Time-course Changes in the Relationship between Serum and Milk β-carotene Concentrations in Mid-lactation Dairy Cows following a Shift from Drylot Feeding to Time-limited Grazing

Satoshi HAGA[1,3]*, Kazuhiro MATOBA[1,4], Hiroshi ISHIZAKI[1,3], Yasuko TOGAMURA[1,3], Yasuko UEDA[2,5] and Yoshihiro KARIYA[1]

[1] Grazing System Research Team, NARO Institute of Livestock and Grassland Science (Nasushiobara, Tochigi 329-2793, Japan)

[2] Intensive Grazing Research Team, NARO Agricultural Research Center for Hokkaido Region (Sapporo, Hokkaido 062-8555, Japan)

Abstract

Grazing cows ingest large quantities of β-carotene (BC) from fresh pasture. The absorbed BC is carried to the mammary glands by the bloodstream and is subsequently transferred to the milk. This study was undertaken to assess the time-course changes in the relationship between serum and milk BC concentrations by calculating the rate of serum and milk BC concentration increase and estimating the mammary transfer of BC efficiency rate in mid-lactation dairy cows following a shift from drylot to time-limited grazing. For the first experiment, dairy cows were allocated to two treatment groups (each $n = 3$). The experimental grazing durations were 4 h (4hG) or 7 h (7hG) daily for 3 weeks. The serum BC concentration in the 7hG group was higher than that in the 4hG group. Nevertheless, no difference in milk BC concentration was found between the 4hG and 7hG groups. To investigate whether a difference exists in the rate of serum and milk BC increase in cows with time-limited grazing of more than 7 h daily, we conducted a second experiment for which the designated grazing time was 8 h daily for 4 weeks ($n = 8$). The rate of serum BC increase was greater than that of milk BC during days 14 to 28 of grazing. During the first 3 weeks of grazing, the estimated mammary extraction rate of BC significantly decreased, and the values at days 14 and 21 were significantly lower than those in drylot-feeding cows. These results suggest that the serum BC concentration strongly reflects the daily grazing time; however, the change in milk BC concentration is apparently delayed compared with that in the serum in cows grazing more than 7 h daily, and the relationship between the serum and milk BC concentrations changes following a shift from drylot feeding to time-limited grazing.

Disciplines: Animal industry, Grassland
Additional key words: carotenoid, extraction rate, mammary gland transfer, rate of increase

Introduction

The availability of pasture and the time spent at pasture are often restricted by various environmental factors such as small pasture area, heavy rainfall, short day length, and low temperature (Pérez-Ramírez et al. 2009); nonetheless, Sairanen et al. (2006) reported that time limited-grazing may enable increased milk production while decreasing the need for supplemental protein concentrate. Furthermore, fewer hours of bovine

access to pasture per day have also been shown to reduce N pollution in the pasture (Aarts et al. 2000) and increase grazing efficiency (Kristensen et al. 2007; Kennedy et al. 2009). For these reasons, the time-limited grazing system has been regarded as an effective grazing management method for lactating cows.

Carotenoids, natural functional pigments that are widely distributed in fresh forage, cannot be synthesized *de novo* by animals, but cattle are able to ingest carotenoids directly from forage. Beta-carotene (BC) is the main carotenoid accumulated in bovine tissues and blood (Yang et al. 1992) and transferred from blood into milk through the mammary glands (Nozière et al. 2006a, b). Milk from cows fed a grass-based diet, especially pasture, contains higher levels of BC than that from cows fed a diet based on concentrate or conserved forage (Butler et al. 2008; Mitani et al. 2011). In addition, BC in milk may contribute to improvement of the nutritional value of milk and dairy products for human consumption and plays a central role in stabilizing oxidizable compounds in milk (Havemose et al. 2004). However, the effective daily grazing time for increasing milk BC concentrations, especially during the period following a shift from drylot feeding to grazing, remains unclear.

Calderon et al. (2007) reported that the BC concentration in plasma is highly dependent on the amount of daily BC intake (low, middle, and high); however, the increase in milk BC concentration in cows fed a high-BC diet did not differ from that in cows fed a middle-BC diet following a shift from hay to grass silage-based diets. This result suggested that the relationship between blood and milk BC concentrations might be affected by the daily BC intake levels during a shift from low- to high-BC diet. Therefore, we hypothesized that in the grazing system, the increase in blood BC concentration is dependent on the daily grazing time (pasture intake), but that an increase in the milk BC concentration does not reflect that in the blood BC concentration (changes in the relationship between blood and milk BC status) following a shift from drylot feeding to grazing. In order to evaluate this hypothesis and obtain the fundamental knowledge for the research of production of BC-rich milk in the grazing system, the aim of the present study was to assess the time-course changes in the relationship between the blood and milk BC statuses, concentration and rate of increase, and the mammary BC extraction rate of mid-lactation dairy cows following a shift from drylot feeding to time-limited grazing.

