

Functional Characterization of Mammary Gland of Holstein Cows under Humid Tropical Summer Climates*

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ABSTRACT : Physiological parameters were measured on six primiparous, non-pregnant Holstein cows prior to peak lactation over a 3-month summer season in southwestern Taiwan. The objectives were to characterize heat stress-induced change in functionality of mammary gland under natural climates of tropical summer and to establish physiological indices applicable to this environment in referring to this change. Environmental and physiological readings, milk and blood samples were taken at 15:00 h biweekly for totally five time points during the study. Climate readings showed that the afternoon humidex value reached the highest (53.5) around mid summer. Rectal temperature of cows taken simultaneously varied between 38.26°C and 40.02°C in parallel to humidex. Milk production declined drastically from 29.2 to 22.2 kg/d the first month entering summer but leveled up at end of the summer season suggesting effects exerted by heat stress rather than stages of lactation. Lactose content decreased linearly ($p < 0.05$) with times in summer, from 4.69 to 4.38%. On the other hand, activity of N-acetylglucosaminidase (NAGase) in milk increased linearly to over two folds ($p < 0.05$) during the same intervals. Elevations of fractional constituent of BSA in whey protein and serum cortisol level were also noticed in the course. Measurement of arteriovenous concentration (A-V) difference across the mammary gland demonstrated net uptake of glucose and net release of urea throughout the study period. The amount of urea released from mammary gland increased ($p < 0.05$) progressively from 1.54 to 7.76 mg/dl during summer. It is concluded that gradual regression of mammary gland occurred along the humid tropical summer season. This regression is likely initiated through elevation of body temperature, which is irreversible above certain point. The increased release of urea from mammary gland during heat stress suggests its potential role as an early indicator of suboptimal mammary function. (*Asian-Aust. J. Anim. Sci.* 2003, Vol 16, No. 7: 988-995)

Key Words : Tropics, Holstein Cow, Lactation, N-Acetylglucosaminidase, Urea

INTRODUCTION

The adverse effects of heat stress on dairy production are most extensively studied in southwestern and southeastern United States. In summary, heat stress increases rectal temperature and maintenance energy requirement, reduces dry matter intake, milk yield and partial efficiencies of milk production in dairy cows (Collier et al., 1981; Staples et al., 1988; West et al., 1991; West, 1994). A temperature-humidity index table developed for dairy cattle (Armstrong, 1994) indicates that, even under low humidity, it is beyond the comfort zone of high producing cows when ambient temperature exceeds 27°C. Decrease in yields of milk true protein and casein and

increase in milk urea were reported recently (Muroya et al., 1997) for cows confined at 28°C and 80% relative humidity for 14 d. Nevertheless, environmental conditions such as ambient temperature, relative humidity, radiation and day length of tropical and subtropical countries are even more extreme for dairy production. Chronic effect of natural climates of these areas on lactation and physiology of Holstein cows is still unrevealed.

Blood urea is not considered of nutritive importance. However, its level is capable of reflecting different aspects of physiology. Imbalanced production of ammonia and fermentable carbon in rumen, as might result from improper ratio of protein and energy intake or inadequate degradation of dietary nitrogen, tends to elevate plasma urea (Roseler et al., 1993; Gonda and Lindberg, 1994). Besides, during euglycemic hyperinsulinemic clamp, the decline of plasma urea level is thought due to an overall improved efficiency of amino acid utilization, involving sparing amino acid use for oxidation and net protein accretion (Grinari et al., 1997).

Mammary gland of lactating animal is a site of extensive synthesis and degradation for both constitutive and milk proteins (Bequette et al., 1998). During heat stress or in declining phase of lactation, as the demand for milk protein drops, it is hypothesized that surplus amino acids are directing to oxidation and degradation, therefore, urea production within mammary gland is presumably elevated accordingly.

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The present study was designed to chronically monitor dairy cows on selected lactation traits and physiological parameters under natural climates of tropical summer. The objectives were to characterize long-term heat stress-induced adjustment in function of mammary gland when special effort is required for homothermous, and to establish physiological indices suitable to this environment that can refer to the functionality change in cow mammary gland.

