 2007; Sivakumar et al., 2010; Chaiyabutr et al., 2011; Tanaka et al., 2011; Matsui, 2012). There was a negative correlation between Tr and plasma Asc concentration in dairy cows (Tanaka et al., 2011) and the depleted Asc was proposed to affect the mammary gland function (Chaiyabutr et al., 2011). The Asc supplementation has been used to increase the plasma Asc in lactating cows (Matsui, 2012) and this could be supplemented through diet, water, and intravenous injection (Chaiyowittayakun et al., 2002; Sivakumar et al., 2010; Matsui, 2012). The use of intravenous Asc could increase plasma Asc level whereas the low stability of Asc in diet, water, and rumen could limit the efficacy of the supplement (Chaiyowittayakun et al., 2002; Matsui, 2012). In humans, the use of intravenous Asc at high dose has been used to manage oxidative stress and this supplement possibly alter the oxidative stress of the mammary gland in ruminants similarly to humans (Chaiyowittayakun et al., 2002; Gegotek and Skrzydlewska, 2022). There are two forms of Asc presented in plasma including Asc and dehydroascorbic acid. The major form in plasma is Asc, and their transporter is sodium-dependent transporter which is located on the epithelium cell membrane (Rivas et al., 2008). This transporter was found in many organs including the kidney and mammary gland. Besides, the effect of this supplement on plasma electrolytes and body fluid had been reported in rats, sheep, and humans (Sakurai et al., 1997; Dubick et al., 2005; Rizzo et al., 2016). Even though Asc is an acid, the high dose supplement did not alter blood gas parameters including blood pH, PCO_2, and blood HCO_3 of healthy humans (Chen et al., 2022). Regarding the cellular response, the Asc supplement directly depleted ROS and MDA in cells, organs, and plasma (Matsuda et al., 1993; Dubick et al., 2005; Muthuvel et al., 2006; Koc et al.,

2008; Sun et al., 2019). With a decreased MDA, the GPx activity in the organ was altered when the rat was supplemented with Asc (Muthuvel et al., 2006; Koc et al., 2008). Indeed, the study in rats and cows showed that the mammary gland oxidative stress could alter the plasma (systemic) oxidative stress (Eslami et al., 2015; Nedic et al., 2019). These observations suggested that there might be a link between systemic and organ oxidative stress including mammary gland while the high dose Asc supplement might alter this link in the presence of HTa.

Figure 1 Conceptual framework



CHAPTER III

MATERIALS AND METHODS

This current study planned to investigate whether the mammary gland function could be changed when the acid-base imbalance, electrolyte imbalance, and oxidative stress are compromised in the presence of HTa. To investigate these objectives, high DCAD and high dose Asc were supplemented to dairy goat during the summer seasons (March-July). However, the protocol of Asc injection is not available in goats and the used protocol in dairy cows needs to be tested. Then, this current study was separated into two main experiments. The first experiment aimed to test the effect of high dose Asc on blood gas, blood electrolytes, and systemic antioxidants of non-lactating goats. The second experiment aimed to investigate the effect of high DCAD and high dose Asc on acid-base balance, electrolyte balance, oxidative stress, and mammary gland function. The previous study in dairy ruminants showed that the effect of DCAD was observed after 4 weeks of the supplementation while the negative effect of HTa was prominently compromised after 8 weeks of the DCAD supplement. According to these observations, the second experiment lasted for 8 weeks, and the sample collection was carried out on both the 4th and 8th week of the experiment. The data from the 4th week was used to monitor the effect of DCAD while the 8th week was used to investigate the main hypothesis of the current study.

Experiment 1: The effects of the ascorbic acid administration on electrolyte balance, acid-base balance, and antioxidant capacity of non-lactating goats

The first experiment was planned to investigate the effect of Asc on 1) blood electrolytes, 2) blood gas parameters, and 3) systemic oxidative stress of dairy goat. In this experiment, non-lactating dairy goats were included to minimize the interference of lactation. We hypothesized that the Asc supplement could affect these three parameters during the daytime HTa.

Animals

The six non-lactating dairy goats (n = 6) were included in this experiment and were fed in one big cage (5 x 4 m. shaped pens opened top with the plastic floor). The concentrate was fed to goats twice a day while pangola hay and water were fed ad libitum in the cage.

Experiment protocol

The first experiment consisted of 9 days which had two periods. The first seven days of this period were used for acclimatization and the last two were used for the experiment. On the 8th and 9th day of this experiment, all goats were intravenously injected with vehicle and Asc according to supplement protocol (Figure 2). Besides, the Ta, RH, THI, Tr, and RR were measured at 0600, 1300, and 1800 during day 8th and 9th of the experiment.

Supplement and sample collection protocol.

The Asc (45 mg/kg body weight (BW)) was intravenously injected into the jugular vein at 0800 and 1300 while an equal volume was applied to the vehicle. Blood samples (5 ml) were collected from the jugular vein at 1500 on both sampling days (Figure 3). Blood sample was then separated into three parts to measure blood gas parameters, to harvest plasma, and red blood cell lysate. The protocol of separation was detailed in the blood collection procedure. Both plasma samples and red blood cell lysate were used for GPx activity and MDA measurement while the cortisol level was measured using a plasma sample.

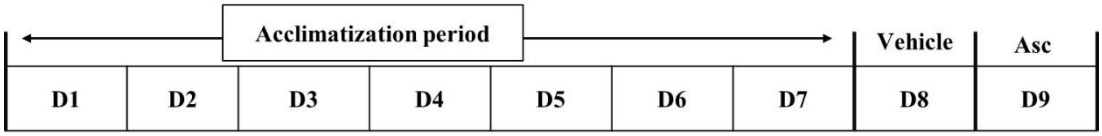


Figure 2 Experimental design of the first experiment.

Time	Experimental day		Sample collection (D8 & D9)
	D8	D9	
0800	Vehicle	Ascorbic acid	-
1300	Vehicle	Ascorbic acid	-
1500	-	-	Blood (5 ml)

Figure 3 Experimental procedure of 8th and 9th day of the first experiment.

Experiment 2: The effect of high DCAD and high dose ascorbic acid on mammary gland function.

The second experiment was planned to investigate the effect of high DCAD and Asc on the acid-base balance, electrolyte balance, and antioxidant capacity of lactating goats fed under HTa of the summer season. The second experiment lasted for 8 weeks, and the sample collection was carried out during both the 4^{th} and 8^{th} week of the experiment. The data from the 4^{th} week was used to monitor the effect of DCAD while the 8^{th} week was used to investigate the main hypothesis of the current study.

Animals

Twelve early lactating Saanen goats (n = 12) aged 3 years were included in this study and all goats were at second lactation. After parturition, all goats were fed in individual pens (90 × 200 cm) and were milked in the milking barn twice a day at 0600 and 1400. The concentrate was individually fed to goats twice a day after milking. The total amount of concentrate was calculated based on the MY, while water and pangola hay were individually provided ad libitum.

Experiment protocol

After the first two weeks of postpartum, all goats were randomly assigned into two groups, control group (n = 6) and the DCAD group (n = 6), where dairy goats were fed with a control and a high DCAD diet for 8 weeks. The Ta, RH, THI, Tr, and RR were measured three times a day while the concentrate intake, roughage intake, DMI, and WI were measured daily. These parameters were weekly averaged and used to evaluate the effect of DCAD (Figure 4). On the 4^{th} and 8^{th} week of the experimental period, the protocol of Asc supplement was applied on the last two days (45 mg/kg BW, (Chaiyowittayakun et al., 2002)), while both blood and urine samples were collected at 1500 to measure blood gas analysis, plasma cortisol, plasma electrolyte, and renal function (Figures 5 and 6). The blood gas and plasma electrolyte were including pH, PCO_2, HCO_3, glucose, Hct, plasma Na, plasma K, and plasma Cl. Regarding renal function, the plasma creatinine, blood urea nitrogen (BUN), endogenous creatinine clearance, net acid excretion (NAE), and fractional excretion of electrolytes were included. Besides, the plasma GPx

activity, plasma MDA, and Milk compositions were additionally measured on the 8th week of the experimental period.

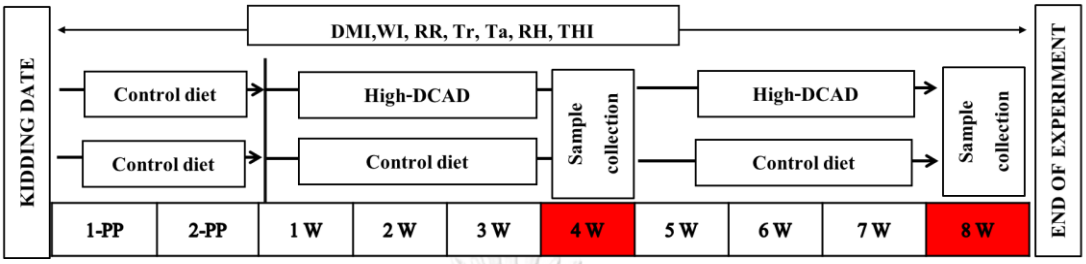


Figure 4 Experimental design of the second experiment

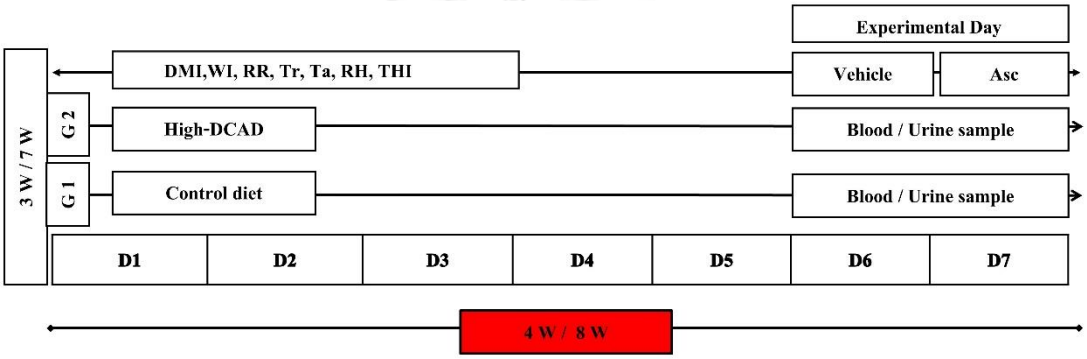


Figure 5 Experimental procedure in 4th and 8th week of experimental periods (2nd experiment).

Time	Experimental day		Sample collection (D6 & D7)
	D6	D7	
0800	Vehicle	Ascorbic acid	-
1300	Vehicle	Ascorbic acid	-
1500	-	-	Blood (5 ml) / Urine

Figure 6 The procedure of the experimental day (2nd experiment)

Feeding protocol

Sodium bicarbonate ($NaHCO_3$) and potassium carbonate (K_2CO_3) were mixed with the concentrate mixture to make a high DCAD diet. The percentage of $NaHCO_3$ and K_2CO_3 in the high DCAD diet were 0.62 and 1.24 g/100g DM, respectively while the control diet was a concentrate mixture without supplements. The total amount of concentrated was calculated based on the MY, while water and pangola hay were individually provided ad libitum. The chemical compositions of both pangolar and concentrate were presented in table1. The DCAD level in control and DCAD group were calculated based on the average hay intake and average concentrate intake of dairy goat during the 4^{th} and 8^{th} week of experiment. The calculated DCAD level in the diet of control and DCAD group were +15 and +40 milliequivalent per 100 DM (Figure 7).

