



## Relation of Calcium Activity in Milk and Milk Production of Holstein Cows in Hot Season

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**ABSTRACT :** The content of Ca in milk exceeds the typical saturation level of Ca salts, which is necessary for neonate growth. This calcium is distributed between the casein micelles in the colloidal and aqueous phases. Information on the properties of calcium activity in the aqueous phase is limited compared with that on the properties of bound or sequestered calcium. The objectives of this study were to evaluate the changes in calcium activity in fresh milk using an ion-selective electrode and to assess the relationship between calcium activity and milk production in hot season. Milk samples collected from 10 cows at the National Agricultural Research Center for Kyushu Okinawa Region in June to October (Min-Max: 7.2-35.2°C, 24.3-100% RH) were analyzed on total calcium concentrations and calcium activity. We observed that the rectal temperature of the cows increased according to elevation of ambient temperature but that the pH of the collected milk ( $6.61 \pm 0.01$  (Mean $\pm$ SEM)) was not significantly influenced by rectal and ambient temperature. Total calcium concentrations and calcium activity in fresh milk decreased in July (Min-Max: 21.1-33.5°C, 48.9-100.0% RH) compared with the values after August (Min-Max: 18.1-35.0°C, 26.5-96.2% RH) ( $p < 0.05$ ); however, there was no significant correlation between the two parameters. The ratio of calcium activity to total calcium concentration decreased after August compared with the values in June and July ( $p < 0.05$ ). The calcium activity in fresh milk was positively correlated with milk yield ( $r = 0.45$ ,  $p < 0.01$ ) and negatively correlated with milk lactose content ( $r = -0.53$ ,  $p < 0.01$ ). These results suggest that the calcium activity in milk could be affected by ambient temperature and might be associated with milking production in hot season. (**Key Words :** Calcium Activity, Dairy Cows, Hot Season, Milk Production)

### INTRODUCTION

Calcium is the major mineral in bovine milk with a total concentration in excess of 25 mM. The calcium in bovine milk is distributed between the colloidal casein micelles and aqueous phases (Holt and Jenness, 1984; Neville et al., 1994). Casein is composed of a complex micelle structure containing approximately 25,000 phosphorylated monomers (Farrell, 1988), which can react with calcium phosphate complexes in the milk to bind from 20 to 40 moles of calcium per mole of casein (Jenness, 1979; Holt and Jenness, 1984; Neville et al., 1994). More than 65% of calcium in bovine milk is associated with casein (Griffin et al., 1988). Calcium in the aqueous phase of milk exists as ionic calcium, calcium phosphate and calcium citrate. The concentration of calcium ions in mammary glands is

thought to be associated with the integrity of mammary tight junctions during lactation (Neville and Peaker, 1981), suggesting that the calcium ion concentration in mammary glands plays a role in regulating the physiological function of milk production. The calcium concentration in milk is affected by environmental and physiological conditions. It has been reported that a high ambient temperature decreases the total calcium and other minerals concentrations in milk (Kume et al., 1990; Sevi et al., 2004). These results seemed to be derived from a decreasing absorption through digestive tract of these minerals and an increasing of their losses to the outside (Kume et al., 1989). However, the influence of high ambient temperature on calcium ion concentration in milk is unclear. The concentration of calcium ions in milk can be measured directly using a calcium ion-selective electrode (Allen and Neville, 1983). Calcium activity was defined as an uncorrected direct analysis using ion selective electrode and showed lower value than repeated addition, ultrafiltration and equilibration dialysis methods (Silanikove et al., 2003). Calcium activity could be measured by a simple and convenient protocol, however the

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changing profile and significance of calcium activity in milk production was unclear. The objectives of this study were to clarify the effects of ambient temperature on calcium activity in bovine milk using an ion-selective electrode, and the relationship between calcium activity and milk production in hot season.

## MATERIALS AND METHODS

### Animals and milk samples

From June to October, we used 10 Holstein cows (parity,  $2 \pm 1.1$ ; age,  $4.3 \pm 1.7$  years) that were born and raised at National Agricultural Research Center for Kyushu Okinawa Region in south-west area in Japan. All cows calved more than one month before. These cows were fed total mixed ration (TDN = 70.5-71.1% of DM, CP = 13.6-14.5% of DM) twice a day. During the daytime, each dairy cow was fed in a tie stool equipped with fans and a periodic misting system, whereas at night time they were fed in a paddock where they could have free access to feed and water. Rectal temperature was recorded every morning (09:00-09:30) throughout the experimental period. Fresh milk samples from evening milking were stored at 4°C in a sealed tube, subsequently mixed with next morning milk on the day as shown in Table 1, and analyzed within a day. Milk yield was recorded using a WIKATO milk meter (Hamilton, New Zealand) at each milking time. All procedures were performed in accordance with the guidelines for the care and use of laboratory animals of the National Agricultural Research Center for Kyushu Okinawa Region.