MATERIALS AND METHODS

Animals and management

This study was conducted in the dairy station of Chai Yi University in the southwestern plain of Taiwan island (23.5 N and 120.5 E). Six primiparous, non-pregnant Holstein cows without udder infection were selected with similar age, body weight, stage of lactation and milk production. The average days in milk was 90 ± 24 days (49-120 days). The test-day milk yield before commencement of the study averaged 27.8 ± 5.5 kg/d and somatic cell counts (SCC), based on a DNA-ethidium bromide binding assay (Somacount 300 Flow, Bentley Instruments Inc. Chaska, MN, USA), were lower than 5×10^5 cells/ml milk.

Animals were kept in free stalls with wood pulp bedding on concrete floor. An unsheltered sandy exercise area was adjoined to. The cows were fed automatically (GM 2000, Gascoigne Melotte Co., Emmeloord, Holland) on a commercial concentrate according to individual milk production 6 to 8 times daily of totally 8 to 12 kg/d. This concentrate consisted of soybean meal, corn kernel, wheat bran and molasses (crude protein 13.5%). Bermuda grass and drinking water were provided for ad lib consumption. Milking was performed twice daily at 06:00 and 17:00.

Experimental procedure and sample collection

This study was conducted in summer season covering June, July and August. The monthly temperature averaged (range) over previous 10 years for June, July and August was 27.5 (23.9-32.1), 28.4 (24.8-32.9) and 27.8 (24.5-32.1)°C, respectively. The average relative humidity for each month was 83, 81 and 84%, respectively. Air temperature and the relative humidity under the barn, along with the rectal temperature of experimental cows were recorded around 15:00 for the totally five sampling dates at biweekly intervals. Humidex was calculated accordingly.

Blood were sampled following each recording. Arterial blood of the coccygeal vessel and venous blood of the subcutaneous abdominal vein were aseptically collected by needle puncture. To calculate uptake or release of metabolites by the mammary gland, coccygeal vessels were chosen to represent arterial supply because it is simple for sampling. Arterial blood is considered be sufficiently mixed so that it may be obtained from any source. Also,

arteriovenous concentration (A-V) differences across the tail were assumed to be negligible and thus composition of either venous or arterial blood from the tail was assumed to be as good as to be representative of that of the mammary arterial supply (Cant et al., 1993a). Abdominal venous vein was used to represent the total venous effluent from the udder. There is extreme anastomosing of veins within the udder and the valvular incompetence in the pudic vein is less prevalent in primiparous cows (Linzell, 1974). A-V difference between the simultaneously collected coccygeal and abdominal vein was calculated to show the net uptake or production by mammary gland. Composite milk sample was taken from the subsequent evening milking following blood sampling.

Milk production was measured individually in selected test days for each month from May to September according to the standard procedure of Dairy Herd Improvement Association by Taiwan Livestock Research Institute.

Chemical analysis

Plasma of heparin-NaF blood was used for analysis of glucose content based on coupled reactions of hexokinase and glucose-6-phosphate dehydrogenase (Sigma Co., Ltd, St. Louis, MO, USA). Serum collected at 4°C was stored as aliquots at -20°C for analysis of urea, total protein, cortisol, thyroxine and triiodothyronine within 2 weeks. Urea was analyzed with Merck AutoAnalyzer (Merck Corp., Frankfurter, German), while hormones were assayed immunochemically using ACS: 180 Automated Chemiluminescence System (Ciba Corning Corp., Medfield, MA, USA).

Major milk composition was analyzed on the Bentley-2000 Infrared Milk Analyzer (Bentley Instruments Inc, MN, USA). Total whey protein, the supernatant of milk centrifuged at $15,000 \times g$, 40 min followed by acidification to pH 4.6 to precipitate casein, was fractioned by a commercial pre-cast gel electrophoresis system at pH 8.6 and 90 mV and constituent proportion quantified by a densitometer (Helena Laboratories, Beaumont, TX, USA). From cathode to anode the fractions were γ -globulin, α -lactalbumin, β -lactoglobulin, and bovine serum albumin (BSA).