Table 1 The chemical composition of diet

Feed composition (%)	Hay	Concentrate	
		Control	DCAD
Dry matter	92.68	91.26	87.76
Protein	4.2	16.1	15.5
Crude Fat	1.0	3.9	2.6
NDF	78.9	-	-
ADF	48.4	-	-
Ash	7.7	7.1	7.1
Ca	0.8	1.2	1.4
P	0.1	0.5	0.6
Na	14	1	13
K	36	32	57
Cl	9	15	17
S	12	12	8
DCAD† (mEq/100g DM)	29	6	45

†DCAD in milliequivalents of (Na+K) - (S+Cl)/ 100 g of DM.

DCAD, dietary cation anion difference

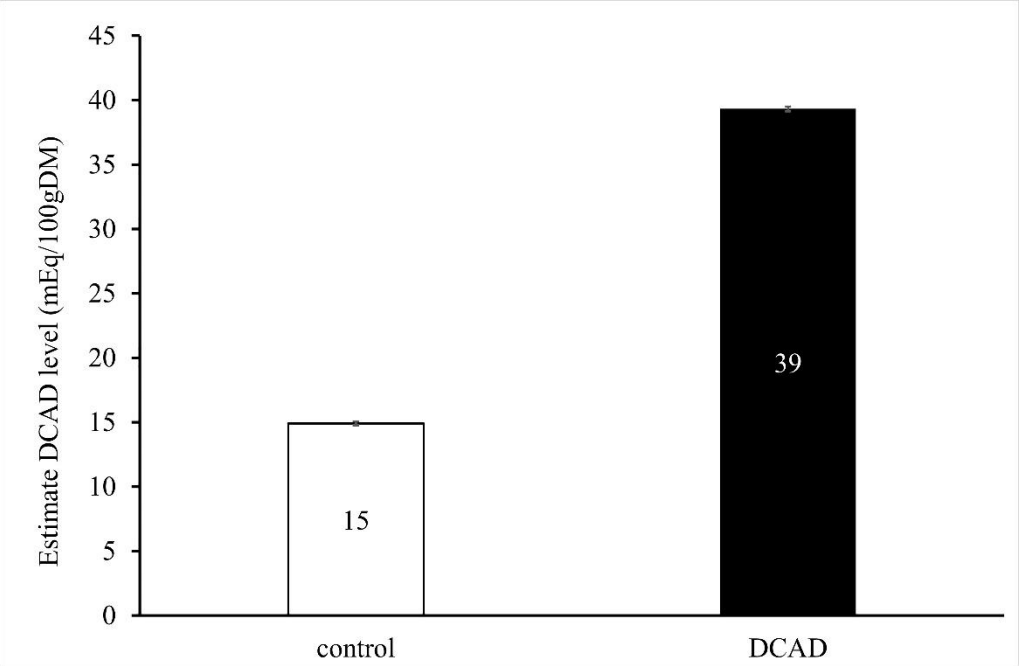


Figure 7 The calculated total DCAD level in the diet of the control and DCAD group

Note: Estimated DCAD level = (% DMI of Hay × DCAD level in Hay) + (% DMI of Concentrate × DCAD level in Concentrate)

Sample collection and measurements

Feed sample collection

Feed samples were collected for five consecutive days before the experiment day. Both concentrate and roughage were subsampled in the morning and stored at -20°C. Then, all samples were pooled to analyze feed composition.

Blood collection procedure

On both experimental days, blood samples (5 ml) were collected from the jugular vein in the afternoon (1500) and were separated into two parts. The first part was used to measure pH, PCO_2, HCO_3, and Hct by using an iSTAT blood gas analyzer (Abbott, New Jersey, USA) within 30 min of collection time. To obtain this part, blood was drawn using a 1 mL heparinized syringe with a cap and placed on crushed ice until analysis. The second part contained 4 mL blood stored in a lithium-heparin blood tube and this part was centrifuged at 1200 × g (4°C, 10 minutes) to harvest the plasma. To harvest red blood lysate, the blood sample (1 ml) of the second part was

washed three times with isotonic normal saline and subsequently lysed with sterile water. These samples including plasma and erythrocyte lysate were aliquoted and stored at – 80°C.

Urine collection procedure

Urine samples were collected via foley catheter connected to a urine collection bag (Figure 8). This urine bag was filled with liquid paraffin to preserve anaerobic condition during 0600-1500. To collect urine samples from the urine bag, urine was poured into a plastic bottle and weighed to determine the urine volume. The urine sample (250 ml) was aliquoted to the transfer bottle which was previously filled with mineral oil. Transfer bottles were placed on ice and transported to the laboratory within 30 minutes. The urine pH was measured using a pH meter (Digicon PH-218, Sangchai Meter Co. Ltd., Thailand) and the sample was stored at – 20 ºC for further analysis. These samples were used to measure the urine electrolyte, creatinine, and NAE.

Milk sample collection

To study the effect of daytime HTa on milk production, the yield of milk during the afternoon (0600-1400) was individually weighted and the yield of each composition was calculated based on afternoon milk yield. A milk sample (50 ml) was collected from each goat, and it was aliquoted into 2 plastic bottles and stored at -20°C until analysis. The first bottle was used for milk composition analysis by Milko Scan (FT2; Foss, Hilleroed, Denmark) and the parameters were fat, protein, lactose, citrate, and FFA. The yield of compositions was calculated using the 8-hr milk volume and this volume was used to calculate the 4% fat corrected milk (4%FCM; 8hr, 0600 -1400) based on the dairy goat formula (Mavrogenis and Papachristoforou, 1988). Milk from the second bottle was centrifuged at 300 x g, 4°C (Thermo Sorvall Legend XTR refrigerated centrifuge; Thermo Scientific, Osterode am Harz, Germany) for 10 minutes to obtain skim milk. Then, the skim milk was deproteinized by adding 6 N HCL and centrifuged for 5 minutes at 10,000 x g, 4°C. After discarding the pellet, the sample was neutralized by 6 N NaOH and centrifuged again for 5 minutes at 10,000 x g 4°C. The supernatant from this step was used for both glucose and G6P measurement.

Figure 8 The urine collection equipment with goat harness

Measurement of climate

The environmental temperature and relative humidity were recorded daily. The environment temperature was measured by the wet and dry bulb at 0600, 1300, and 1800 for the whole period. The temperature of the dry bulb is an environmental temperature while the relative humidity was calculated based on the difference between wet and dry bulb temperature. Then, THI was calculated based on the following equation (NRC, 1971).

$$THI = ((T_{db} + T_{wb}) \times 0.72) + 40.6$$

Where: T_{db} = dry bulb temperature

T_{wb} = wet bulb temperature

Measurement of HTa response

The HTa effect on physiological responses including Tr, RR, DMI, and WI was measured daily during the 4th and 8th week of experimental periods, and these were weekly averaged to monitor the effect of DCAD. The thermometer (digital clinical thermometer C202, Terumo, Tokyo, Japan) was placed against the rectum wall for 1 minute to measure Tr while the RR was measured by counting the movement of the chest. These parameters were measured at 0600, 1300, and 1800. To determine feed intake and WI, the weight of feed and water was subtracted by the weight of refusal. The value of DMI was calculated based on the dry matter feed.

Measurement of blood gas parameters and blood metabolites

The blood gas parameters include pH, PCO_2, HCO_3, glucose, and Hct using an iSTAT blood gas analyzer with an EG8+ iSTAT cartridge (Abbott, New Jersey, USA). Both creatinine and BUN were measured by using an automated analyzer (Mindray BS-800, Shenzhen Mindray Bio-Medical Electronics Co., Ltd., Shenzhen, China) while the plasma electrolytes including Na, K, and Cl were measured using a flame photometer (Flame photometer 410C, Ciba Corning Inc., Phoenix, AZ, USA) and a chlorimeter (chloride 925, Ciba Corning Inc., Phoenix, AZ, USA). Lastly, both plasma osmolarity and anion gap were calculated based on the following formulas (Stevens et al. 1994).

$$\text{Osmolarity} = 1.86 \times [Na^+ + K^+] + [\text{Glucose}/18] + [\text{BUN}/2.8]$$

$$\text{Anion gap} = [Na^+ + K^+] - [HCO_3^- + Cl^-]$$

Measurement of plasma cortisol concentration

The cortisol concentration was measured using an enzyme-linked immunosorbent assay (ELIZA) kit (CBS-E18048G, CUSABIO, Houston, USA). In principle, the plasma cortisol competed with the pre-coated antigen and the strong reaction indicated a lower amount of plasma cortisol. To measure cortisol, 50 μL of each standard and sample were mixed with an equal amount of antibody in wells. This mixture was incubated at 37°C for 40 minutes and the wells were washed three times using a washing buffer. To visualize, HRP-conjugated (100 μL) was

added to each well and the reaction was incubated again at 37°C for 40 minutes. The second washing step was repeated as a previous protocol. Then, 90 µL of a substrate (TMB) was added and incubated at 37°C for 20 minutes. Lastly, the reaction was ended by adding a stop solution and the optical density of each reaction was determined by a spectrophotometer at 450 nm.

Measurement of creatinine clearance

The creatinine clearance of lactating goats was calculated based on the urine volume, plasma, and urine creatinine concentration. The concentration of creatinine in both urine and plasma samples was determined by an automated analyzer (Mindray BS-800, Shenzhen Mindray Bio-Medical Electronics Co., Ltd., Shenzhen, China). Then, the endogenous creatinine clearance was calculated using the following equation (Do Nguyen et al., 2022).

Equation: Creatinine clearance = $(U_{cr} \times V) / P_{cr}$

Where; U_{cr}= Urinary creatinine concentration (mmol/L)

P_{cr}= Plasma creatinine concentration (mmol/L)

V = Urine volume (ml/min)

Measurement of fraction excretion

The fractional excretion (FE) was calculated using the concentration of electrolytes and creatinine in both urine and blood sample. The concentration of potassium and sodium in urine was measured by a flame photometer (Flame photometer 410C, Ciba Corning Inc., USA). The chloride and creatinine were determined by a chlorimeter (Chloride 925, Ciba Corning Inc., USA) and an automated analyzer (Mindray BS-800, Shenzhen Mindray Bio-Medical Electronics Co., Ltd., Shenzhen, China). Then, FE of sodium, potassium, and chloride were calculated based on the following equations (Do Nguyen et al., 2022).

Equation 1: $FE_{Na}(\%) = [(U_{Na} \times P_{cr}) / (P_{Na} \times U_{cr})] \times 100$

Equation 2: $FE_{K}(\%) = [(U_{K} \times P_{cr}) / (P_{K} \times U_{cr})] \times 100$

Equation 3: $FE_{Cl}(\%) = [(U_{Cl} \times P_{cr}) / (P_{Cl} \times U_{cr})] \times 100$

Where; U_{Na}= Urinary sodium concentration

U_{K} = Urinary potassium concentration

U_{Cl}= Urinary chloride concentration

U_{cr}= Urinary creatinine concentration

P_{Na}= Plasma sodium concentration

P_K = Plasma potassium concentration

P_{Cl}= Plasma chloride concentration

P_{cr}= Plasma creatinine concentration

Unit of both P_x , U_x ; mmol/L

Measurement of urinary acid excretion

The acid excretion was determined by the concentration of titratable acid, and ammonium. The urine sample (1 ml) was mixed with 0.1 N HCL and boiled in water for 2 min to remove CO_2. This mixture was titrated by 0.1 N NaOH to pH 7.4 at 37ºC. while the blank (distilled water) was repeated with the same procedure. The volume of NaOH used for the sample was subtracted from the blank and its result was a titratable acid (TA). The titrated sample was further added with 1 ml of 8% formaldehyde and was titrated again by NaOH. Again, all procedure was repeated with the blank as the control. The difference in the volume of NaOH between the sample and the blank was acid excretion. TA was subtracted by acid excretion to calculate a concentration of ammonium excretion (NH_4). Then, the concentration of TA and NH_4 was calculated as the following equation while the summation of these values was a net acid excretion (NAE) (Chan, 1972).