### Sample analysis

The percentage of fat, protein and lactose in the milk were measured using an automatic analyzer (Milko-Scan 133B; Foss Japan, Tokyo, Japan).

The total calcium concentration in each milk sample was determined using an automatic biochemical dry

analyzer (Dri-Chem 3500V; Fuji-Film Co., Tokyo, Japan), which showed good correlation with atomic absorbance results on total calcium contents in milk. After measuring the pH in milk, the calcium activity of the milk was analyzed using a calcium ion-selective electrode (Calcium ionplus Combination ISE; Thermo Fisher Scientific Inc. Beverly, USA). The electrode was calibrated using 0.5 mM and 5 mM standard calcium solutions. The conductance of the standard solution was adjusted to 5 mS by adding KCl because most milk samples showed approximately the same conductance. The slope of the standard line showed the range of 25 to 30 according to the instruction manuals of the electrode. The sample of 30-ml milk in tube was maintained with gentle mixing at 38°C, which was near average body temperature of cows, during the analysis. The value after a 5-min measurement was recorded as the calcium activity in each milk sample. Additional one-point calibration using a 1.25 mM calcium standard solution was performed before each analysis.

### Statistical analyses

The values obtained from each cow during the experimental period were expressed as the means  $\pm$  standard error of mean (SEM). Statistical analysis was performed using SPSS for Windows 16.0J. Multiple comparisons of the results were performed using the Tukey's test. The *p*-values below 0.05 in correlation analyses and difference tests were considered statistically significant.

## RESULTS

The monthly averages of ambient temperature and relative humidity varied in June (23.7°C, 77.4%), July (27.0°C, 83.1%), August (28.2°C, 74.9%), September (25.3°C, 69.8%), and October (18.4°C, 74.2%), respectively. During the experimental period, the average milk yield of cows was 33.6 kg/d and rectal temperature was

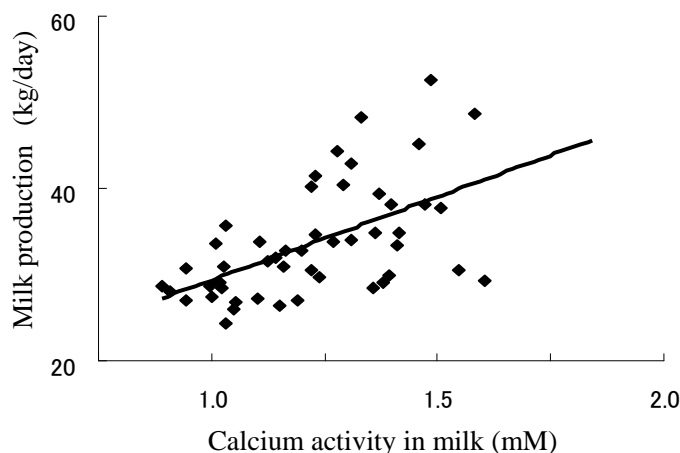
**Table 1.** Changes of rectal temperature, calcium activity and total calcium concentration in milk, and ratio of calcium activity to total calcium concentration in hot season

		24/JUN	8/JUL	22/JUL	5/AUG	19/AUG	9/SEP	30/SEP	21/OCT
Rectal temperature (°C)	Mean	38.20 <sup>ab</sup>	38.30 <sup>ab</sup>	38.66 <sup>ab</sup>	38.85 <sup>a</sup>	38.79 <sup>a</sup>	38.34 <sup>b</sup>	38.41 <sup>ab</sup>	38.28 <sup>ab</sup>
	SEM	0.20	0.20	0.13	0.15	0.14	0.11	0.08	0.16
Total calcium (mM)	Mean	25.00 <sup>ab</sup>	22.50 <sup>b</sup>	22.75 <sup>b</sup>	28.43 <sup>a</sup>	27.38 <sup>a</sup>	28.63 <sup>a</sup>	29.08 <sup>a</sup>	30.20 <sup>a</sup>
	SEM	0.72	0.42	0.59	0.79	0.48	1.28	0.82	0.84
Calcium activity (mM)	Mean	1.28 <sup>ab</sup>	1.31 <sup>ab</sup>	1.17 <sup>b</sup>	1.13 <sup>b</sup>	1.21 <sup>ab</sup>	1.28 <sup>ab</sup>	1.34 <sup>ab</sup>	1.40 <sup>a</sup>
	SEM	0.07	0.06	0.05	0.05	0.07	0.07	0.07	0.05
Ratio <sup>1</sup>	Mean	5.67 <sup>a</sup>	5.90 <sup>a</sup>	5.66 <sup>a</sup>	4.02 <sup>b</sup>	4.45 <sup>ab</sup>	4.52 <sup>ab</sup>	4.63 <sup>ab</sup>	4.64 <sup>ab</sup>
	SEM	0.35	0.29	0.27	0.22	0.25	0.23	0.23	0.18