The fluorescence method developed by Kitchen et al. (1978) adopting 4-methylumbelliferyl-N-acetyl- β -D-glucosaminide as an artificial substrate was used to measure N-acetylglucosaminidase (NAGase) activity on milk sample.

Data analysis

Simple means and standard errors were calculated considering cow and time in summer main factors of the model. GLM procedure of SAS (1988) was used to test the effects of month in summer on levels of blood metabolites, A-V differences, the rectal temperature, hormones and milk

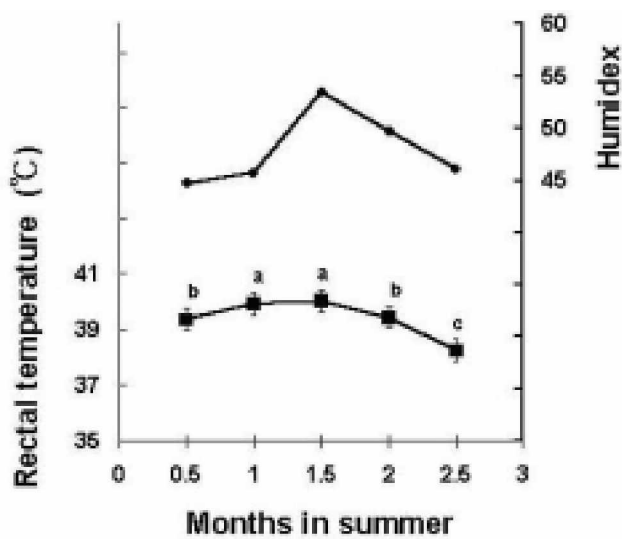


Figure 1. Progressive changes in ambient humidex value and rectal temperature of primiparous Holstein cows during a humid tropical summer. Vertical bar represents standard error of six cows.

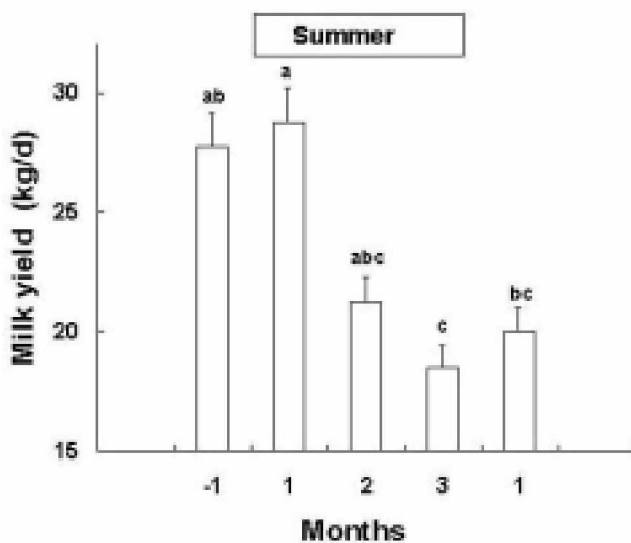


Figure 2. Average milk yield of primiparous Holstein cows for the month before summer season (-1), the three summer-months (1, 2, 3) and the month after summer season (1). Monthly test-day milk yield was determined according to standard Dairy Herd Improvement procedure. Vertical bar represents standard error of six cows.

components. Duncan's test was used for multiple range tests. Linear and quadratic regression analysis (SAS, 1988) was used to determine serial effect of month in summer. Regression analyses were also conducted between pairs of arterial concentration and corresponding A-V difference to assess the mechanism of mammary uptake or release.