Concentration (mmol/L) = d V_{NaOH} × 0.1 N NaOH mol/L × mmol/Eq.

NAE (mEq /day) = (U_{NH4} × V) + (U_{TA} × V)

Where: d V_{NaOH} = difference in a volume of NaOH between sample and blank

U_{NH4} = concentration of NH_4^+

U_{TA} = concentration of titratable acid

V = urine volume (8-hour)

Measurement of malondialdehyde concentration

MDA is a product of lipid peroxidation which is an oxidative stress marker. The concentration of MDA was determined using a colorimetric assay (ab118970, Abcam, Oregon,

USA). The high plasma MDA formed a high level of colored product (MDA-Thiobarbituric acid adduct) which was measured by a spectrophotometer. The sample (20 µL) was mixed with 500 µL of 42 nM H_2SO_4 and 125 µL of the phosphotungstic acid solution. This solution was mixed using vertex and incubated at room temperature for 5 minutes. To separate pellets, this mixture was centrifuged at 10,000 rpm for 3 minutes and the harvested pellets were suspended with 100 µL ddH_2O. The volume of suspension was adjusted to 200 µL by ddH_2O. The 200 µL of the prepared sample was mixed again with 600 µL of TBA reagent in a microcentrifuge tube. This mixture was incubated at 95°C for 60 minutes and cooled with an ice bath for 10 minutes. The change of color was measured by a spectrophotometer at 532 nm.

Measurement of Glutathione peroxidase activity

The activity of GPx was determined by colorimetric assay (ab102530, Abcam, Oregon, USA). In principle, the GPx reduces ROS and oxidized glutathione. This oxidized glutathione is then reduced by glutathione reductase and NADPH. These reactions could be visualized by measurement of the depleted NADPH. Briefly, 50 µL of the sample was mixed with 40 µL of the reaction mixture. This reaction mixture consisted of 33 µL assay buffer, 3 µL 40 mM NADPH solution, 2 µL glutathione reductase solution, and 2 µL glutathione solution. The reaction was incubated at room temperature for 15 minutes and the cumene hydroperoxide solution was added. The first absorbance (A1) of the reaction was read at 340 nm and the same reaction was incubated again at room temperature for 5 minutes. The second absorbance (A2) was measured at 340 nm. The GPx activity was stepwise calculated as the following equation and the reduction rate of the substrate of this kit represented this enzyme activity.

Equation 1: ΔA340 nm = ((sample A1 - sampleA2) – (blank A1 – blank A2))

Equation 2: B (nmol) = (ΔA340 nm – intercepts) / slope

Equation 3: GPx activity (nmol/minute/ml) = [B / ((T1-T2) × V)] × D

Where: A= Absorbance

B = NADPH amount that was decreased between T1 and T2 (nmol)

T1 = Time of first reading (A1, minutes)

T2 = Time of second reading (A2, minutes)

V = Pretreated sample volume added into the reaction well (ml)

D = Sample dilution factor

Measurement of glucose concentration

The concentration of glucose was measured by colorimetric assay (EnzyChromTM Glucose assay kits (EBGL-100); BioAssay System, Hayward, USA). In principle, free glucose was changed to D-gluconate and hydrogen peroxide by glucose oxidase. Then, the reaction between hydrogen peroxide and peroxidase reagent turned substrates into quinonimine dye. In brief, 20 µL of standards and samples were added into separate wells. The reaction buffer (80 µL) which consists of assay buffer, enzyme mix, and dye reagent was added into well and incubated at room temperature for 30 minutes. The developed color was measured by spectrophotometer at 570 nm and the glucose concentration was calculated as this equation.

Equation: Glucose (µM) = $[(A_{sample} - A_{Blank}) / \text{slope } (\mu M)] \times N$

Where; A = Absorbance

N = Sample dilution factor

Measurement of glucose-6-phosphate concentration

The concentration of G6P was determined by colorimetric assay (EnzyChromTM Glucose-6-Phosphate assay kit (EG6P-100); BioAssay System, Hayward, USA). In principle, G6P reacts with G6P dehydrogenase and forms NADPH. The produced NADPH was coupled with formazan (WST8) chromogen and this colored product was measured by spectrophotometer at 460 nm. The milk serum sample (20 µL) was mixed with a working reaction (80 µL) in a well. This working reaction consisted of assay buffer, NADP/WST8, enzyme A, and enzyme B. After mixing, this reaction was incubated at room temperature for 20 minutes and this incubation was protected from the light. Then, the absorbance was measured at 460 nm and the concentration of G6P was calculated based on the following equation.

Equation: G6P (µM) = $[(A_{sample} - A_{Blank}) / \text{slope } (\mu M)] \times N$

Where; A = Absorbance

N = Sample dilution factor

Measurement of DCAD level of diet

The DCAD level was calculated based on the milliequivalent of Na, K, Cl, and sulfate in both concentrate and pangola hay. The total amount of each element in the concentrate mixture, pangola hay was used to calculate the milliequivalent per each 100 g dry matter (DM). The calculation of the milliequivalent of each element per 100 g of DM was based on the following equation.

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

Statistical analysis

The result presented in mean ± standard error. THI, DMI, WI, RR, and Tr reported as a weekly mean. All data were statistically analyzed by GraphPad Prism 8. In the first experiment, the effect of high dose Asc supplement on RR and Tr during the daytime were analyzed with repeated measure two-way analysis of variance while the effect of this supplement on blood gas parameters, plasma electrolyte, plasma oxidative stress, and cortisol in the afternoon was tested by student's paired t-test. To evaluate the effect of DCAD on HTa response, the RR and Tr during the daytime were tested with repeated measure two-way analysis of variance while the effect of this supplement on DMI and WI was tested by student's t-test. Then, the effect of both high DCAD and high dose Asc supplement on blood gas, plasma electrolytes, plasm oxidative stress, renal function, MY, and milk composition in the afternoon was analyzed by repeated measure two-way analysis of variance. The Dunnett's test was used for post-hoc analysis of RR and Tr while other parameters were tested with Bonferroni test. The significance was declared at P value < 0.05.

CHAPTER IV

RESULTS

Experiment 1: The effect of high dose Asc supplement on HTa responses, plasma oxidative stress, plasma electrolytes, and plasma cortisol of non-lactating dairy goats.

1.1 Ambient condition and the effect of high dose Asc supplement on RR and Tr during daytime HTa

On the vehicle-supplemented day, Ta, RH, and THI in the morning (0600) were 24°C, 92%, and 73, while these parameters in the afternoon (1300) were 32°C, 70%, and 82. In the evening (1800) of this day, Ta, RH, and THI were 33°C, 68%, and 85. On the Asc supplemented day, Ta at 0600, 1300, and 1800 was 26°C, 36°C, and 38°C while the RH was 92%, 59%, and 34%. The calculated THI of this day at 0600, 1300, and 1800 were 73, 82, and 85, respectively. In terms of HTa response, HTa significantly affected both Tr and RR while the high dose Asc supplement did not affect these parameters (Figure 9 and 10). Both Tr and RR at 1300 and 1800 were significantly higher when compared to 0600.

1.2 The effect of high dose Asc supplements on blood gas parameters, serum chemistries, plasma electrolytes, plasma, and red blood cell lysate oxidative stress.

According to the results, the high dose supplement did not affect most of parameters but the PCO_2 was affected by this supplement (Table 2). These unaffected parameters were blood pH, blood HCO_3, anion gap, plasm sodium, plasma potassium, BUN, blood glucose, Hct, and plasma cortisol level (Table 2 and 3). Interestingly, this high dose Asc supplement tended to affect the plasma Cl and Hct (Table 3). Both PCO_2 and Hct were decreased while plasma chloride concentration was increased by the high dose Asc supplement. However, the high dose of the antioxidant Asc supplement did not significantly change GPx activity and MDA level in both plasma and red blood cell lysate (Table 4).

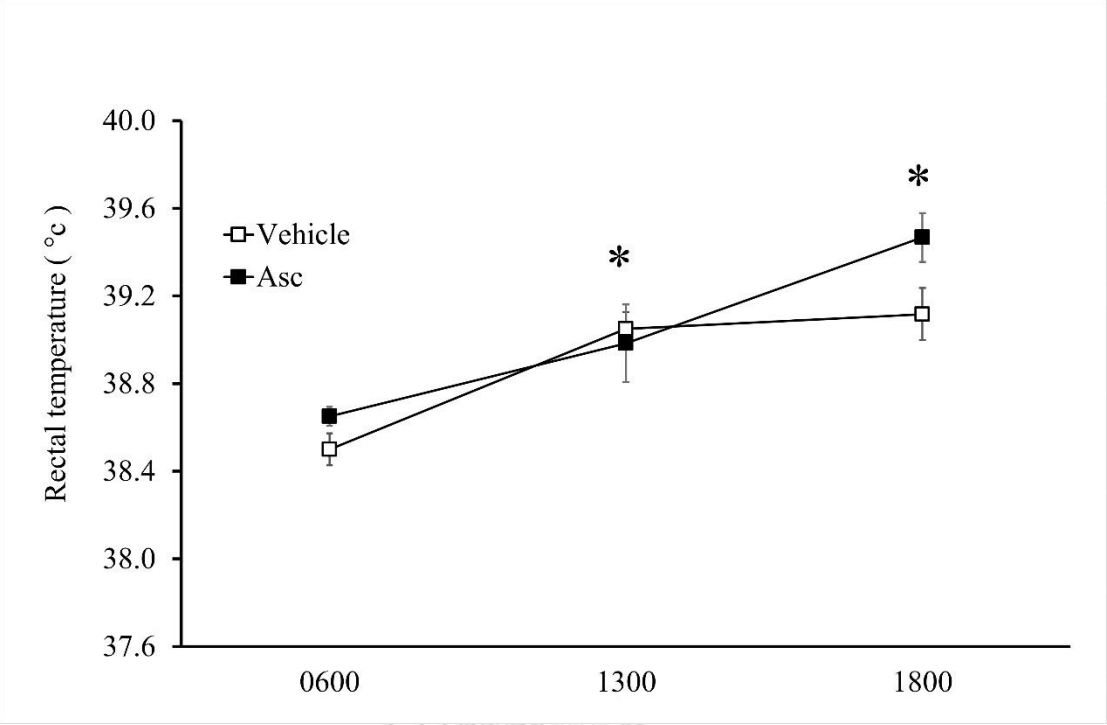


*Figure 9 The effect of high dose Asc on Tr of non-lactating dairy goat during the daytime HTa. Note: The open and close square indicated the vehicle and Asc supplemented groups, respectively. * The significant difference in Tr compared to morning time point (0600, P<0.05).*