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<sup>1</sup> Calcium activity/total calcium concentration.

Different letter showed statistically significant differences in same row (Tukey *p*<0.05).



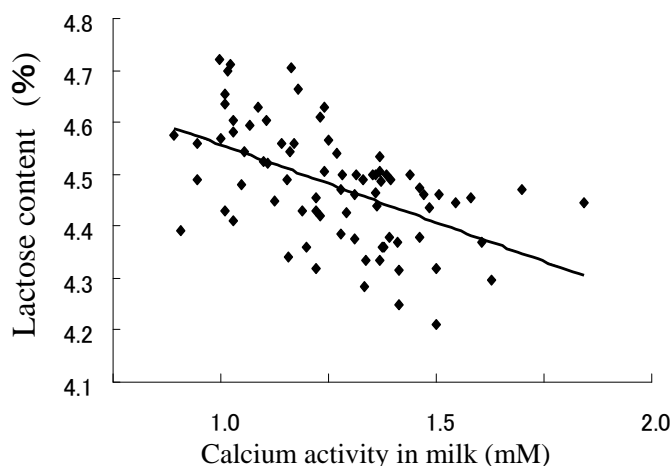
**Figure 1.** Relationship of milk production and calcium activity in milk during hot season ( $n = 80$ ), with correlation coefficient of 0.45 ( $p < 0.01$ ).

$38.58 \pm 0.06^\circ\text{C}$ . The rectal temperature at each sampling time was shown in Table 1. A maximum rectal temperature of  $38.82 \pm 0.15^\circ\text{C}$  was recorded in August. The average percentages of milk fat, protein and lactose during the experimental period were  $3.60 \pm 0.07\%$ ,  $3.13 \pm 0.03\%$  and  $4.47 \pm 0.01\%$ , respectively. There were no significant changes in these components among sampling times. The average pH value of milk during the experimental period was  $6.61 \pm 0.01$ . This value did not show any significant changes during the experimental period. The total calcium concentration in milk through the entire period was  $26.23 \pm 0.44$  mM. The concentration in July decreased significantly compared with concentrations measured after August ( $p < 0.05$ , Table 1). The average of calcium activity in milk from June to October was  $1.26 \pm 0.02$  mM and showed a significant decrease from July to August compared with that collected before July ( $p < 0.05$ ) (Table 1). No effect of the time course after parturition on the calcium activity was

observed in this study. The ratio of calcium activity to total calcium concentration in milk through the experimental period was  $4.92 \pm 0.11\%$  and decreased significantly in August ( $p < 0.05$ ) (Table 1). Although we observed no correlation between calcium activity and total calcium concentration, we did observe a positive correlation between milk yield and calcium activity ( $p < 0.01$ ) (Figure 1), and a negative correlation between percentage of milk lactose and calcium activity ( $p < 0.01$ ) (Figure 2).

## DISCUSSION

The mean rectal temperature of cows increased in August to  $38.8^\circ\text{C}$  (range,  $38.2$  to  $39.6^\circ\text{C}$ ), which indicates that the cows were exposed to high environmental temperature. It has been reported that high environmental temperature decreases the percentage of fat, protein and lactose in milk (Kadzere et al., 2002; Kamiya et al., 2005,



**Figure 2.** Relationship of lactose content and calcium activity in milk during hot season ( $n = 80$ ), with correlation coefficient of -0.53 ( $p < 0.01$ ).

2006); however, in the present study, these components were not significantly affected by heat stress. Therefore, the heat stress to the cows in this study may have been relatively mild.