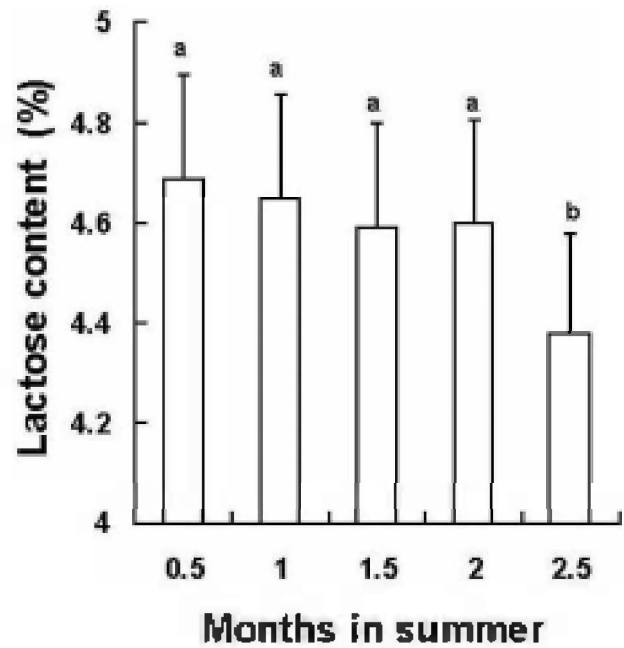


Figure 3. Lactose content in milk of primiparous Holstein cows during a humid tropical summer. Composite evening milk samples were analyzed on an infrared milk autoanalyzer. Vertical bar represents standard error of six cows.

RESULTS

Surroundings and body temperature

In the beginning of the 3-month summer season, air temperature of the immediate surrounding of experimental cows was 31.5°C when read at 15:00, it elevated to 34.0°C in mid summer and then dropped to 29.4°C near the end. The meanwhile relative humidity fluctuated between 73 to 98%. The humidex value, calculated by combining the two climatic readings, started with 44.64, reached a peak value of 53.48 in mid summer and then returned to 46.11 in the third month of the study (Figure 1). Rectal temperature of cows, recorded at the same time as the climate factors, averaged 39.36°C in the beginning, increased ($p < 0.05$) to 40.02°C in mid summer. Rectal temperature decreased ($p < 0.05$) since then to 39.44°C and further down to 38.26°C during the third month (Figure 1).

Milk production and quality

Milk yield (Figure 2) remained similar ($p > 0.05$) for May (27.8±3.4 kg/d) and June (28.8±3.2 kg/d), it dropped drastically to 21.3±1.8 kg/d in the second month of summer. Milk yield decreased further ($p < 0.05$) down to 18.50 kg/d for the third month in summer. Milk yield leveled up in September (20.2 kg/d) after the ending of official summer season. Throughout the summer season, somatic cell counts of experimental cows remained less than 5×10^5 cells/ml.

Content of lactose declined linearly with advancing



Figure 4. Electrophoresis pattern of whey proteins from Holstein cows during a humid tropical summer. Whey protein was prepared as supernatant of milk centrifuge at 10,000×g followed by acidification to pH 4.6. Electrophoresis was conducted on a commercial pre-cast agarose gel at pH 8.6, 90 mV. Lanes 1 and 10 are standards, lanes 2 to 8 are samples of representative cows.

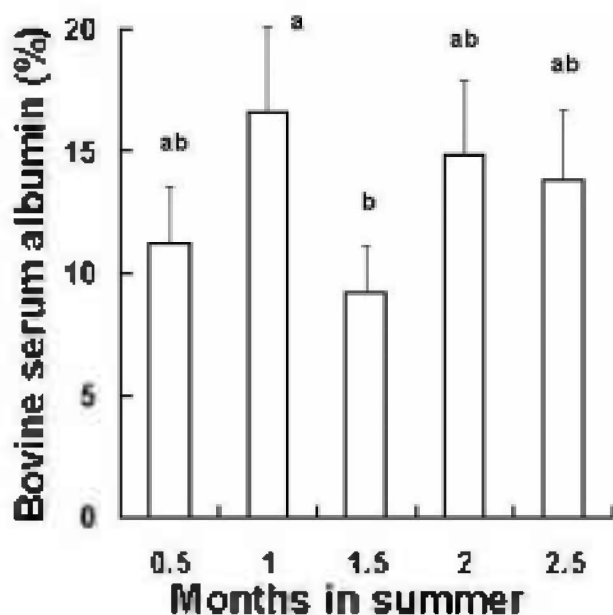


Figure 5. Fractional content of BSA in whey of primiparous Holstein cows during a humid tropical summer. Composite evening milk samples were electrophoresis on a commercial pre-cast gel at pH 8.6. Gels were scanned with a densitometer. Vertical bar represents standard error of six cows.

times in summer ($r^2=0.23$, $p=0.015$). Overall, lactose decreased from 4.69 to 4.38% ($p<0.05$, Figure 3) and daily production of lactose reduced about 40 % over the period of summer. Contents of milk protein and fat remained unchanged ($p>0.05$) among various intervals of the summer season.

