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Milk Protein Production and Plasma 3-Methylhistidine Concentration in Lactating Holstein Cows Exposed to High Ambient Temperatures

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ABSTRACT : This experiment was performed to examine the influences of high ambient temperature on milk production, nutrient digestibility, energy and protein sufficiency ratio, and plasma metabolites concentration in lactating cows. In a 2×2 crossover design, four multiparous lactating Holstein cows were maintained in a chamber under treatment of constant moderate (18°C) ambient temperature (MT) or high (28°C) ambient temperatures (HT). The DMI and milk protein yield were significantly lower in HT ($p<0.05$). The milk yield, milk lactose yield, and milk SNF yield tended to be lower in HT ($p<0.10$). No statistical differences for 4% fat-corrected milk and milk fat yield were observed. Rectal temperatures were significantly higher in HT than MT ($p<0.05$). The apparent DM, OM, ether extract, CF, and ash digestibility did not differ between treatments. On the other hand, the apparent CP digestibility was increased significantly ($p<0.05$) and nitrogen free extract tended to increase ($p<0.10$) in HT. The sufficiency ratio of ME and DCP intake for each requirement tended to be lower in HT than in MT ($p<0.10$). Concentrations of total protein (TP), albumin, and urea nitrogen in plasma did not differ between treatments. Plasma 3-methylhistidine (3MH) concentration as a marker of myofibrillar protein degradation tended to be higher in HT ($p<0.15$). In conclusion, high ambient temperature was associated with a lower energy and protein sufficiency ratio, and decreased milk protein production, even though the body protein mobilization tended to be higher. (**Key Words :** Dairy Cows, Heat Stress, Milk Production, Energy, Protein, Protein Degradation)

INTRODUCTION

In southwestern Japan, climatic conditions are such that the hot season is long and the average temperature is higher than the upper critical temperature of lactating Holstein cows during summer. Shibata (1983) reviewed the influence of a hot environment in lactating dairy cows, and reported that milk protein concentration was decreased even though the reported effects of heat stress on milk fat and lactose concentrations differ. Previous experiment (Kamiya et al., 2005) also showed that milk protein production was greatly decreased by high ambient temperature treatment. During early lactation, body compartments are mobilized to support milk production in lactating dairy cows because they cannot consume sufficient nutrients to meet the production requirements (Komaragiri and Erdman, 1997). Therefore, the rate and extent of tissue compartment mobilization also would affect the milk protein production at low nutritional status such as that of heat-stressed cattle. For this reason, we examined the relationships between milk protein production and body protein mobilization. This study investigated milk production, nutrient digestibility, energy and protein sufficiency, and plasma markers of myofibrillar protein degradation at the treatment of high ambient temperature.

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MATERIALS AND METHODS

Cows and treatments

Four multiparous lactating Holstein cows were assigned to either of two ambient-temperature groups in a 2×2 crossover experimental design: constant moderate ambient temperature (18°C; relative humidity: 60%) or high ambient temperature (28°C; relative humidity: 60%). At the beginning of the experiment, the average time in milk was 59 days, the average parity was 2.5 calves, and the average age was 5.4 yr. This experiment was consisted of two 13-day experimental periods, each comprising 8 days of

Table 1. Ingredients and chemical composition of TMR

Ingredients (%)	
Sudangrass hay	35.0
Beet pulp	10.5
Soybean hulls	5.0
Soybean meal	6.5
Corn	20.0
Concentrate mix	16.5
Cotton seed	2.0
Fish meal[1]	2.0
Calcium salts of fatty acids	1.5
$Ca_3(PO_4)_2$	1.0
Chemical composition (%)	
TDN[2]	73.4
CP	14.6
EE[3]	3.4
CF	17.8
Ash	7.2
NFE[4]	57.0
ADF	21.4
NDF	35.1

[1] Ministry of Agriculture, Forest and Fisheries of Japan has forbidden the use of fish meals for cattle feed since 2001.

[2] TDN estimated according to the National Agricultural Research Organization Standard Tables of Feed Composition in Japan (2001).

[3] Ether extract.

[4] Nitrogen free extract.

* The vitamin supplement contained (per gram): 10,000 IU of vitamin A, 2,000 IU of vitamin D_3 and 10 mg of D, L-tocopheryl acetate.