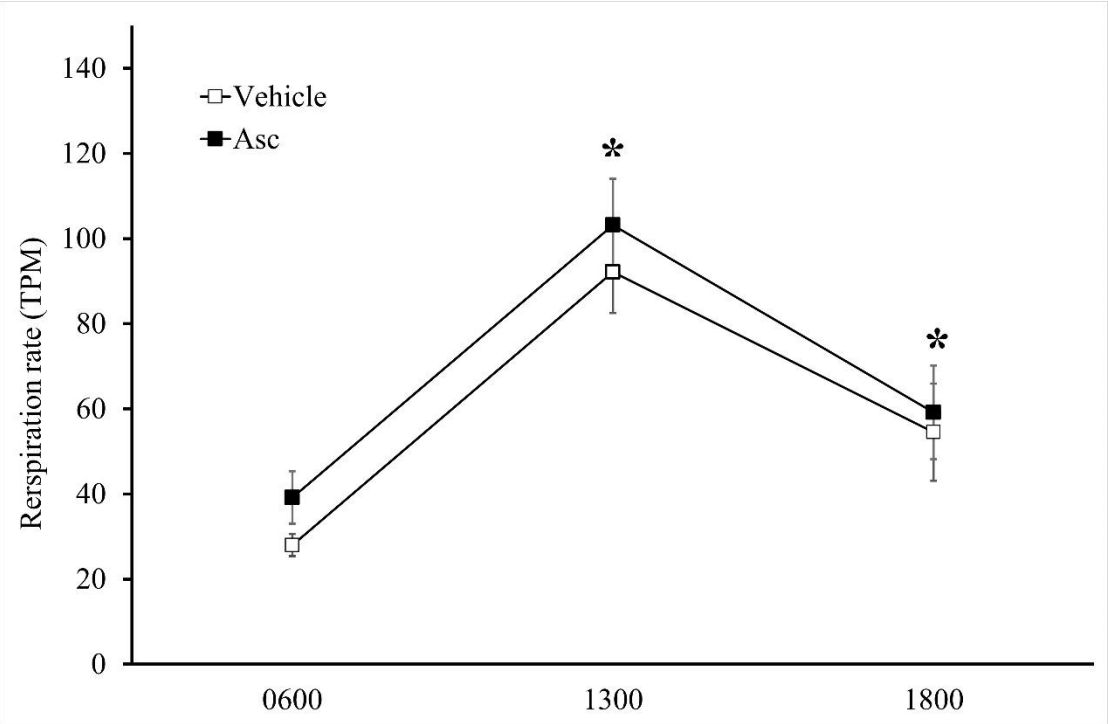


*Figure 10 The effect of high dose Asc on RR of non-lactating dairy goat during the daytime HTa. Note: The open and close square indicated the vehicle and Asc supplemented groups, respectively. * The significant difference in RR compared to morning time point (0600, P<0.05).*

Table 2 The average blood gas parameters of the first experiment

Items	Treatment		P-Value
	Vehicle	Asc	
pH	7.44±0.01	7.46±0.01	0.238
HCO_3 (mmol/L)	26±0.9	25±0.7	0.362
PCO_2 (mmHg)	39±1.4	35±0.8	0.024
Anion Gap (mmol/L)	30±4.7	25±3	0.298

Table 3 The averaged plasma electrolytes and blood chemistries of the first experiment.

Items	Treatment		P-Value
	Vehicle	Asc	
Sodium (mmol/L)	150±2.8	148±2.1	0.695
Potassium (mmol/L)	4.2±0.14	4.1±0.15	0.382
Chloride (mmol/L)	97±2.6	103±0.8	0.079
BUN (mg/dL)	12±1.01	10±0.52	0.108
Blood Glucose (mg/dL)	73±1.6	74±2.2	0.788
Osmolarity (mOsm/kg)	294±5.4	291±4.4	0.605
Hct (%)	21±0.7	19.3±0.3	0.076
Cortisol (ng/mL)	92±24.5	101.3±18.6	0.522

Table 4 The average GPx activity and MDA in plasma and red blood cell of the first experiment.

Items	Treatment		P-Value
	Vehicle	Asc	
Plasma oxidative stress			
• GPx activity (nmol/min/ml)	130.69±9.91	131.26±8.03	0.949
• MDA (nmol/ml)	19.63±1.01	19.98±0.71	0.636
RBC lysate oxidative stress			
• GPx activity (nmol/min/Hct)	11.79±0.52	14.39±1.93	0.146
• MDA (nmol/gHb)	0.25±0.04	0.25±0.01	0.933

Experiment 2: The effect of high DCAD and high dose Asc supplement on HTa response, plasma oxidative stress, acid-base balance, electrolyte balance, and mammary gland function

2.1 Ambient conditions and the effect of high DCAD on HTa response

On the 4th week of the experimental period, the average Ta and THI were increased while the RH was decreased during the daytime (Table 5). The highest Ta was observed at 1300 and the difference in Ta (ΔTa) between the morning (0600) and afternoon (1300) was 10°C. An increased Ta significantly affected both Tr and RR of lactating goats by this week. These parameters at 1300 and 1800 were significantly higher when compared to those parameters at 0600 (Figure 11 and 13). Regarding DCAD, this supplement did not affect the HTa response excluding RR (Table 6, Figure 11 and 13). The RR of the DCAD group at 1300 was higher when compared to the control group at the same time.

On the 8th week of the experimental period, the changing pattern of Ta, RH, and THI during the daytime was similar to the ambient condition of the 4th week of the experimental period. Again, the highest Ta was observed at 1300 and ΔTa was 7°C. During the daytime, an increased Ta did affect both Tr and RR. Like the 4th week of experimental period, these parameters at 1300 and 1800 were significantly higher when compared to morning (0600; Figure 12 and 14). However, the effect of DCAD on RR and other HTa responses was not observed on the 8th week of the experimental period (Table 6, Figure 12, and 14).

Table 5 The average Ta, RH, and THI of the 4th and 8th week of the second experimental period.

	Time of the day		
	0600	1300	1800
4th week of the experimental period			
Ta (°C)	25±0.5	35±0.6	34±0.9
RH (%)	92±0.9	62±0.5	66±3.1
THI	76±0.7	87±0.8	85±0.8
8th week of the experimental period			
Ta (°C)	25±0.3	32±1.0	31±0.7
RH (%)	91±0.5	70±2.2	74±2.0
THI	76±0.4	83±1.1	83±0.7

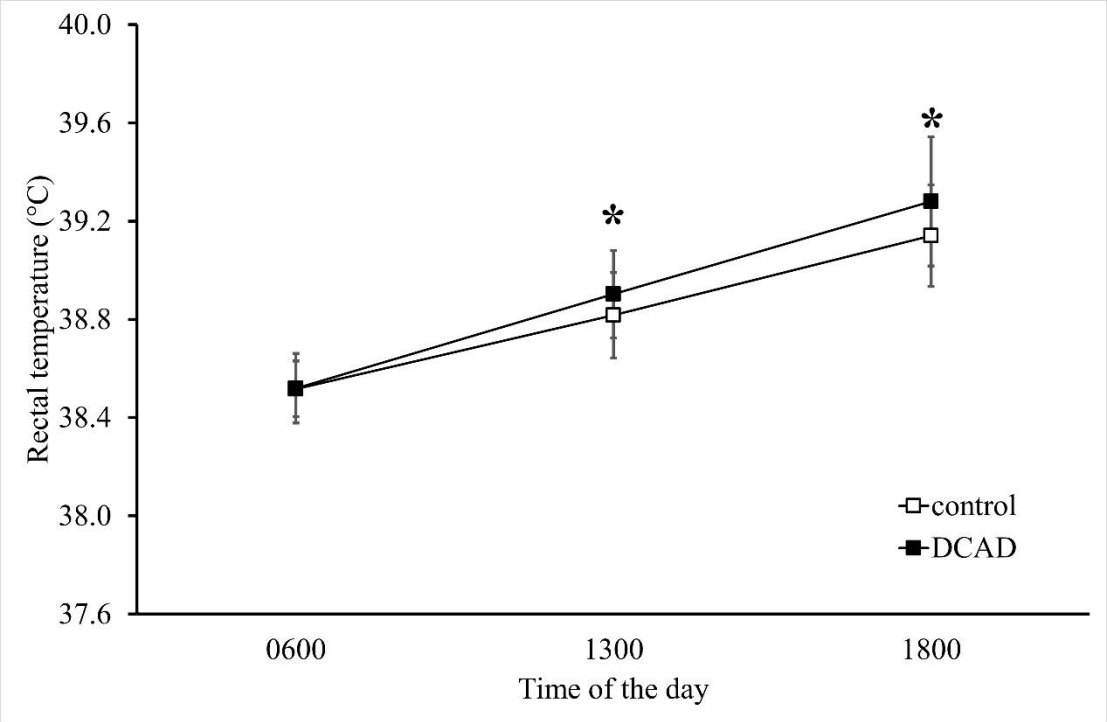


Figure 11 The effect of DCAD on Tr during the daytime HTa of 4th week of second experimental period.

Note: The open and close square indicated the control and DCAD groups, respectively.

** The significant difference in Tr compared to morning time point (0600, P<0.05).*

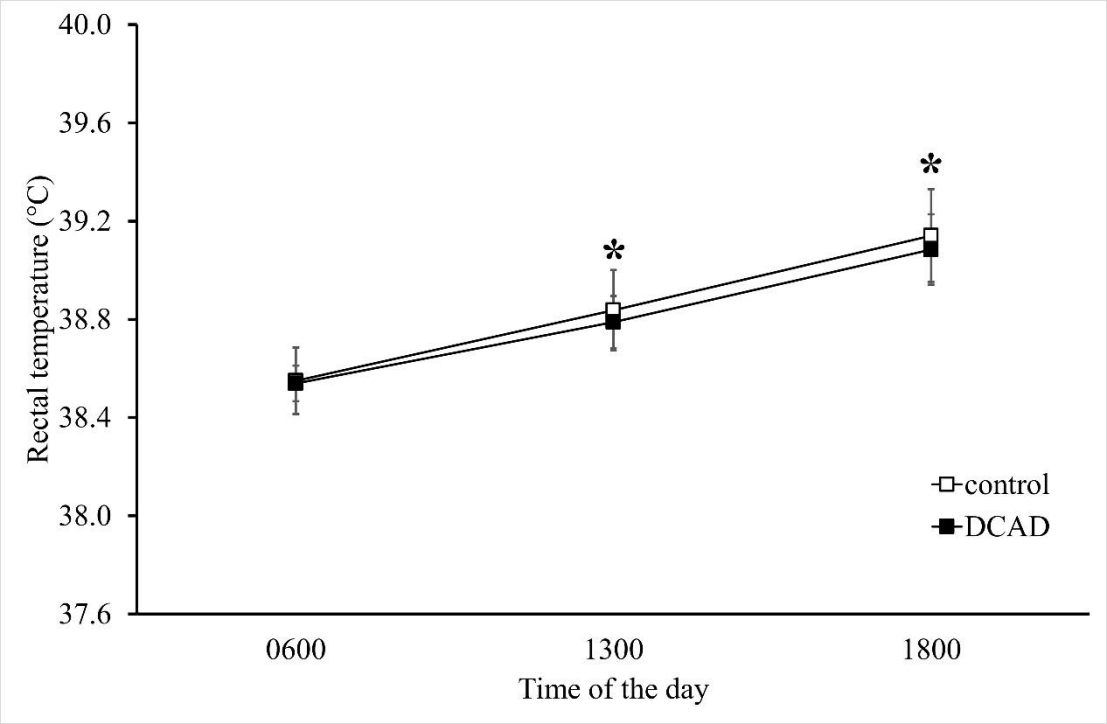


Figure 12 The effect of DCAD on Tr during the daytime HTa of 8th week of second experimental period.

Note: The open and close square indicated the control and DCAD groups, respectively.