Cows were homozygous (Figure 4) for the genetic variants β -lactoglobulin A (lane 7) or β -lactoglobulin B (lanes 3, 4, 6, 8) or heterozygous (lanes 2, 5). The present

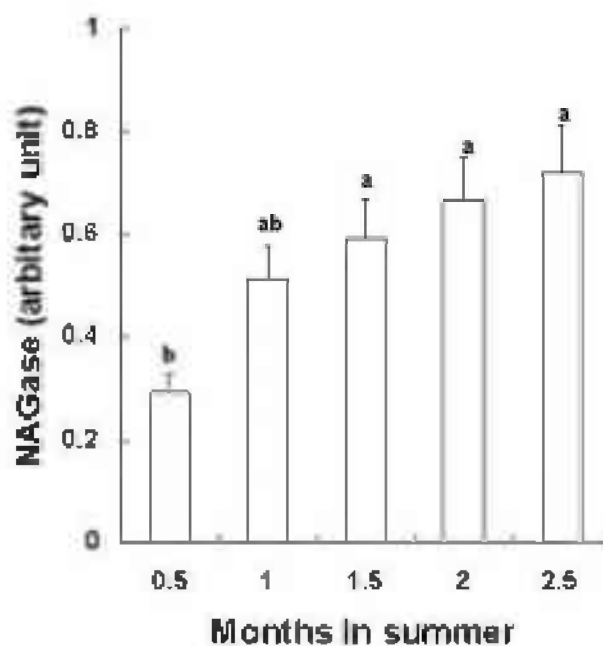


Figure 6. Activity of N-acetylglucosaminidase (NAGase) in milk of primiparous Holstein cows during a humid tropical summer. Results from composite evening milk samples. Vertical bar represents standard error of six cows.

electrophoresis system was suitable to distinguish among those. Whey constituted a constant portion of milk total protein during summer ($p>0.05$), from 4.88 to 5.29 g/l milk. The relative proportion of the four whey proteins were: β -lactoglobulin 43.4 to 49.4%, α -lactalbumin 24.2 to 29.4%, BSA 9.2 to 16.6%, and γ -globulin 7.8 to 10.8%, respectively. These proportions remained similar during summer except the proportion of BSA ($p<0.05$, Figure 5) between month 1 ($16.6\pm 0.84\%$) and month 1.5 ($9.2\pm 0.45\%$) in summer.

Activity of NAGase in milk increased linearly ($r^2=0.46$, $p=0.0002$) with the progress of summer season. Overall, by the end of the 3-month period, activity of milk NAGase increased up to over two folds ($p<0.05$, Figure 6).

Blood chemistry and mammary uptake

Concentrations of thyroxine and triiodothyronine in serum remained no change ($p>0.05$) along the summer season. The ratio thyroxine:triiodothyronine were not different among various intervals of summer either. However, concentration of serum cortisol transiently increased ($p<0.05$, Figure 7) from 0.128 to 0.404 $\mu\text{g/dl}$ during the first month of summer and decreased thereafter to the starting level.

Arterial concentration of glucose and urea did not change along the summer season ($p>0.05$, Figure 8). Across the mammary gland, it is calculated to obtain positive glucose A-V difference and negative urea A-V difference