** Mineral blocks contained (per kilogram): Fe 1,232 mg; Cu 150 mg; Co 25mg; Zn 500 mg; Mn 500 mg; I 50 mg; Ser 15 mg; Na 380 g and vitamin E 2,000 IU.

adaptation period and 5 days of sampling period. All cows were fed *ad libitum* and water was available at all times. The composition and characteristics of the total mixed ration (TMR) are given in Table 1. The TMR was offered at 08:30, 10:30, 16:00 and 18:00 h to both treatment groups. The diet was top-dressed with a vitamin supplement (7 g/day) and was given at the 10:30 h feeding. Mineral blocks were available free choice. At the 1st day of first experimental period, four cows were housed individually in open circuit respiration chamber with stanchion stall, water cup, feed box, air conditioner, air flow system, and gas analysis systems. The feces and urine separator was installed in each basement. Ambient temperature and relative humidity of two chambers were computer-controlled by air conditioner system at 18±1°C and 60±7% of humidity (moderate ambient temperature) from day one to day 13 of first experimental period. Another two chambers were computer-controlled by air conditioner system at 28±1°C and 60±7% of humidity (high ambient temperature) from day one to day 13 of first experimental period. Apparent digestibility and methane production were determined with 5 days samples (from day 9 to day 13). The operation of these chambers was described elsewhere (Kurihara et al., 1989; Mukai et al., 1989). Second experimental period was immediately started after first experimental period. Ambient temperature and relative humidity of each chamber in second experimental period were set contrary to first experimental period, respectively. Other procedures were the same as those in first experimental period.

Experimental procedure and laboratory analysis

Feed refusals and feces were collected daily at 10:30 h before feeding for 5 days (from day 9 to day 13 of each experimental period) and were pooled for analysis. Samples of feed, refusals, and feces were dried at 60°C in a forced air oven and ground through a 1-mm screen in a mill before analysis. The DM, CP, CF, ether extract, and ash of feed, refusals, and feces were determined using a standard procedure (AOAC, 1990). The NDF and ADF of feed were analyzed according to the procedure of Van Soest et al. (1991). The cows were milked twice daily at 08:30 and 18:00 h and the milk yield was recorded. Sample collected at each milking were analyzed for fat, protein, lactose and solids-not-fat (SNF) contents using a Foss Milko-Scan 133B (N. Foss Electric A/S, Hillerød, Denmark). Urine was collected daily at the 10:30 h for 5 days (from day 9 to day 13) and was pooled for analysis. The gross energy of all samples was measured using an adiabatic bomb calorimeter (CA-4P; Shimadzu Corp., Kyoto, Japan). Milk and urine samples were placed in polyethylene bags, freeze-dried, and then burnt using calorimeter (Itoh and Tano, 1977). Body weight was measured at 10:30 h on days 0 and 13. Rectal temperature was measured daily at 8:30 hr.

At the end of the each experimental period, blood samples were taken from the jugular vein using a heparinized tube. Plasma was separated by centrifugation at 1,500 g for 15 min and stored at -20°C until analysis. The TP, albumin, and urea nitrogen in plasma were measured using an automatic analyzer (7080 Clinical Analyzer; Hitachi Ltd., Tokyo, Japan). The 3MH in plasma was measured using HPLC method after converting the fluorescamine derivative in the acidic condition at 80°C for 1 h (Wassner et al., 1980). The HPLC system (Hitachi Ltd, Tokyo, Japan) was consisted of a pump (model L-7100), syringe-loading sample injector containing a 5-μl loop, a column oven (model L-7300) and a fluorescence detector (model L-7480). The analytical reversed-phase column was a LiChrospher 100 RP-18 (e) (particle size 5 μm, 250×4 mm i.d.) protected by guard column (10×4 mm i.d.) containing with the same material (both obtained from Kanto Chemical, Co., Inc., Tokyo, Japan). The column temperature was maintained at 40°C. The isocratic mobile phase was composed of 24% acetonitrile/76% 10 mM sodium phosphate buffer (pH 6.5, vol/vol). The flow rate was 1.2 ml/min. Detection was performed at 360 nm (excitation wavelength) and 485 nm (emission wavelength).

Table 2. Bodyweight, feed intake, milk production, and rectal temperature in lactating dairy cows

	18°C	28°C	Pooled SE	p value
Body weight (kg)	620	607	7	0.28
Feed intake				
DMI (kg/day)	19.3	15.6	0.5	0.02
DMI/BW (kg/kg)	0.032	0.026	0.001	0.01
Milk production (kg/day)				
Milk yield	36.3	31.7	1.1	0.06
4% FCM[1]	35.4	32.9	2.1	0.46
Fat yield	1.41	1.31	0.09	0.47
Protein yield	1.08	0.86	0.05	0.04
Lactose yield	1.69	1.47	0.06	0.07
SNF[2] yield	3.13	2.64	0.11	0.05
Milk composition (%)				
Fat	3.91	4.16	0.19	0.41
Protein	2.97	2.68	0.07	0.06
Lactose	4.66	4.63	0.02	0.31
SNF	8.63	8.31	0.09	0.08
Rectal temperature (°C)	38.6	39.3	0.1	0.01

[1] 4% Fat-corrected milk. [2] Solids-not-fat.