** The significant difference in Tr compared to morning time point (0600, P<0.05).*

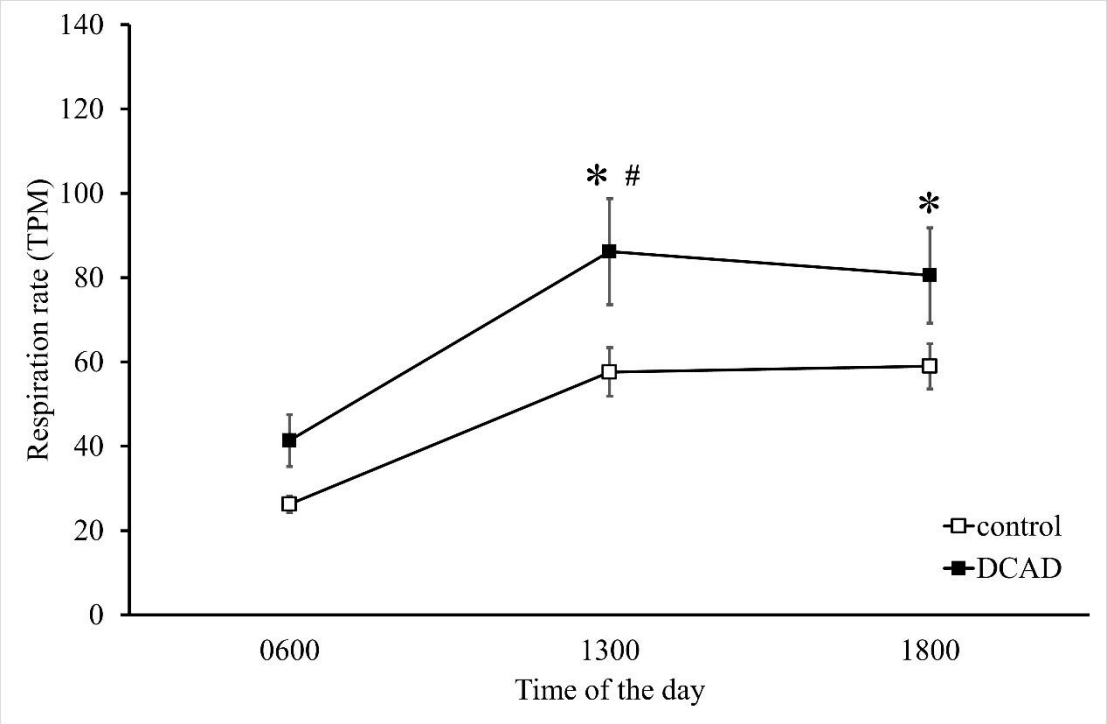


Figure 13 The effect of DCAD on RR during the daytime HTa of 4th week of second experimental period.

*Note: The open and close square indicated the control and DCAD groups, respectively. * The significant different RR when compared to morning time point (0600, P<0.05). # This indicates the significant difference between DCAD and control group at each time point.*

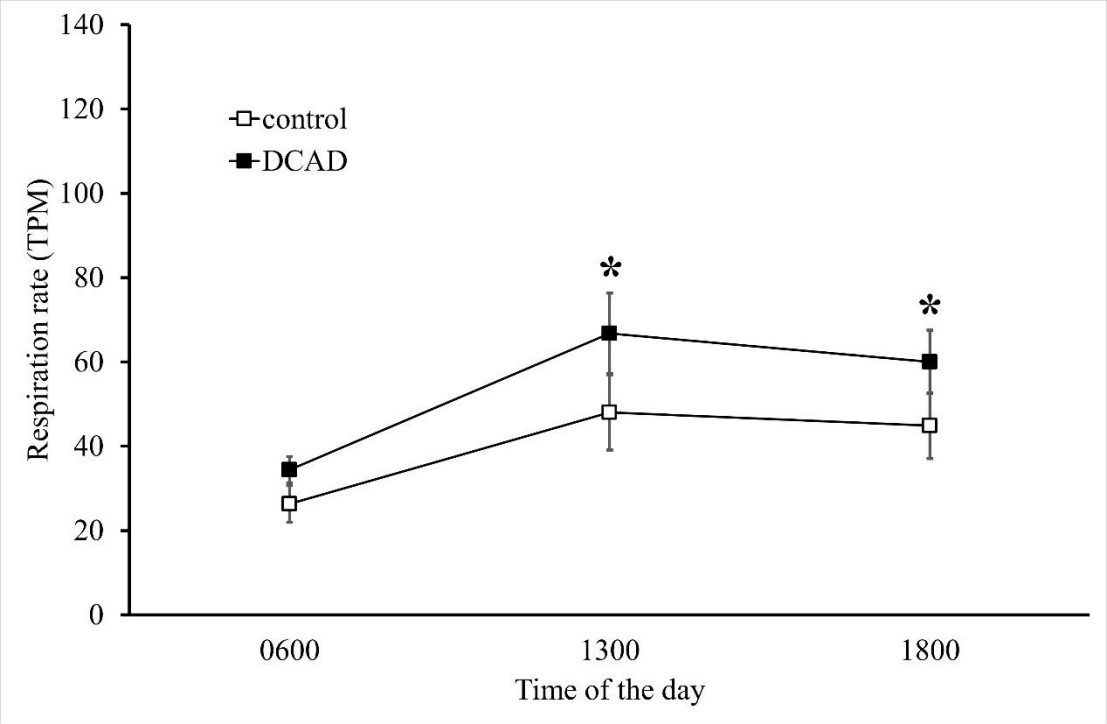


Figure 14 The effect of DCAD on RR during the daytime HTa of the 8th week of second experimental period.

Note: The open and close square indicated the control and DCAD groups, respectively.

** The significant different RR when compared to morning time point (0600, P<0.05).*

Table 6 The effect of DCAD on average concentrate intake, roughage intake, dry matter intake, and water intake of the 4th and 8th week of the second experimental period.

Items	Treatment		P-Value
	Control	DCAD	
4th week of the experimental period			
Concentrate Intake (%kgBW)	2.0±0.2	2.2±0.3	0.641
Roughage Intake (%kgBW)	1.2±0.2	1.3±0.1	0.820
DMI (%kgBW)	3.3±0.3	3.5±0.2	0.615
WI (ml/kgBW)	164.0±20.4	157.5±17.6	0.812
8th week of the experimental period			
Concentrate Intake (%kgBW)	2.0±0.3	2.1±0.2	0.629
Roughage Intake (%kgBW)	1.3±0.1	1.5±0.1	0.335
DMI (%kgBW)	3.3±0.3	3.6±0.2	0.454
WI (ml/kgBW)	132.5±26.7	131.4±16.9	0.975

2.2 The effect of high DCAD and high dose Asc supplement on acid-base balance

On the 4th week of the experimental period, blood pH was significantly affected by a high DCAD supplement, and this supplement tended to affect blood HCO_3 (Table 7). Both blood pH and HCO_3 of the DCAD group were higher than the control group, while other blood gas parameters were not changed. Regarding Asc, blood gas parameters were not affected but the high dose Asc supplement tended to affect urine pH (Table 7).

On the 8th week of the experimental period, the significant effect of DCAD was no longer observed on blood gas parameters but it tended to affect the urine pH (Table 7). The presence of high DCAD increased the urine pH. In contrast, the high dose Asc supplement significantly affected urine pH (Table 7), and the presence of high dose Asc decreased urine pH. Moreover, there was an interaction effect on urine pH where the lowest urine pH was founded in the Asc-treated control group. The percentage reduction of urine pH by Asc was in line with the interaction effect where the higher reduction was founded in the control group (Figure 15).

Table 7 The average blood gas parameters and urine pH at 1500 of the 4th and 8th week of the second experimental period.

	Control	DCAD	Vehicle	Asc	Control		DCAD		SEM	P-Value		
					Vehicle	Asc	Vehicle	Asc		DCAD	Asc	DCAD×Asc
4th week of the experimental period												
Blood pH	7.44 [b]	7.50 [a]	7.46	7.47	7.43	7.44	7.50	7.49	0.03	0.02	0.69	0.24
PCO_2 (mmHg)	34.59	34.28	34.89	33.98	35.37	33.82	34.42	34.15	2.54	0.85	0.40	0.55
HCO_3 (mmol/L)	23.02	26.39	25.32	24.09	23.35	22.68	27.28	25.50	2.65	0.09	0.28	0.62
Urine pH	8.11	8.10	8.14	8.07	8.17	8.06	8.13	8.08	0.1	0.9	0.06	0.47
8th week of the experimental period												
Blood pH	7.48	7.50	7.47	7.49	7.47	7.48	7.48	7.50	0.03	0.46	0.18	0.74
PCO_2 (mmHg)	35.01	36.16	35.73	35.43	35.02	35.0	36.45	35.87	1.57	0.36	0.65	0.67
HCO_3 (mmol/L)	25.86	27.67	26.40	27.13	25.48	26.23	27.32	28.02	1.70	0.31	0.32	0.97
Urine pH	8.13	8.27	8.26 [a]	8.14 [b]	8.22 [a]	8.04 [b]	8.31 [a]	8.24 [a]	0.05	0.05	0.0003	0.025

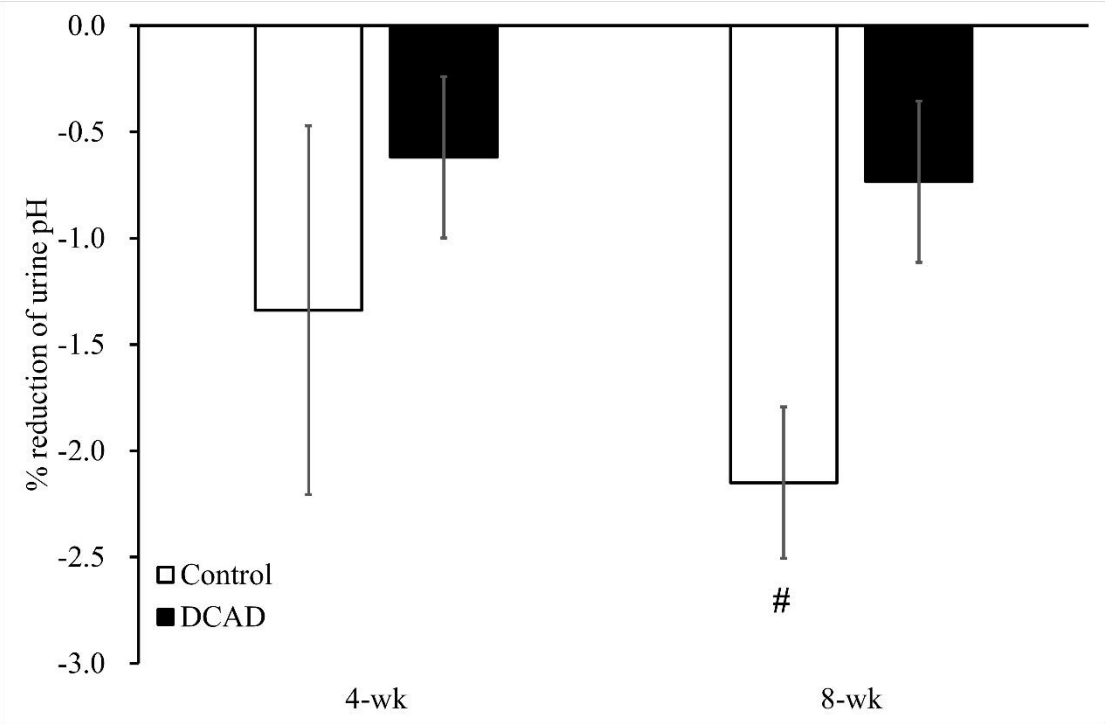


Figure 15 The effect of DCAD on % reduction of urine pH by high dose Asc supplement of 4th and 8th week of second experimental period.

Note: White and black bar indicated the control and DCAD groups, respectively. # The significant difference between control and DCAD which was determined using Student's t-test (P<0.05).

2.3 The effect of high DCAD and high Asc supplement on electrolyte balance and renal function

On the 4th week of the experimental period, a high DCAD supplement had no effect on plasma electrolyte and serum chemistries while the high dose Asc supplement did significantly affect the anion gap and blood glucose (Table 8). The anion gap of Asc treated group was significantly higher than the vehicle treated group, while the blood glucose of Asc was significantly lower when compared to the vehicle treated group. Regarding renal function, high DCAD significantly affected TA and NAE, where the parameters of DCAD groups were lower than the control group (Table 10). With Asc, the high dose supplement did significantly affect the urine volume, TA, and NAE. These parameters of the Asc-treated group were higher than the vehicle treated group and there was an interaction effect between these supplements on TA and NAE (Table 10).