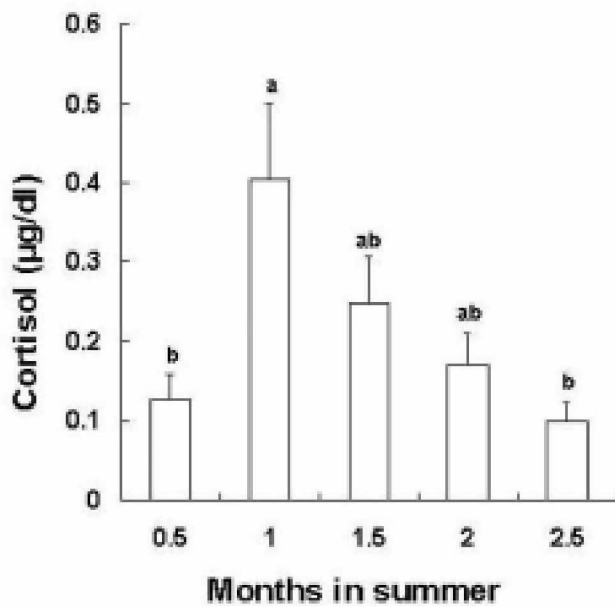


Figure 7. Concentration of serum cortisol of primiparous Holstein cows during a humid tropical summer. Vertical bar represents standard error of six cows.

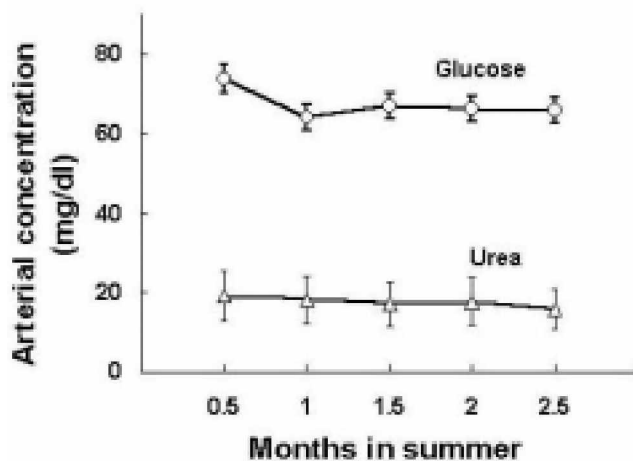


Figure 8. Progressive changes in blood concentrations of glucose and urea of primiparous Holstein cows during a humid tropical summer. Vertical bar represents standard error of six cows.

throughout the summer (Figure 9). Furthermore, the absolute value of A-V difference for urea increased ($p < 0.05$) from 1.54 mg/dl in the beginning to 7.76 mg/dl in the third month.

A positive linear ($r^2 = 0.72$, $p < 0.0001$) relationship was found between the arterial concentration of glucose and the A-V difference of glucose across the mammary gland. The linear equation predicting the latter parameter from the former was $Y = -60.49 + 1.023X$ in the glucose concentration range 55.0 to 88.1 mg/dl of the present study. On the other hand, the relationship between A-V difference of urea and

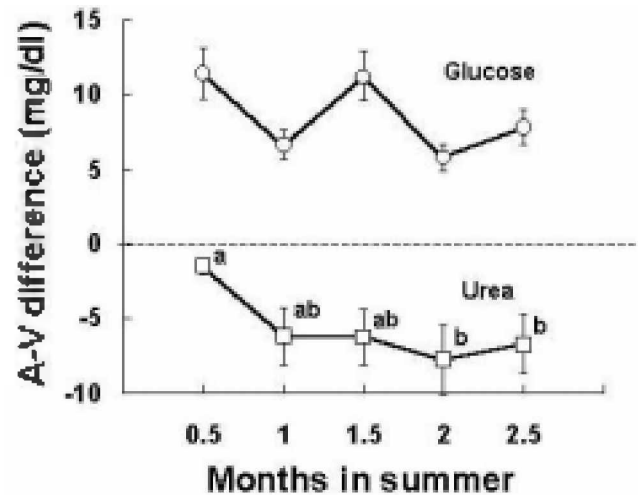


Figure 9. Progressive changes in arteriovenous concentration (A-V) differences of glucose and urea across mammary gland of primiparous Holstein cows during a humid tropical summer. Blood samples were taken from coccygeal vessel and subcutaneous abdominal vein simultaneously. Vertical bar represents standard error of six cows.

the coccygeal concentration of urea lacks significance ($p = 0.25$).