Our results that total calcium concentration in milk decreases in the summer season were consistent with previous studies (Kume et al., 1990; Sevi et al., 2004). Total calcium concentration decreased in July (Table 1) but without any simultaneous changes of rectal temperature. A change in rectal temperature might indirectly affect milk calcium concentration during the hot season. Since the total milk calcium concentration in various species is positively correlated with the growth of their neonates (Neville et al., 1994), a decrease in total calcium concentration in milk during the hot season might affect calcium intake and the growth of calves.

There have been several reports on calcium ion concentration in bovine milk. Silanikove et al. (2003) reported that direct measurement of milk calcium ion concentration using an ion-selective electrode, which was defined as calcium activity, showed lower values than those using ultrafiltration and equilibration dialysis methods. The calculated value of milk calcium ion concentration reported by Holts et al. (1981) was 2.0 mM, whereas Silanikove et al. (2003), using an ion-selective electrode in equilibration dialyzed and ultrafiltered samples, reported that the calcium ion concentration in bovine milk was approximately 3.0 mM. The latter authors also reported a value of 1.8 mM obtained as a calcium activity in bovine milk. Our results showed that the average of calcium activity in milk was 1.26 mM. Difference between the two results might be derived from environmental temperature and physiological condition of the cows and also on the difference of temperature during the calcium activity analysis. Thus, a consensus on the calcium activity in bovine milk has not yet been obtained. Our results showed that calcium activity decreased in the hot season in a different manner to total calcium concentration. The calcium activity (the uncorrected direct measurement of calcium ion concentration) in this study has advantages on the point of less artificial treatment of milk and convenient procedures, particularly comparing the relative changes in calcium ion condition with the physiological state of cows. Calcium activity measured using an ion-selective electrode is, however, affected by changes in pH (Allen and Neville, 1983). The pH of milk in this study was 6.6, which is slightly lower than the 6.7 recorded by Neville (2005). The values of calcium activity obtained in this study did not appear to be influenced by the change in milk pH. Indeed, calcium activity was stable up to 48 hours after sampling under sealed conditions at 4°C (data not shown). The temperature of the milk sample and the electrode are

important factors in the measurement of ion calcium activity. It has been reported that calcium ion concentrations at 37°C decrease by 2% to 5% compared with those at 25°C (Landenson and Bowers, Jr. 1973). Accordingly, in this study, the temperature of milk samples and the electrode were maintained at 38°C in order to eliminate any thermal disturbances and to feedback the value to cow physiology.

The calcium activity in milk was observed to be 4% to 6% of the total calcium concentration. Holts et al. (1981) reported that the ratio of ionic calcium to total calcium was approximately 6.9% by calculation. The reason for the difference between these two sets of results might include different analysis methods. A decrease of calcium ion concentration around mammary epithelial cells influences the integrity of occluding junctions in mammary glands. It has been reported that decreasing calcium ion concentrations in mammary glands lumen of goats, induced by the injection of citrate, led to increases in the milk concentrations of sodium and chloride and decreases in potassium and lactose, which suggests the important role of calcium ions in the integrity of the mammary epithelium during lactation (Neville and Peaker, 1981; Stelwagen et al., 1995). Our results showed a positive correlation between milk yield and calcium activity (Figure 1). Pitelka et al. (1983) reported that occluding junction structure and permeability in cultured epithelial sheets depended on calcium ion concentration. Although similar information on calcium activity in dairy cows was limited, a decrease in calcium activity in the hot season might be possibly associated with milk productivity and the integrity of the mammary epithelium. Calcium activity in milk during this experimental period was negatively correlated with lactose concentration ( $p < 0.05$ ) (Figure 2). The lactose concentration in milk has been shown to be positively correlated with the energy status of dairy cows (Reist et al., 2002). Increase of dry matter intake means improvement of the energy status and milk production of cows in general. It was suggested that the reasons for decreasing milk calcium content in summer season had been derived from decreasing of dry matter intake, which showed calcium intake deficiency (Kume et al., 1898). In this study, cows in higher milk production, expected higher dry matter intake, showed higher calcium activity. However cows higher lactose content, expected higher dry matter intake, showed lower calcium activity. The reasons of this discrepancy are not clear at the present time. The calcium activity in milk might not be associated with only energy status of cows during the hot season. The precise relationship between calcium activity, casein and lactose in milk in hot season, however, remains to be clarified.

Calcium activity in bovine milk was decreased in summer, and the relation of calcium activity and milk

production was obtained in this study at first time. Accumulation of further information on the calcium activity in milk and their relationship with cow physiology is necessary.

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