On the 8th week of the experimental period, a significant effect of DCAD was only found on BUN where this parameter of the DCAD group was lower than the control group (Table 9). With Asc, the high dose supplement did affect the plasma creatinine, and Hct (Table 9). These parameters were lower in the Asc-treated group when compared to the vehicle-treated group. The interaction of both supplements did significantly affect the creatinine (Table 9). The high dose Asc did decrease plasma creatinine in both the control and DCAD groups, but this effect was more pronounced in the presence of high DCAD supplement. In terms of renal function, the high DCAD supplement significantly affected FE_{Na}, FE_K, TA, and NAE while the effect of high dose Asc supplement was found on urine volume, creatine clearance, FE_K, TA, NH_4, and NAE (Table 11). With high DCAD supplement, FE_{Na} and FE_K were higher than the control group, while both TA and NAE in the DCAD group were lower when compared to the control group. In contrast, the high dose Asc supplement did increase TA, NH_4, and NAE, while this supplement decreased FE_K. Besides, both urine volume and creatinine clearance were higher in the Asc-treated group when compared to the vehicle-treated group. There was no interaction effect on renal function (Table 11).

Table 8 The effect of high DCAD and high Asc supplement on averaged plasma electrolyte and serum chemistries of the 4th week of the second experimental period.

	Control	DCAD	Vehicle	Asc	Control		DCAD			P-Value		
					Vehicle	Asc	Vehicle	Asc	SEM	DCAD	Asc	DCAD×Asc
Na (mmol/L)	141.5	144.8	142.8	143.4	145.35	148.27	145.03	144.47	4.13	0.092	0.744	0.516
K (mmol/L)	3.38	3.06	3.29	3.15	3.32	3.45	3.27	2.85	0.35	0.079	0.338	0.079
Cl (mmol/L)	99.83	97.36	98.83	98.36	100.00	99.67	97.67	96.20	2.57	0.454	0.676	0.902
Anion Gap (mmol/L)	27.38	25.51	24.37[b]	28.51[a]	25.38	29.37	23.35	27.74	3.14	0.239	0.012	0.903
Osmolarity (msOm/kg)	288.00	283.10	284.80	286.30	285.23	290.69	284.33	281.92	7.35	0.276	0.623	0.219
Creatinine (mg/dL)	0.78	0.69	0.75	0.72	0.80	0.77	0.70	0.68	0.05	0.339	0.209	0.664
BUN (mg/dL)	13.67	12.75	13.33	13.08	13.50	13.83	13.17	12.33	2.53	0.709	0.814	0.585
Glucose (mg/dL)	65.88	65.68	68.01[a]	63.56[b]	67.80	63.97	68.21	63.14	2.03	0.921	0.0003	0.474
Hct (%)	26.9	25.4	25.9	26.3	26.4	27.3	25.5	25.3	2.58	0.557	0.758	0.592

Table 9 The effect of high DCAD and high Asc supplement on averaged plasma electrolyte and serum chemistries of the 8th week of the second experimental period.

	Control	DCAD	Vehicle	Asc	Control		DCAD			P-Value		
					Vehicle	Asc	Vehicle	Asc	SEM	DCAD	Asc	DCAD×Asc
Na (mmol/L)	149.10	149.70	147.90	150.90	148.02	150.08	147.75	151.65	4.49	0.666	0.135	0.626
K (mmol/L)	3.49	3.63	3.48	3.63	3.38	3.60	3.58	3.67	0.25	0.548	0.170	0.526
Cl (mmol/L)	100.8	100.7	100.3	101.3	100.83	100.83	99.67	101.67	3.11	0.946	0.449	0.449
Anion Gap (mmol/L)	14.55	13.46	15.00	13.01	14.57	14.53	15.43	11.48	2.63	0.526	0.093	0.098
Osmolarity (msOm/kg)	271.50	272.60	173.10	271.00	270.94	272.15	275.18	269.94	3.80	0.685	0.223	0.065
Creatinine (mg/dL)	0.73	0.65	0.73^{a}	0.66^{b}	0.75^{a}	0.72^{bc}	0.70^{ab}	0.60^{c}	0.03	0.415	0.001	0.010
BUN (mg/dL)	14.42^{a}	10.50^{b}	12.50	12.42	14.50	14.33	10.50	10.50	0.97	0.034	0.838	0.838
Glucose (mg/dL)	66.36	65.54	66.43	65.47	66.98	65.75	65.88	65.20	2.21	0.420	0.313	0.767
Hct (%)	24.5	23.1	24.4^{a}	23.2^{b}	25.2	22.5	23.7	22.5	1.18	0.410	0.031	0.933

Table 10 The effect of high DCAD and high dose Asc supplement on renal function of the 4th week of the second experimental period.

	Control	DCAD	Vehicle	Asc	Control		DCAD		SEM	P-Value		
					Vehicle	Asc	Vehicle	Asc		DCAD	Asc	DCAD×Asc
Urine Volume (8-hr, ml/min)	1.38	1.13	1.06[b]	1.45[a]	1.16	1.61	0.96	1.30	0.42	0.534	0.042	0.746
Creatinine clearance (ml/min)	71.88	52.48	54.65	69.71	60.52	83.23	48.78	56.18	34.25	0.323	0.306	0.596
Fractional excretion, %												
• Na	1.39	2.16	1.77	1.78	0.67	2.10	2.87	1.46	1.90	0.352	0.991	0.096
• K	69.54	119.60	104.20	84.94	60.77	78.31	147.64	91.57	66.26	0.076	0.493	0.204
• Cl	0.17	0.31	0.26	0.23	0.18	0.16	0.34	0.08	0.15	0.540	0.575	0.771
Acid excretion (mmol/L)												
• TA	-129.70[a]	-227.70[b]	-197.90[b]	-159.50[a]	-130.00[a]	-129.33[a]	-265.75[b]	-189.67[b]	39.38	0.047	0.038	0.041
• NH_4	1.08	1.00	1.25	0.83	1.50	0.67	1.00	1.00	0.58	0.913	0.111	0.111
• NAE	-128.6[a]	-266.70[b]	-196.6[b]	158.7[a]	-128.50[a]	-128.67[a]	-264.75[b]	-188.67[b]	32.29	0.044	0.039	0.039

Table 11 The effect of high DCAD and high dose Asc supplement on renal function of the 8th week of the second experimental period.

	Control	DCAD	Vehicle	Asc	Control		DCAD			P-Value		
					Vehicle	Asc	Vehicle	Asc	SEM	DCAD	Asc	DCAD×Asc
Urine volume (8-hr, ml/min)	1.09	0.99	0.95[b]	1.13[a]	1.06	1.13	0.84	1.14	0.19	0.770	0.046	0.166
Creatinine clearance (ml/min)	53.15	52.19	49.05[a]	56.29[b]	51.39	54.91	46.71	57.67	6.49	0.894	0.021	0.190
Fractional excretion, %												
• Na	0.55[b]	1.59[a]	1.00	1.13	0.53	0.56	1.48	1.71	0.21	0.006	0.162	0.254
• K	55.75[b]	79.00[a]	75.07[a]	59.68[b]	63.03	48.47	87.12	70.88	9.38	0.002	0.002	0.831
• Cl	0.14	0.20	0.18	0.16	0.17	0.11	0.19	0.21	0.07	0.431	0.535	0.216
Acid excretion (mmol/L)												
• TA	-141.00[a]	-264.60[b]	-221.40[b]	-184.20[a]	-167.17	-114.83	-275.67	-253.50	40.85	0.005	0.049	0.387
• NH_4	1.83	0.33	0.58[b]	1.58[a]	1.17	2.50	0.00	0.67	1.06	0.068	0.044	0.461
• NAE	-139.20[a]	-264.30[b]	-220.80[b]	-182.60[a]	-166.00	-112.33	-275.67	-252.83	42.23	0.004	0.046	0.381

2.4 The effect of high DCAD and high Asc supplement on plasma oxidative stress, plasma cortisol, and mammary gland function.

On the 8th week of the experimental period, the GPx activity tended to be affected by the high DCAD supplement while the other parameters were not affected by this supplement (Table 12). Regarding the Asc effect, the high dose supplement did significantly affect the plasma GPx activity and MDA level (Table 12). These parameters were decreased when dairy goats were supplemented with high dose Asc, while the effect of interaction was found on the plasma MDA level. The plasma MDA of the Asc-treated DCAD group was the lowest concentration when compared to vehicle-treated control, Asc-treated control, and vehicle-treated DCAD group. The % reduction of plasma MDA level by Asc supplement was in line with the interaction effect and the significantly higher % reduction was found in the DCAD group (Figure 16). In terms of mammary gland function, the high dose DCAD supplement did not affect these parameters while the high dose Asc supplement did significantly affect the concentration of milk protein, citrate, and FFA (Table 13). The concentration of protein and citrate were higher in the Asc-treated group when compared to the vehicle-treated group. In contrast, FFA concentration of the Asc-treated group was lower than the vehicle-treated group. Even though these compositions were affected, only the yield of citrate and FFA were significantly changed by the high dose Asc supplement (Table 14). These results were in line with the effect of Asc on concentration (Table 14).

Table 12 The effect of high DCAD and high dose Asc supplement on plasma oxidative stress and cortisol level on the 8th week of the second experimental period.

	Control	DCAD	Vehicle	Asc	Control		DCAD			P-Value		
					Vehicle	Asc	Vehicle	Asc	SEM	DCAD	Asc	DCAD×Asc
GPx activity (nmol/min/ml)	128	156	159[a]	125[b]	144	111	175	138	22.08	0.06	0.003	0.80
MDA (nmol/ml)	13.6	13.5	14.8[a]	12.2[b]	13.7[a]	13.4[ab]	15.9[a]	11.1[b]	2.1	0.93	0.014	0.022
Cortisol (ng/mL)	69.8	67.8	68.4	69.3	75.8	63.9	60.9	74.6	38.3	0.927	0.955	0.434

Table 13 The effect of high DCAD and high dose Asc supplement on milk composition of the 8th week of the second experimental period.

	Control	DCAD	Vehicle	Asc	Control		DCAD			P-Value		
					Vehicle	Asc	Vehicle	Asc	SEM	DCAD	Asc	DCAD×Asc
Fat (%)	4.70	4.93	4.86	4.77	4.68	4.73	5.05	4.81	0.27	0.63	0.42	0.21
Protein (%)	2.71	2.64	2.64[b]	2.70[a]	2.68	2.74	2.61	2.67	0.05	0.46	0.01	0.89
Lactose (%)	4.24	4.25	4.24	4.24	4.25	4.23	4.24	4.26	0.06	0.89	0.83	0.43
Citrate (%)	0.129	0.104	0.112[b]	0.122[a]	0.125	0.133	0.098	0.110	0.01	0.28	0.01	0.57
FFA (mmol/L)	0.541	0.824	0.726[a]	0.639[b]	0.568	0.513	0.883	0.766	0.07	0.15	0.01	0.29
Glucose (μM)	71	87	74	83	70	72	79	95	14.13	0.61	0.15	0.27
G6P (μM)	174	178	168	184	166	182	170	186	29.44	0.83	0.21	0.97
4%FCM (kg/8hrs)	0.51	0.53	0.52	0.52	0.50	0.53	0.55	0.52	0.04	0.789	0.854	0.120

Table 14 The effect of high DCAD and high dose Asc supplement on yield composition of the 8th week of the second experimental period.