DISCUSSION

The present study characterized profiles of several physiological parameters that were associated with lactation performance of cows along an entire humid tropic summer. We reported evidence of irreversible regression in functionality of mammary gland during this period. The results are unique in that they were obtained from an extended study period under natural climates of low latitude. It was designed to maintain experimental animals in a less disturbed state with low frequency of sampling and the same nutrition and management regimens throughout the study. Recording and blood sampling by needle puncture at fixed-hour (15:00) of the day at fixed interval (biweekly) of the summer season was applied rather than a continuous catheter-sampling scheme. Variation in body temperature or concentration of blood metabolite and hormone caused by nycthemeral rhythm or feeding and milking would be minimized. Also, profiles of parameters along the season were obtained in addition to the absolute values.

According to the temperature-humidity index table constructed by Armstrong (1994) for dairy cows, at humidity as high as 70%, animal would suffer mild heat stress at ambient temperature above 24°C and severe heat stress at ambient temperature above 28°C. The recorded barn temperature during the study season varied between 29.4 to 34.0°C and barn humidity from 73 to 98%. Body

temperature 38.6°C is generally regarded as neutral for cow of normal lactation, above which milk yield decreases, partly attributable to the elevation of maintenance energy cost (West, 1994). Body temperature above 40°C was previously encountered in cow when somatotropin was used to increase milk production under a heat stress environment (West et al., 1990; West et al., 1991; Elvinger et al., 1992). It is apparent that cows in the experimental area have experienced very severe heat stress throughout the summer course. Rectal temperature of experimental cows were higher than 38.6°C for most of the time in summer. The highest rectal temperature exceeded 40°C around mid summer. It is estimated that in the study area, the degree of heat stress in mid summer is similar to the added actions of somatotropin and the summer climates of southern United States to cow. Different rectal temperature of cow in the course of this humid tropic summer implies that cows were able to adjust to the changing heat stress with the most severe degree of the table of Armstrong (1994).

Record of milk yield in the present study showed an abrupt decline around mid summer. No further reduction was observed the month after summer. Lactation of Hostein cow usually encompasses summer season, it is difficult to segregate the effects on milk yield between heat stress and stage of lactation. However, heat stress should play a major role in decreasing milk yield in this study because milk yield stopped declining at the end of summer season.

The steady decline in lactose content throughout the study demonstrates regression of mammary function along the summer season. Lactose serves as the major osmotic regulator in milk. Water is drawn into Golgi vesicles of the secretory cell where lactose is formed inside to maintain a constant osmotic pressure (Mepham, 1983). Because of the micelle nature of casein and the distinct synthesis and secretory route for milk fat, their final concentrations in milk relate primarily to the secretion of lactose. Unfavorable physiological conditions often reflect in the reduction of lactose content. The lactose concentration in milk decreased in goat on severe heat exposure for 4 d (Sano et al., 1985). Intramammary infection reduced lactose content while milk contents of fat and protein increased in compensation to the decrease in milk yield (Shuster et al., 1991). High air temperature during late pregnancy and the early postpartum period resulted in low lactose colostrum (Nardone et al., 1997). Also, since decrease in lactose content in the present study continued after mid summer, two phases of mammary regression was postulated. Prior to mid summer, lactose declined when body temperature and ambient temperature were increasing, the depression in activity/number of secretory cell was presumably initiated by elevated body temperature. The continuing decline after mid summer, however, represented the second phase of regression which was spontaneous and independent of body

temperature. Extent and duration of heat stress might be critical in determining whether or not the regression of mammary gland is reversible. Therefore, early stress index for mammary gland is important for prognosis and reverting undergoing regression.

In this study, the fractional content of BSA in whey protein (9.2-16.6%) was high compared to that normally found for Holstein cows (7-8%, Prosser et al., 2000). Furthermore, a transient elevation in BSA in the first month of summer was found without concomitant SCC change. It has been well documented that heat stress exerts effects on the content and distribution of milk protein. Casein changes reciprocally with whey protein and true protein and non-protein nitrogen elevate in heat stressed cows (Muroya et al., 1997). Serum albumin is thought enter milk via paracellular route through loosening tight junction and/or in association with neutrophil diapedesis, not necessarily under secretory cell damage. Higher milk BSA was found during intramammary infection (Shuster et al., 1991) and reducing milking frequency (Stelwagen and Lacy-Hulbert, 1996). NAGase activity in milk is believed to be a direct indicator of secretory cell damage. Milk accumulation increased BSA and SCC but not NAGase activity in milk (Stelwagen and Lacy-Hulbert, 1996). Steady elevation in milk NAGase activity found in this study suggests a gradual deterioration of mammary epithelial barrier under this summer climates. Not only tight junction was loosening but also detachment of epithelial cells from the basement.