All procedures used in this study were examined and approved by the Annual Research Project Examination Committee of the National Agricultural Research Center for Kyushu Okinawa Region.

Statistical analysis

The data were analyzed according to a crossover design using the general linear model procedure of SAS (1999), with cows and treatments as main effects.

RESULTS AND DISCUSSION

The body weight (BW), feed intake, milk production and composition, and rectal temperature are shown in Table 2. No differences between BW of groups were observed. The cows in HT treatment had significantly higher rectal temperatures ($p<0.05$) than that in MT. The DMI and DMI/BW were decreased ($p<0.05$) in HT. The milk protein yields were significantly lower in HT cows ($p<0.05$). The milk yield, milk lactose yield, and milk SNF yield tended to be lower in HT ($p<0.10$), which may be due to lower DMI. On the other hand, no statistical differences of 4% FCM and milk fat yield were observed. In addition, concentrations of protein and SNF in milk tended to be lower in HT cows ($p<0.10$). However, concentrations of fat and lactose in milk did not differ between treatments. Milk yield was generally reduced during hot seasons (Lee et al., 2004), which supported our result. On the other hand, Mazumder and Kumagai (2006) reported that milk fat concentration was lower in hot season, which disagreed with our result. Because heat stress treatment in this study was relatively short period, the effect of milk fat concentration would be different between long-term and short-term heat stress.

Table 3. Digestibility of feed in lacatating dairy cows

	18°C	28°C	Pooled SE	*P*-value
Apparent digestibility, %				
DM	66.0	67.4	0.7	0.23
OM	68.6	70.0	0.7	0.26
CP	61.8	64.0	0.4	0.03
EE[1]	78.0	78.4	2.0	0.90
CF	56.9	54.0	2.8	0.51
Ash	32.1	33.5	1.1	0.43
NFE[2]	73.2	75.8	0.8	0.09

[1] Ether extract. [2] Nitrogen free extract.

Terada and Shioya (1998) reported that high ambient temperatures reduce the milk protein yield and concentration in lactating dairy cows. In addition, it is known that energy and nitrogen intake affect milk protein production (Emery, 1978). Consequently, the decrease in milk protein production would be greater than that of other milk components. Furthermore, the reduction of milk protein content observed in summer (Bernabucci et al., 2002), indicating that long-term heat stress also lowered milk protein production.

Results of digestion trials (Table 3) show that the apparent DM, OM, ether extract, CF, and ash digestibility did not differ between treatments. On the other hand, the apparent CP digestibility was significantly higher ($p<0.05$) and nitrogen free extract digestibility tended to be higher ($p<0.10$) in the HT treatment cows than in those of moderate temperature treatment (MT). Hirayama and Katoh (2004) and Hirayama et al. (2004) reported increased CP, NDF, and ADF digestibility in goats during heat exposure. On the other hand, Bernabucci et al. (1998) reported that feed digestibility was higher in heifer exposed to short-term heat stress than thermal comfort condition or long-term heat stress, suggesting the adaptive response of digestive tract to heat stress. Therefore, the effect of heat stress on feed digestibility might be different between short-term treatment in this study and long-term treatment. Furthermore, the results of the present experiment were not in accordance with previous experiment (Kamiya et al., 2005), which showed the influence of high ambient temperature or feed restriction on the apparent CP digestibility were not observed. Therefore, future studies are needed to clarify the precise influence of heat stress, including long-term exposure, on feed digestibility in lactating dairy cows.