	Control	DCAD	Vehicle	Asc	Control		DCAD			P-Value		
					Vehicle	Asc	Vehicle	Asc	SEM	DCAD	Asc	DCAD×Asc
Fat (g)	21.69	22.92	22.38	22.23	20.98	22.42	23.76	22.02	1.96	0.72	0.85	0.07
Protein (g)	12.65	12.60	12.37	12.88	12.16	13.11	12.54	12.65	0.92	0.98	0.19	0.29
Lactose (g)	19.83	20.33	19.86	20.29	19.34	20.33	20.40	20.26	1.40	0.89	0.47	0.36
Citrate (g)	0.583	0.517	0.517[b]	0.583[a]	0.54	0.61	0.50	0.56	0.06	0.65	0.02	0.99
FFA (mmol)	0.252	0.358	0.323[a]	0.288[b]	0.257	0.247	0.386	0.328	0.04	0.18	0.03	0.10
Glucose (µmol)	32	44	35	41	30	32	39	49	9.20	0.48	0.13	0.32
G6P (µmol)	81	87	80	88	76	86	84	91	14.30	0.73	0.18	0.83

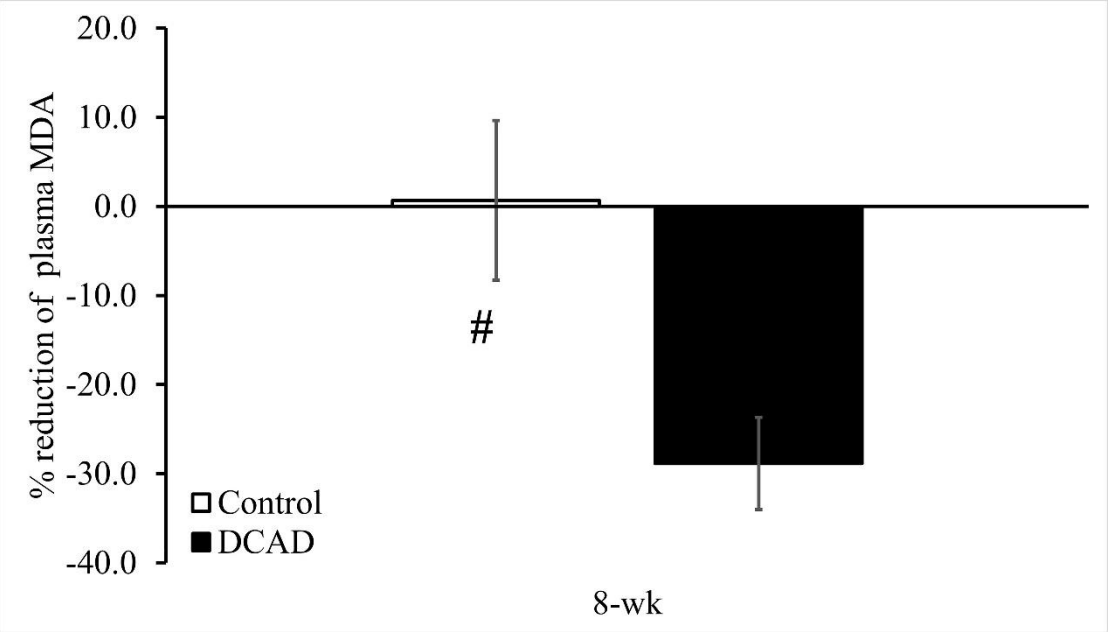


Figure 16 The effect of DCAD on % reduction of plasma MDA by high dose Asc supplement on 8th week of second experimental period.

Note: The white and black bar indicated the control and DCAD groups, respectively. # The significant difference between control and DCAD which was determined using Student's t-test (P<0.05).

CHAPTER V

DISCUSSION

Experiment 1: The effect of high DCAD supplement on HTa and blood gas parameters, serum chemistries, plasma electrolyte of non-lactating dairy goat fed under HTa

The first experiment aimed to investigate the effect of high dose Asc supplement on blood gas, electrolytes, and oxidative stress in dairy goats. During the daytime HTa, the high dose Asc supplement did not affect blood gas parameters, plasma electrolytes, and oxidative stress. However, the decreased Hct by high dose Asc supplement might indicate depleted plasma oxidative stress.

The ΔTa of first experiment was 9°C while both Tr and RR were increased during the daytime. The RR at 1300 was three times higher than the morning, while Tr was increased by 0.4-0.7°C when compared to the morning. These HTa responses were in line with our previous publication. We previously reported that the ΔTa at 5-10°C was enough to increase Tr by 0.3-0.7°C (Nguyen et al., 2018; Nguyen et al., 2019; Saipin et al., 2020a; Do Nguyen et al., 2022). The RR at 1300 of goats in the previous study were two to three time higher than at morning (Nguyen et al., 2018; Saipin et al., 2020; Do Nguyen et al., 2022). With this HTa of previous publication, an increase in RR decreased PCO_2 without altering other blood gas parameters (Do Nguyen et al., 2022). These observations supported that the HTa condition of the first experiment was enough to activate heat dissipation and affect blood gas parameters. Besides, other studies in goats reported that HTa decreased PCO_2 while it increased both plasma Cl and Hct (Minka and Ayo, 2007; Ayo et al., 2009; Sivakumar et al., 2010). These negative effects could be alleviated by the high dose Asc supplement, and this effect was proposed to be mediated by a decreased in plasma cortisol and oxidative stress (Minka and Ayo, 2007; Ayo et al., 2009; Sivakumar et al., 2010). On the contrary, the effect of high dose Asc on PCO_2, plasma Cl, and Hct in the first experiment was not observed with the change of plasma cortisol and oxidative stress. Besides, the different degree of heat dissipation between vehicle and Asc supplemented day might confound the Asc effect on PCO_2 and plasma Cl. However, a decrease in Hct suggested depleted oxidative

stress during the Asc supplement. This observation has been observed in other species (Sakurai et al., 1997; Tanaka et al., 2000; Soltany and Al Aissami, 2022). These studies showed that an increased plasma Asc depleted ROS and this response could shift the water toward the intravascular compartment (Sakurai et al., 1997; Tanaka et al., 2000; Soltany and Al Aissami, 2022). It is possible that the oxidative stress was alleviated by Asc supplement, and it shifted water toward the intravascular compartment. Interestingly, this Asc effect was not observed with the change in both plasma GPx activity and MDA level. The effect of Asc on oxidative stress was varied among the levels of oxidative stress and this might explain the absence of Asc effect on these parameters in the current study. With the oxidative above the physiological range, Asc supplement decreased oxidative stress in both plasma and cell (Dubick et al., 2005; Muthuvel et al., 2006; Koc et al., 2008; Sun et al., 2019; Soltany and Al Aissami, 2022). In contrast, Asc supplement did not affect intracellular MDA at physiological range of oxidative stress (Gegotek et al., 2017; Gegotek et al., 2019). In dairy goat, the activation of HTa response at ΔTa below 10°C was not involved in stress response while the plasma MDA in summer was not higher than in winter (Saipin et al., 2020; Do Nguyen et al., 2022). Like in dairy goat, there was only a slight correlation between the Tr and plasma MDA of dairy cow fed in summer (Tanaka et al., 2007) and the high dose Asc supplement did not change plasma antioxidants (Chaiyowittayakun et al., 2002).

These retrieved data from the first experiment indicated that the Asc supplement did not affect blood parameters in non-lactating goats, but it could alleviate the oxidative stress during the natural HTa exposure. Therefore, this protocol was further used in the second experiment.

Experiment 2: The effect of high DCAD and high dose Asc supplement in lactating dairy goats fed under HTa

Experiment 2.1: The effect of high DCAD on HTa response

This part of the second experiment demonstrated the ambient condition and the effect of high DCAD supplement on HTa response. With daytime HTa, the ambient condition increased both Tr and RR of lactating dairy goats while only RR was affected by high DCAD supplement.

The average ΔTa from the 4th and 8th week of the experimental period was around 8°C. With this HTa, the respiration at 1300 was higher than at 0600 by doubled, while Tr was increased by 0.4°C when compared to the morning. In general, body temperature was balanced by heat load and heat dissipation (Mercer, 2001; Hall, 2015). The imbalance resulted in a change in core body temperature. With HTa, heat load overwhelmed heat dissipation and increased core body temperature (Mercer, 2001; Maia et al., 2016). In goats, the evaporative heat dissipation was a main heat loss mechanism, and this mechanism was included as the adaptive mechanism of lactating goat fed under tropical condition (Robertshaw, 2006; Maia et al., 2016; Saipin et al., 2020a). This mechanism could be activated by daytime HTa (ΔTa at 5 – 10°C) which increased Tr and RR by 0.4-07°C and 2-3 times, respectively (Nguyen et al., 2018; Nguyen et al., 2019; Saipin et al., 2020a; Do Nguyen et al., 2022). The milk production of both dairy goats and cows was compromised when they were fed under this HTa (Saipin et al., 2020a; Thammacharoen et al., 2022). With an increase in Tr and RR, the response of lactating goats in the current study was in accordance with previous publications. This indicated that the dairy goats in this study were fed under tropical condition and the heat dissipation mechanism was activated.

Regarding the DCAD supplement, the current study demonstrated that this supplement did increase RR but it did not affect concentrate, roughage, and water intake. During the 4th week of the experiment, the RR of the DCAD group at 1300 was higher than the control group. An increased RR by DCAD was in accordance with previous study on dairy goats and cows (Escobosa et al, 1984; Nguyen et al., 2018). In lactating goats, this response was observed with a decreased percentage change of Tr while both DMI and WI were increased (Nguyen et al., 2018). The presence of DCAD increased body water and increased heat dissipation, which eventually enhanced the heat dissipation (Nguyen et al., 2018). However, the supplement of DCAD at high levels also shifted the acid-base balance toward metabolic alkalosis in dairy cows (Hu and Murphy, 2004). It is questioned whether an increased RR in this study involved acid-base imbalance. In general, the metabolic alkalosis is compensated by a decreased RR while an excess heat dissipation was observed with a decrease in both RR and PCO_2 (Halse and Webster, 1967; Halse, 1969; Olsson and Dahlborn, 1989). Since all blood gas parameters were kept within

normal range (Stevens et al. 1994), an increased RR in this study would suggest an increased heat dissipation. On the contrary, this response was not observed with an increased WI and DMI in this study. To alter WI, the change in plasma osmolarity by high DCAD was previously proposed to mediate this effect (Tucker et al., 1988). This effect was observed during the early phase of supplement, but it was less observed in long-term DCAD supplement (Tucker et al., 1988). This observation was possible to explain the absence effect of DCAD on WI in this current study. Since the WI was not significantly increased by DCAD in this study, this response may be responsible for the unchanged Tr and DMI. The data from this part suggested that DCAD increased heat dissipation and the shift body water among compartments would be responsible for evaporation rather than an increased WI.

Experiment 2.2: The effect of supplements on acid-base balance

This part of the second experiment demonstrated the effect of high DCAD and high dose Asc supplement on HTa response. With daytime HTa, the high DCAD supplement did increase RR while the blood gas parameters were altered within the normal range of healthy goats.