Measurement of metabolite A-V difference across mammary gland is useful in estimating efficiency of milk synthesis. Among blood precursors, glucose is most abundantly taken by mammary gland of cows during lactation (Miller et al., 1991; Cant et al., 1993b; Guinard et al., 1994). This prominent uptake of glucose is prone to decrease at declining phase of lactation as the requirement for milk synthesis is decreasing, such as heat stress-induced reduction of milk yield (Sano et al., 1985) and following milk stasis (Chang et al., 1996). In the present study, mammary uptake of glucose is obvious and stable throughout summer although the extraction rate was lower than those of other reports (Cant et al., 1993b; Guinard et al., 1994). It was also found in this study that mammary uptake of glucose is dependent on ($r^2=0.72$) its plasma concentration which was opposite to the findings of other workers (Miller et al., 1991; Cant et al., 1993b) where factors such as gland biosynthetic capacity, availability of other nutrients instead of plasma concentration were suggested in determining uptakes of glucose. There is a possibility that the regulatory mechanism of glucose uptake by mammary gland during heat stress might be different from that of thermoneutral cow.

In the present study, there was a significant urea release from the mammary gland of lactating cows throughout the

summer season. Moreover, this estimated release of urea increased with advancing summer. The source of urea is most likely related to the catabolism of essential amino acid arginine in mammary tissue. Formation of urea by the mammary gland of goats has been previously reported (Mephram and Linzell, 1967). The presence of arginase enzyme in cow mammary tissue was also demonstrated (Clark et al., 1975). Mephram (1982) categorized arginine foremost the amino acid taken up by mammary gland of lactating cows in excess of the milk output. The work of Cant et al. (1993a) measured A-V differences across mammary gland for urea as well as for amino acid in dairy cows fed fat and reported negligible A-V difference for urea. Trottier et al. (1997) using lactating sows reported significant arginine retention in mammary gland. They also reported negative A-V difference for urea but didn't provide accurate value. Despite the prevail recognition of relation between arginine metabolism and urea production in mammary gland, their implication to mammary gland function have not been fully discussed. It is apparent that the release of urea from mammary gland can represent to some extent the efficiency of amino acid utilization within. Using hyperinsulinemic-euglycemic clamp casein-infusion, Grinari et al. (1997) reported increase in milk protein yield, suggesting an improved efficiency during the clamp of amino use for milk protein synthesis. They regard the gradual decline in plasma urea as a proof of decreased amino acid oxidation. Although their study did not restrict to mammary gland, it is pioneer in relating plasma urea level to efficiency of milk protein synthesis. Bequette et al. (1998) pointed out in a review that all amino acids in mammary gland are extensively channeled through an intermediary protein pool or pools that have rapid turnover rate. Amino acids metabolize actively serving for structural, functional and milk protein synthesis. It is assumed that as the production of milk protein decreases, the contribution of non-milk protein turnover to total protein synthesis in mammary gland increases in accompany with accelerated amino acid catabolism and urea release. Measurement of mammary A-V difference of urea is suitable for dynamically and routine monitoring of the efficiency of mammary gland. It can be used as an early stress index if a threshold level is established.

In conclusion, lactating Holstein cows raised in a humid tropics was capable of adjusting their body temperature in parallel to the humidex value during the summer season. The highest rectal reading reached above 40°C during summer indicating heat stress. Changes in lactation traits accompanying heat stress during summer including milk yield, lactose content, whey BSA content, milk NAGase activity and urea release from mammary gland demonstrate some functional deterioration of mammary gland which is

irreversible and spontaneous beyond certain extent of heat stress.

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