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Sumpun Thammacharoen

Siripen Komolvanich

Somchai Chanpongsang

Narongsak Chaiyabutr

Short Communication

Respiratory Hypocapnia at Different Stages of Lactation during Long-term Exogenous Bovine Somatotropin in Crossbred Holstein Cattle in the Tropic

Sumpun Thammacharoen[1*] Siripen Komolvanich[1] Somchai Chanpongsang[2]
Narongsak Chaiyabutr[1]

Abstract

The aim of this study was to investigate the influence of long-term administration of recombinant bovine somatotropin (rbST) on the acid-base homeostasis of lactating crossbred cows under hot and humid condition of Thailand. Two groups of five animals each of primiparous crossbred dairy cattle were supplemented either with rbST or with control vehicle every 14 days throughout the period of lactation. The blood gas parameters, milk yield and feed intake from both groups were recorded at each stage of lactation. Cows supplemented with rbST showed mild degree of acid-base imbalance during early to mid lactation. The lower partial pressure of carbon dioxide (p_aCO_2) and plasma bicarbonate ($[HCO_3^-]a$) from arterial blood were apparent at early stage of lactation as compared with those of the control cows. The present results can conclude that rbST supplementation influences acid-base homeostasis. The supplementation of rbST may cause the respiratory hypocapnia under prolonged moderate heat exposure and high milk production.

Keywords: acid-base homeostasis, dairy cattle, food intake, growth hormone, milk

[1] *Department of Veterinary Physiology* [2] *Department of Animal Husbandry, Faculty of Veterinary Science, Chulalongkorn University, Bangkok 10330, Thailand*
Corresponding author E-mail: *sprueksagorn@hotmail.com*

บทคัดย่อ

ภาวะความดันย่อยของแก๊ซคาร์บอนไดออกไซด์ต่ำเนื่องจากการหายใจที่เกิดขึ้นจากการเสริมฮอร์โมนโซมาโตโทรปินในลักษณะต่อเนื่องยาวนานในโคนมพันธุ์ผสมโฮสไตน์ที่ถูกเลี้ยงในเขตร้อน

สัมพันธ์ ธรรมเจริญ [1*] ศิริเพ็ญ โกมลวานิช [1] สมชาย จันทร์ผ่องแสง [2] ณรงค์ศักดิ์ ชัยบุตร [1]

วัตถุประสงค์ของการทดลองนี้คือเพื่อสืบค้นความเกี่ยวข้องของการเสริมฮอร์โมนโซมาโตโทรปินที่สังเคราะห์ด้วยวิธีรีคอมไบแนนท์ (recombinant bovine somatotropin, rbST) ในลักษณะยาวนานต่อการธำรงดุลของกรดและด่างในโคนมพันธุ์ผสมภายใต้ภาวะอากาศร้อนชื้นของประเทศไทย โคนมพันธุ์ผสมท้องแรก 2 กลุ่ม กลุ่มละ 5 ตัว ได้ถูกนำมาใช้ในการทดลองโดยได้รับ rbST หรือสารเคมีควบคุมทุก 14 วันตลอดช่วงการให้ผลผลิตน้ำนม ในช่วงการทดลองมีการเก็บตัวอย่างเลือดจากโคทั้งสองกลุ่มเพื่อวัดดัชนีบ่งชี้แก๊ซในเลือด (blood gas) และการบันทึกปริมาณน้ำนมและการกินอาหารในแต่ละช่วงของการให้นม ผลการทดลองบ่งชี้ว่าโคนมที่ได้รับการเสริม rbST มีสภาพการธำรงดุลของกรดและด่างในเลือดที่เปลี่ยนไปเล็กน้อยในช่วงต้นถึงช่วงกลางของการให้นม จากผลการทดลองพบว่าค่าความดันย่อยของแก๊ซคาร์บอนไดออกไซด์ในเลือดแดง (p_aCO_2) และความเข้มข้นของไบคาร์บอเนตในพลาสมาจากเลือดแดง ([HCO_3^-]a) ในโคกลุ่มที่ได้รับ rbST น้อยกว่าในกลุ่มควบคุมในช่วงต้นของการให้น้ำนม ผลการทดลองที่นำเสนอในปัจจุบันนี้สามารถสรุปได้ว่าการเสริม rbST จะส่งผลกระทบต่อการธำรงดุลของกรดและด่างของร่างกาย โดยการเสริม rbST ให้แก่โคนมที่ถูกเลี้ยงภายใต้ภาวะความร้อนระดับปานกลางในลักษณะต่อเนื่องอาจจะเป็นสาเหตุให้เกิดภาวะความดันย่อยของแก๊ซคาร์บอนไดออกไซด์ต่ำเนื่องจากการหายใจในช่วงที่แม่โคให้น้ำนมสูง