The respective GE, DE, and ME intakes were significantly lower ($p<0.05$) in HT (Table 4). The sufficiency ratio of ME intake for its requirement tended to be lower in HT than MT ($p<0.10$). The CP and DCP intake were lower ($p<0.05$) in HT treatment. The sufficiency ratio of DCP intake for its requirement also tended to be lower in HT than in MT ($p<0.10$). Therefore, the energy and protein status for nutritional conditions in lactating dairy cows

Table 4. Energy and protein intake in lactating dairy cows

	18°C	28°C	Pooled SE	p value
Energy intake (Mcal/day)				
GE	85.4	69.1	2.5	0.02
DE	56.2	46.6	1.6	0.02
ME	50.2	41.5	1.7	0.03
Protein intake (g/day)				
CP	2,923	2,356	67	<0.01
DCP	1,805	1,501	45	0.02
Sufficiency ratio (intake/requirement[1] ×100)				
ME	80.0	70.8	2.5	0.08
DCP	80.2	72.8	1.9	0.07

[1] Requirement is calculated from Japanese Standard for Dairy Cattle (MAFF, 1999).

would be lower for cows in HT than those in MT. Mazumder and Kumagai (2006) reported that DMI in lactating dairy cows was lower in hot season, indicating that ME and DCP intake were also decreased by long-term heat stress. On the other hand, the sufficiency ratio of ME and DCP intake for its requirements might be higher in long-term heat stress than short-term, because it was expected that the extent of decreases in BW and milk production was higher in long-term heat stress compared with short-term.

Concentrations of plasma metabolites (Plasma TP, albumin, UN, 3MH) were unaffected by HT treatment (Table 5). On contrary, Muroya et al. (1997) reported higher concentrations of urea nitrogen in milk from cows maintained under a high temperature condition. The concentrations of urea in plasma and milk are generally associated closely (Oltner and Wiktorsson, 1983; Broderick and Clayton, 1997). Bunting et al. (1996) observed higher ruminal concentrations of ammonia nitrogen in calves during summer than in winter. But contrary to our expectation, BUN levels were not affected due to HT treatment.

The concentration of plasma 3MH tended to be higher in HT ($p<0.15$) indicating more endogenous protein mobilization when energy intake was decreased. The concentration of 3MH in blood has been used as an index of myofibrillar protein degradation in dairy cows (Blum et al., 1985), mouse (Yoshizawa et al., 1997), goat (Nagasawa et al., 1996) and rat (Nagasawa et al., 1998). The 3MH is an amino acid produced by post-translational methylation of specific histidine residues on actin and myosin molecules. Neither a corresponding tRNA nor an oxidative pathway exists for this amino acid, making it a marker of actin and myosin degradation (Young et al., 1972; Thompson et al., 1996). Ndibualonji et al. (1997) showed that plasma 3MH concentration was increased by an energy underfeeding treatment. Regarding the relationships between heat stress and muscle protein degradation, Baracos et al. (1984) reported greater protein degradation at 42° than at 33°C in isolated rat muscle incubated at these temperatures. Heat stress has also been shown to increase protein oxidation and myofibrillar proteolysis in chick myotubes (Nakashima et al., 2004). Therefore, high ambient temperatures are inferred to accelerate the degradation of myofibril protein in lactating dairy cows. On the other hand, negative relationships between milk protein yield and a plasma marker of myofibrillar proteolysis were observed in this study. These results indicate that, although body protein mobilization increases at high ambient temperatures, it would be insufficient to supply the mammary gland with adequate amino acids for milk protein production in long run. On the other hand, the duration of heat stress treatment in this study was shorter than long-term heat stress such as summer season in Japan. In this study, lactating dairy cows successively exposed to constant 28°C 60% from day 1st to 13th. On contrary, environment temperature gradually increases from spring to summer in Japan, and high environment temperature keeps for several months. Therefore, the rate and extent of myofibrillar protein degradation would be different between short- and long-term heat stresses. During summer season in Japan, myofibrillar protein mass in lactating dairy cows might be gradually decreased by heat stress for several months. In any case, it would be insufficient to supply the mammary gland with adequate amino acids for milk protein production during short- or long-term heat stress.

Table 5. Plasma metabolites concentrations in lactating dairy cows

	18°C	28°C	Pooled SE	p value
Plasma concentrations				
TP (g/100 ml)	8.3	8.5	0.2	0.54
Albumin (g/100 ml)	4.3	4.4	0.1	0.50
Urea nitrogen (mg/100 ml)	13.4	15.7	2.5	0.55
3-methylhistidine (nmol/ml)	6.18	8.78	0.89	0.13

In conclusion, high ambient temperatures lower the energy and protein sufficiency ratio. Furthermore, plasma 3-methylhistidine concentrations, as a marker of myofibrillar proteolysis, increased in lactating Holstein cows. Milk protein yield and concentrations were much lower, indicating that milk protein production might not be strongly supported by muscle protein mobilization in lactating dairy cows at high ambient temperature.

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