On the 4th week of the experiment, blood pH of the DCAD group was higher than the control group and their blood HCO_3 were tended to be increased by DCAD. Other blood gas parameters and urine pH were not affected by both DCAD and Asc supplement during the 4th week of experimental period. With prolonged period, the effect of DCAD on blood pH was no longer observed on the 8th week of experiment, while the high dose Asc supplement did decrease urine pH. In fact, the significant decrease in urine pH on the latter week was only found in control group. This response was in accordance with the percent reduction of urine pH by Asc supplement in the same period. In goats, the natural HTa at ΔTa 5-8°C did increase RR and drive acid-based balance toward alkalosis during the daytime (Do Nguyen et al., 2022). This type of acid-base imbalance was compensated by increased HCO_3 excretion (Barker et al., 1957; Arbus et al., 1969). High DCAD, an alkali salt, has been used to increase HCO_3 in dairy cows and the excess alkali was excreted though urine (Hu and Murphy, 2004; Hu et al., 2007). An increased level of DCAD did increase blood pH in dairy cows but this effect was less pronounced in long-

term supplement (Wildman et al., 2007a,b; Hersom et al., 2010). Since kidney plays a major role in both acid-base and electrolyte balance, the alteration of kidney function was proposed to be an adaptive mechanism of dairy cow fed long-term DCAD supplement (Hu et al., 2007; Wildman et al., 2007a, b; Khelil-Arfa et al., 2014). Regarding the Asc, the supplement has been reported to alleviate the acid-base imbalance when dairy goat exposure HTa (Sivakumar et al., 2010). This response was proposed to be mediated through decreased function of stress response (Sivakumar et al., 2010). Interestingly, the human study showed that a high dose Asc supplement was not sufficient to induce acidosis (Chen et al., 2022). With the excess Asc, human and cows excreted Asc through the urine (Padilla et al., 2007; Chen et al., 2022). In the current study, the average ΔTa at 8°C was enough affect acid-base balance while an increased blood HCO_3 in DCAD group indicated an increased buffer capacity during the daytime. The high level of HCO_3 resulted in a transient high blood pH while other parameters were within the normal range of a healthy goat (Stevens et al., 1994). These data were in accordance with the previous study on dairy cows and this confirmed the effectiveness of the high DCAD diet in this current study. In dairy cow, an increased alkali excretion and FE of electrolyte was responsible for the absence of high blood pH in DCAD group, this was obviously observed after 8 weeks of DCAD supplement (Hu et al., 2007; Wildman et al., 2007b). It was possible that a change in kidney function on the 8^{th} week of experimental period was responsible for the effect DCAD on urine pH on this week of the study. On the contrary, an increased plasma Asc in this study did not affect blood gas parameters and this could be explained by the absence of Asc effect on plasma cortisol. The high-level acid supplement was expected to be excreted through urine and decreased urine pH. The reduction of urine pH by Asc supplement seemed to be consistent over the 4^{th} and 8^{th} week of experiment. However, the effect of Asc on urine pH was only observed in the control group on the 8^{th} week of experiment and the percent reduction of urine pH was also showed the same phenomenon. An increased alkali excretion and FE of electrolyte were adaptive mechanism of a long-term supplement, and this might confound the Asc effect on urine pH on the 8^{th} week of experiment. This could be explained by either neutralization or a decreased Asc excretion but the lack of Asc level in urine was the limitation of this current study.

These current data demonstrated that high DCAD increased the buffer capacity of lactating dairy goats fed under HTa. Even though there was no effect Asc on blood gas parameters, the alteration of Asc excretion might enhance the effectiveness of Asc in other aspects.

Experiment 2.3: The effect of supplements on electrolytes balance and renal function.

This part of the second experiment demonstrated the effect of high DCAD and high dose Asc supplement on electrolyte balance and renal function. These supplements could alter electrolyte balance and enhance evaporative heat dissipation. The alteration of kidney function was response for these responses.

On the 4th week of the experiment, high DCDAD did not affect plasma electrolytes, but it decreased acid excretion. The high dose Asc supplement did increase anion gap, urine volume, and acid excretion. In goat, the effect of DCAD on HTa response was proposed to be mediated through the change in body water (Nguyen et al., 2018). The effect of DCAD on WI and extracellular fluid was first observed after 4 weeks of supplement (Nguyen et al., 2018). An increased plasma osmolarity after feeding the high DCAD initiated WI in dairy cows and the high WI returned osmolarity to normal within 2-hours post feeding (Tucker et al., 1988). The high DCAD was an alkali salt and the kidney was mainly responsible for the alkali excretion (Lindinger et al., 2000; Constable, 2014). An increased alkali excretion was observed without an altered FE of electrolyte (Delaquis and Block, 1995a,b) during a short-term DCAD supplement. These observations suggested that an increase in WI after feeding may confound the effect of DCAD on plasma electrolyte, while an increased alkali excretion by the kidney was responsible for a decrease in acid excretion by high DCAD. With high dose Asc, the supplement could shift water toward the intravascular compartment in previous publications and this acid was mainly excreted through the kidney (Sakurai et al., 1997; Tanaka et al., 2000; Padilla et al., 2007; Chen et al., 2022; Soltany and Al Aissami, 2022). The result of the current study was in accordance with these previous publications. The high dose Asc may increase intravascular fluid which resulted in an increased urine volume. An increased anion gap suggested that the current Asc supplement shifted acid-base balance toward acidosis, and it was directly compensated by an increased acid

excretion. Since alkali salt (DCAD) and acid (Asc) affected acid excretion in opposite directions, this explained the interaction effect during the 4th week of experiment.

On the 8th week of the experiment, high DCAD increased FE_{Na} and FE_K but it decreased BUN and acid excretion. With Asc, the high dose supplement decreased plasma creatinine, Hct, and FE_K while urine volume and acid excretion were increased by Asc supplement. An increased FE of electrolyte in the high DCAD group was in accordance with the previous study in dairy cows (Hu et al., 2007; Wildman et al., 2007a,b,c). With long-term high DCAD, a decreased acid excretion by high DCAD was reported with an increased tubular excretion (FE of electrolyte) and this response was an adaptive mechanism of ruminant (Hu et al., 2007; Wildman et al., 2007a,b,c). Even though a decreased BUN could be a result from an increased high NH_4 production or volume expansion, there was no effect of long-term DCAD supplement on the nitrogen balance of dairy goats in our previous study (Nguyen et al., 2020). This previous study also showed that body reserve water, especially extracellular compartment, was increased after 8 weeks of supplement (Nguyen et al., 2018). The data from this current study demonstrated that the adaptation of tubular function was existed in dairy goats and this mechanism was apparent after 8 weeks of DCAD supplement. Regarding the Asc effect, the high dose supplement through intravenous injection could shift body fluid toward intravascular compartment and this response were proposed to be mediated by depleted oxidative stress (Sakurai et al., 1997; Tanaka et al., 2000; Dubick et al., 2005; Soltany and Al Aissami, 2022). An increased intravascular fluid could decrease Hct while the excess water is excreted thorough urine. An increased urine volume affects both creatinine clearance and FE_K (Potter, 1966; Aperia et al., 1975; Hall, 2015). With Asc supplement of current study, it is possible that high dose Asc supplement shifted body fluid toward intravascular compartment and the lowest blood creatinine in the Asc-treated DCAD group indicated the synergistic effect of both supplements on body water. Since the excess Asc is mainly excreted through the kidney, an increased acid excretion in this study was expected. However, the interaction effect of both supplements on acid excretion was no longer observed on the 8th week of the experiment and this might be the result of an increased tubular secretion by this week.

The current observations demonstrated that both DCAD and Asc affected electrolyte balance, and this was mediated through kidney function, especially the tubular part. The presence

of these supplements increased body water which eventually enhance evaporative heat dissipation. In addition, the change of tubular function over the experimental period may be responsible for the effect of DCAD on acid-base balance.

Experiment 2.4: The effect of supplements on oxidative stress and mammary gland function.

The last part of the second experiment demonstrated the effect of high DCAD and high dose Asc supplement on plasma oxidative stress and mammary gland function. The plasma oxidative stress was mainly alleviated by the high dose Asc supplement, and this was observed with a change in mammary gland function.

The high dose Asc supplement did deplete plasma GPx activity and MDA level. The lowest plasma MDA level was found in the Asc-treated DCAD group, while the percentage reduction of MDA level by Asc was also significantly high in the DCAD group. This result indicated the synergistic effect of both Asc and DCAD on plasma oxidative stress. When oxidative stress was compromised by Asc supplement, the alteration of milk composition was observed. It is hypothesized whether the alleviated oxidative stress was involved in mammary gland function. In tropical condition, the energy requirement of a dairy goat was increased to serve both lactation and heat dissipation (NRC, 1981; Maia et al., 2016). The level of metabolic rate was increased to produce high energy, and this resulted in oxidative stress (Sie et al., 2017). An increased oxidative could alter GPx activity and MDA level in both cells and plasma (Dubick et al., 2005; Sani et al., 2007; Eslami et al., 2015; Nedic et al., 2019). With Asc, the supplement depleted intracellular oxidative stress (Rivas et al., 2008) and decreased intracellular MDA (Sun et al., 2019). Plasma MDA was proposed to be produced by high metabolic organs and excreted into the blood circulation (Sani et al., 2007). Among the high metabolic organs of rats, MDA was mostly produced by the kidney, and this may contribute to the plasma MDA level (Sani et al., 2007). An increased plasma MDA could also be a result of high oxidative stress in the mammary gland of dairy cows where their energy requirement was tremendously increased during early lactation (NRC, 1981; Maia et al., 2016; Nedic et al., 2019). In both kidney and mammary gland, there were an Asc receptors which are a sodium-dependent transporter (Rivas et al., 2008). In terms of antioxidant defense, an increased plasma GPx activity was included as the adaptive

mechanism of dairy goats fed under HTa (Saipin et al., 2020a). The study in rats revealed that the plasma GPx was produced by the kidney (Burk et al., 2011) while the Asc supplement depleted intracellular ROS and decreased cellular GPx production (Yun et al., 2012). Like MDA, an increased oxidative stress in the mammary gland of rats and cows was also observed with the change of systemic oxidative stress (Eslami et al., 2015; Nedic et al., 2019; Laliotis et al., 2020). Based on these previous publications, the Asc supplement in this study depleted ROS and this resulted in a decrease in both plasma GPx activity and MDA level. The high sodium in extracellular fluid during high DCAD supplement could enhance Asc receptor and explain the synergistic effect of Asc and DCAD on plasma MDA level. Regarding mammary gland function, the alleviated oxidative stress in this current study altered milk composition and the yield of each composition. Even though the concentration of milk protein, citrate, and FFA were affected, only the yield of citrate and FFA were altered in this current study. In mammary gland, the citrate cannot be excreted into milk by diffusion or paracellular transport and the concentration in milk represented the level in the cytoplasm (Linzell et al., 1976). The citrate was used for both milk fat synthesis and antioxidant defense in mammary gland (Garnsworthy et al., 2006; Anderson et al., 2007). Acute oxidative stress in the mammary gland of a dairy cow significantly decreased milk citrate (Oshima and Fuse, 1981; Silanikove et al., 2014; Silanikove et al., 2016). In milk, FFA was excreted with low concentration, while an increased milk FFA would relate to milk oxidative stress rather than an increased excretion (Chilliard et al., 2003; Van Ardt et al., 2005). Then, an increased milk citrate indicated the preserved antioxidant, while a depleted oxidative stress in a mammary gland could explain the reduction in milk FFA.

These results demonstrated that high dose supplement alleviated plasma oxidative stress and it was enhanced by the presence of high DCAD. The depleted oxidative stress altered mammary gland function under natural HTa without the involvement of stress response.

Conclusion

These observations indicated the link between acid-base imbalance, electrolyte imbalance, and oxidative stress under HTa of tropical condition. High DCAD increased buffer capacity while the presence of both supplement increased body water. With an increased RR, these responses suggested an increased heat dissipation, and this was mediated by the alteration of kidney function. Besides, these supplements synergistically depleted plasma oxidative stress and this depletion altered milk composition. Therefore, the current study showed that the presence of both high DCAD and high dose Asc did synergistically alleviate the effect of HTa on mammary gland function of lactating goat fed under tropical condition.

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