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[1] ภาควิชาสรีรวิทยา [2] ภาควิชาสัตวบาล คณะสัตวแพทยศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย กรุงเทพฯ 10330 ประเทศไทย

***ผู้รับผิดชอบบทความ E-mail:** *sprueksagorn@hotmail.com*

Introduction

It is known that acid-base balance in body fluid is important for the role of enzyme reaction. The pH of cells and tissues should remain constant within narrow limits. The pH of the extracellular fluid particularly that of blood plasma will remain on a constant level. To maintain this acid-base status, the process in the body may be regulated by multi-organ systems, e.g. the buffer system of body fluid (bicarbonate or phosphate buffer), the respiratory system controlling the equilibrium of bicarbonate buffer via elimination of carbondioxide (CO_2) through the lungs, and the kidney controlling an excretion/reabsorption hydrogen ions and bicarbonate ions.

Acid-base regulation is one of the main homeostasis of animal during exposure to high temperatures. In mammal, body heat load is dissipated mainly by water evaporation via either breathing or sweating. It is known that dairy cattle have both capabilities to dissipate the additional heat. However, increased breathing is the primary response to high environmental temperature and humidity because sweating is much less efficient in this specie. Although the high respiratory rate during heat stress involves mainly dead space ventilation rather than alveolar ventilation, the effect of high respiratory rate induced by respiratory hypocapnia and alkalosis was reported in many species including in cattle (Scheider et al., 1988; Comito et al., 2007; Steiss and Wright., 2008).

Bovine somatotropin (ST) has been shown earlier for its galactopoietic effect in dairy cattle. Increased milk production after rbST supplementation appeared to come mainly from the partitioning of nutrients to mammary gland and an increase in mammary gland activities (Bauman., 1992; Etherton and Bauman., 1998; Chaiyabutr et al., 2005). An increase in body heat during nutritional metabolism coinciding with an increase in milk yield was reported in exotic cow treated with recombinant bovine somatotropin (rbST) (West, 1994). In Thailand, a study of the galactopoietic effect of rbST in primiparous crossbred Holstein cattle showed an increase in milk yield (Chaiyabutr et al., 2004; Chaiyabutr et al., 2005) with an increase in respiratory rate in each stage of lactation (Chanchai et al., 2010). However, few data are available about the effect of rbST administration on acid-base balance of body fluid in crossbred Holstein cattle in the tropic although there is an information concerning the effects of bovine somatotropin on acid-base balance of lactating Holstein and Jersey cows during hot, humid weather (West et al., 1991). The objective of the present study was to study the acid-base balance as assessed by the blood gas parameters during long-term supplemented rbST in crossbred Holstein cattle under hot and humid conditions.

Materials and Methods

Animals and management: First lactation of non-pregnant 87.5% crossbred Holstein cattle (n=10) was selected and housed in individual stalls under shaded barn. The maximum temperature of the experimental area at noon was 34±1ºC and the minimum temperature at night was 26±1ºC. The relative humidity was 68±12%. The cattle had *ad libitum* access to water and were fed twice daily to maintain a moderate body score condition at 2.5 (1-5 scale). On each day, the individual animal was milked twice with milking machine at 06.00 am and 05.00 pm and fed with the same concentrated mixture (3.5 kg for each milking time). In addition to the concentrated mixture, we used urea-treated rice straw as roughage supplement. Four kg per day of roughage were offered to the animals 4 times at 08.00, 12.00, 16.00 and 20.00 hour. The chemical compositions of concentrated mixture and roughage used in the present experiment had been described previously (Chaiyabutr et al., 2007). All experiment procedures were approved by the ethic committee of Faculty of Veterinary Science, Chulalongkorn University.

Experimental procedures: The cattle were divided into 2 groups, control group and experimental group of five animals each. In each group, four consecutive measurements were performed, consisting of a pretreatment period at day 45 post partum (45-PP) as control period and at days 105-PP (early lactation), 165-PP (mid lactation) and 225-PP (late lactation) as treatment periods. The hormone supplementation group received 500 mg of rbST per animal (POSILAC, Monsanto, USA. 792 mg of a prolonged-release formulation in sesame oil), while the control group received sesame oil (800 mg). Either rbST or sesame oil was subcutaneously injected to the animal at the tail head depression (the ischiorectal fossa) every 14 days interval throughout the lactating periods. The injection site started at day 60 post partum (60-PP).

On the day of study at 14.00 pm, the arterial blood was collected from the coccygeal artery under anaerobic conditions via the needle (#18) installed tuberculin syringe moistened with heparin solution. The following method was used to prevent environmental disturbances of the acid-base balance before blood sample collection. The cow was first locally anesthetized by caudal epidural injection (Muir and Hubbell, 1989) with 2% lidocaine (Union drug lab Ltd, Bangkok, Thailand). Briefly, the needle (#21) was inserted into the epidural space at the sacrococcygeal joint. After the success of needle insertion, three milliliter of 2% lidocaine was infused into this space. The cow was anesthetized within 2 min and allowed to collect arterial blood from coccygeal artery. The arterial blood that came out from the needle was bright red and showed arterial pulse prominently. The heparinized tuberculin syringe was then connected to the needle and the blood was carefully drawn back in anaerobic conditions. To prevent changes in the pH of the blood, care was taken to exclude all air from the syringes, which were then capped with metal caps, sealed with paraffin paper and immediately kept in crush ice until blood gas measurement. Arterial blood pH (pH_a), partial pressure of oxygen (p_aO_2) and carbondioxide (p_aCO_2) were measured using a blood gas analyzer (Model 348, Buyer Healthcare, MA. USA). The other blood gas parameters including [HCO_3-]a, total carbondioxide ($TotCO_2$) and base excess (BE) were calculated based on the blood gas analyzer.

Statistical analysis: All data from the experiment were analyzed using analysis of variance (ANOVA). Significant main effects were followed up with pair wise comparisons using Bonferroni posttest. All data were presented as mean±SEM.

Results and Discussion

During the experimental period the average day temperature and humidity at the experimental barn were 31.0±1.0ºC and 68.0±12.0%, respectively. The comparable environmental conditions at the same barn were reported previously. On those experiments, the average year round temperature and humidity were 32.3-35.3°C and 51.0-61.8%. Cows from both groups were housed in a barn which had the range of temperature humidity index (THI) around 83.2-85.5 throughout the study period. This condition is considered the upper critical THI of 72 for lactating exotic dairy cows (Armstrong, 1994). Moreover, cows fed under this condition showed high rectal temperature and respiration rate as seen during exposure to high temperatures (Boonsanit et al., 2010; Chanchai et al., 2010). It was assumed that all crossbred Holstein cows from the present experiment were fed under moderate prolong heat stress condition.

During prolonged exposure to high temperatures, body heat dissipation is activated mainly via skin (sweating) and respiratory (breathing) evaporation. The present results of crossbred dairy cattle in the control group showed values of pH_a, p_aO_2, p_aCO_2, [HCO_3-]a, $TotCO_2$ and BE (Table 1) within normal range of blood gas for dairy cattle throughout the lactation (Fisher et al., 1980; Parker et al., 2003; Carlson, 2009). No alteration of the value of p_aCO_2 indicates that respiratory factors under prolonged exposure to high temperatures was not sufficient to decrease p_aCO_2 as compared within the control value, although the level of p_aCO_2 during high respiratory rate is expected to decrease and subsequently results in respiratory hypocapnia (DiBartola and Autran De Morais, 1992; Carlson, 2009). It is also known that an increase in the rate of respiration during heat dissipation is confined primarily to dead space ventilation rather than alveolar ventilation. The study in other ruminating

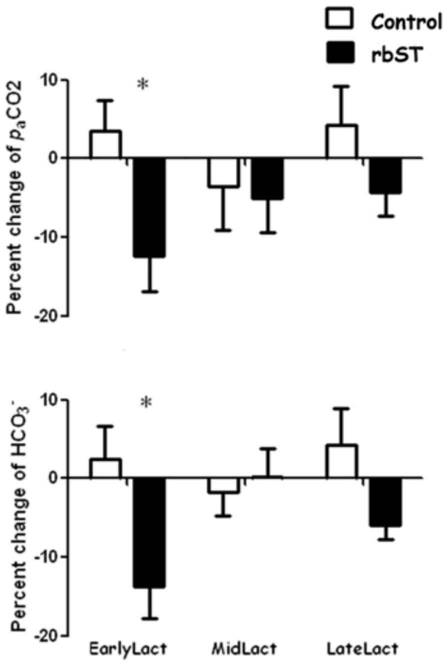

Figure 1 Percentage change of p_aCO_2 and [HCO_3-]a from the pre-treatment period suggesting the pattern of respiratory hypocapnia at the early stage of lactation. *significant difference from the control group (p<0.05).

animal in sheep indicated that a marked increase in respiratory minute volume during heat stress showed to cause proportional increase in dead space ventilation while alveolar ventilation was kept within normal (Robertshaw, 2006).

Somatotropin has been shown to affect acid base homeostasis. Plasma bicarbonate was lower in ST deficiency patient (Dimke et al., 2007). Supplementation of ST increased plasma bicarbonate concentration in NH_4Cl induced metabolic acidosis (Sicuro et al., 1998). In the present study, long-term of rbST supplementation during the early stage of lactation of crossbred Holstein cattle revealed a pattern of respiratory hypocapnia which is characterized by a decreased p_aCO_2 and a compensatory decreased [HCO_3-]a (Table 1). The p_aCO_2 of rbST supplementation group (32.88±2.24 mmHg) was significantly lower than that of the control group (40.11±0.42 mmHg, p<0.01). The other blood gas parameters including [HCO_3-]a, $TotCO_2$ and BE of rbST treatment group were also significantly lower than those of the control group (21.40±1.34 vs. 25.54±0.64 mmol/l, 22.34±1.40 vs. 26.76±0.68 mmol/l and -1.78±1.24 vs. 1.96±0.76 mmol/l, p<0.01, respectively, Table 1). These results are in agreement with studies in lactating *Bos taurus* dairy cows treated with rbST under hot and humid weather which showed reduced blood bicarbonate, base excess, and total CO_2 (West et al.1991). However, rbST supplementation during the early stage of lactation was not sufficient to cause respiratory alkalosis because the values of pHa from both groups were not significantly different (7.43±0.00 vs. 7.42±0.01, p>0.05), which then restored to normal range (Fisher et al., 1980; Parker et al., 2003; Carlson, 2009). Although the supplementations of rbST in this experiment were conducted throughout the lactating periods, the prolonged effect of rbST to acid base balance was compromised in mid and late stages of lactation. The p_aCO_2 from rbST treatment group at the mid and late stages of lactation were 35.42±1.09 and 35.77±0.89 mmHg, which were not different from the control and within the normal values (Fisher et al., 1980; Parker et al., 2003; Carlson, 2009). These differences may be due in part to the patterns of higher milk yield coinciding with feed intake during the early lactation in rbST supplemented cows. An increase in dry matter intake would increase in the metabolic energy expenditure association with high milk production (Tyrell et al., 1988; Manalu et al., 1991). An increasing heat formation would be expected in rbST supplemented dairy cows due to an increase milk production (West, 1994). Dissipation of the heat formation with an increase in the respiratory rate in rbST supplemented animals occurred in early lactation in the present study. The decrease in paCO2 in crossbred cow supplemented with rbST referred to a respiratory effect by a subsequent increase in the respiratory frequency and evaporative heat loss from the upper respiratory tract with alveolar hyperventilation.

Animals in both groups showed no differences in milk yield and feed intake at pre-treatment period (Table 1). After rbST supplementation, the milk yield was significantly higher than the control group in the early lactation (p<0.05). This galactopoetic effect of rbST during the exposure to prolong high THI was in agreement with a previous finding (West, 1994). In addition, the increase of milk synthesis after rbST treatment was not sufficient to increase the feed intake (Table 1). However, previous reports had showed that rbST supplementation increased feed intake during hot and humid condition (West, 1994; Chanchai et al., 2010). It is known that feed intake tends to decrease when the core temperature increases during prolonged heat stress condition and by rbST supplementation. However, increase milk synthesis after rbST supplementation appears to increase feed intake in case that body partitioning of nutrients is not sufficient to account the demand of milk synthesis by mammary gland. Moreover, somatotropin may influence feed intake either by its direct effect on the brain (Bohlooly-Y et al., 2005; Berryman et al., 2006) or indirectly by ghrelin, a peptide hormone that stimulates appetite (Sugino et al., 2002[a]; Sugino et al., 2002[b]; Iqbal et al., 2006; ThidarMyint et al., 2006; Grouselle et al., 2008) or indirectly by leptin, a protein hormone that decreases appetite (Schwartz et al., 2000; Chanchai et al., 2010). Taken together, the effect of rbST on feed intake action in dairy cattle remains to be further investigated.

In conclusion, the present experiment revealed that rbST supplementation in crossbred Holstein cattle under hot and humid condition affected body acid-base homeostasis. Cows supplemented with rbST showed typical pattern of respiratory hypocapnia with normal arterial blood pH. The hypocapnia reported in the present study came from the respiratory factor during heat dissipation rather than the metabolic factor of feed intake. This effect could be seen during transition period from early to mid lactation.

Table 1 Milk yield, feed intake and arterial blood gas parameters from pre- and post-supplementation with rbST in crossbred cattle at different stages of lactation. (n= 5 in each group)

Parameter	Period of measurements	Control group	rbST group	Control vs. rbST group
Milk yield (kg/day/animal)	Pre-treatment	13.00 ± 0.63 [a]	13.40 ± 1.21[a]	NS
	Early	13.10 ± 0.68[a]	16.00 ± 0.94[a]	p<0.05
	Mid	12.94 ± 0.58[a]	14.62 ± 0.85[a]	NS
	Late	11.50 ± 0.40[a]	13.00 ± 0.59[a]	NS
Feed intake (kg/day)	Pre-treatment	11.40 ± 0.31 [a]	12.30 ± 0.36 [a]	NS
	Early	11.60 ± 0.61[a]	13.02 ± 0.75[a]	NS
	Mid	12.18 ± 0.83[a]	13.90 ± 0.58[a]	NS
	Late	12.30 ± 0.74[a]	13.40 ± 0.80[a]	NS
Blood pH	Pre-treatment	7.41 ± 0.01 [a]	7.44 ± 0.01 [a]	NS
	Early	7.42 ± 0.01[a]	7.43 ± 0.00[a]	NS
	Mid	7.43 ± 0.01[a]	7.46 ± 0.01[a]	NS
	Late	7.42 ± 0.00[a]	7.43 ± 0.01[a]	NS
p_aO_2 (mmHg)	Pre-treatment	112.63 ± 8.14 [a]	112.31 ± 1.70 [a]	NS
	Early	123.62 ± 3.75[a]	112.85 ± 1.72[a]	NS
	Mid	121.90 ± 2.44[a]	118.37 ± 3.48[a]	NS
	Late	123.89 ± 3.67[a]	121.49 ± 3.04[a]	NS
p_aCO_2 (mmHg)	Pre-treatment	38.98 ± 1.40 [a]	37.44 ± 0.81 [a]	NS
	Early	40.11 ± 0.42[a]	32.88 ± 2.24[a]	p<0.01
	Mid	37.30 ± 1.02[a]	35.42 ± 1.09[a]	NS
	Late	40.38 ± 0.78[a]	35.77 ± 0.89[a]	NS
TotalCO_2 (mmol/l)	Pre-treatment	26.19 ± 1.05 [a]	25.84 ± 0.42 [a]	NS
	Early	26.76 ± 0.68[a]	22.34 ± 1.40[a]	p<0.01
	Mid	26.31 ± 0.54[a]	25.74 ± 0.57[a]	NS
	Late	26.83 ± 0.50[a]	24.24 ± 0.42[a]	NS
$[HCO_3^-]_a$ (mmol/l)	Pre-treatment	25.07 ± 0.96 [a]	24.74 ± 0.41 [a]	NS
	Early	25.54 ± 0.64[a]	21.40 ± 1.34[b]	p<0.01
	Mid	24.53 ± 0.26[a]	24.72 ± 0.55[a]	NS
	Late	25.97 ± 0.33[a]	23.24 ± 0.39[a]	p<0.05
BE (mmol/l)	Pre-treatment	1.40 ± 0.81 [a]	1.49 ± 0.43 [a]	NS
	Early	1.96 ± 0.76[a]	-1.78 ± 1.24[a]	p<0.01
	Mid	1.25 ± 0.41[a]	1.77 ± 0.53[a]	NS
	Late	2.44 ± 0.28[a]	-0.05 ± 0.40[a]	NS

[a,b] Mean values within a column of each parameter indicated with different superscripts are significantly different. p values indicated the difference between groups. NS indicated no significance.

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