Materials and methods

This study was conducted across two animal experiments according to the Guideline for the NARO Institute of Livestock and Grassland Science and was approved by the Animal Care Committee of the NARO Institute of Livestock and Grassland Science. The grazing experiments were conducted on a perennial ryegrass (*Lolium perenne*) and white clover (*Trifolium repens*) mixed pasture located in Nasushiobara, Tochigi, Japan (latitude 36.92°N, longitude 139.93°E, and altitude 325 m). All cows were milked twice daily at 0830 and 1800 h in the milking parlor near the pasture.

1. Animals and grazing procedure
(1) First experiment

We conducted the first experiment (Exp. 1) to compare the influence of the daily grazing time on the relationship between serum and milk BC concentrations in dairy cows following a shift from drylot feeding to time-limited grazing. Six mid-lactation Holstein dairy cows (mean ± standard error [SE]; body weight [BW] of 591 ± 22 kg, 130 ± 23 days in milk [DIM], age of 3.4 ± 0.2 years, and parity 1.3 ± 0.28) were used. These cows had experience in grazing. During the drylot (DL) treatment (3 weeks, April 1-22, 2007), all cows were fed in a free-stall barn equipped with door feeders (DF-100-B; Orion Machinery Co., Ltd., Nagano, Japan) for individual feed intake. They were allocated randomly into two treatments of three cows each. One treatment entailed 4 h of grazing per day (4hG). The other included 7 h of grazing per day (7hG). This time-limited grazing system was considered rotational strip-grazing, and cows were moved to new paddocks (375 m^2 and 750 m^2 for the 4hG and 7hG treatments, respectively) every day. The grazing period occurred during April 23 to May 13, 2007 (3 weeks). Cows in the 4hG and 7hG treatment groups were allowed access to the pasture after morning milking and grazed 0900-1300 and 0900-1600 h, respectively.
(2) Second experiment

Following Exp. 1, we performed the second experiment (Exp. 2) to ascertain whether a difference exists in the rate of increase of serum and milk BC and estimate the mammary extraction rate of BC with more than 7 h of grazing per day by increasing the number of heads and period (for 4 weeks). Eight mid-lactation Holstein dairy cows (mean BW of 683 ± 22 kg, 128 ± 12 DIM, age of 3.9 ± 0.2 years, and parity 1.9 ± 0.2) were used. These cows also had experience in grazing. The DL treatment was conducted during April 14 to May 4, 2008 (3 weeks), and the experimental grazing treatment during May 5 to June 2, 2008 (4 weeks). During the experimental treatment, the animals were grazed rotationally on 750 m^2 of pasture for 8 h per day (8hG) after morning milking during 0900-1700 h. This condition in 8hG was set as similar with that in 7hG of Exp. 1.

2. Feeding procedure

Throughout both experiments, the cows were fed total mixed ration (TMR) during the DL period (0900, 1300, and 1700 h) and partial mixed ration (PMR) diets in the free-stall barn after grazing. The ingredients and nutritional content of TMR or PMR, which were prepared weekly, were calculated individually according to the Japanese Feeding Standard for Dairy Cattle (NARO 2006) based on the expected dry matter intake (DMI) from the pasture and data for individual milk yield and BW (Table 1). In Exp. 1, the expected DMI of pasture was 0, 4, and 8 kg/day in the DL, 4hG, and 7hG treatment groups, respectively. In Exp. 2, the expected DMI of pasture was 0 and 8 kg/day in the DL and 8hG treatment groups. The pasture allowance was sufficient for the amount of expected pasture intake. All cows had free access to water and mineral salts in the free-stall barn and each pasture.

3. Sampling procedure

In Exp. 1, blood samples were collected by venipuncture using vacuum tubes lacking anticoagulant after the morning milking at days −5, 0, 4, 7, 14, and 21 of the grazing treatments, and individual milk samples were collected quantitatively from the morning and evening milking, synchronized with the blood sampling schedule. In Exp. 2, the blood and milk samples were collected by the same methods of Exp. 1 on the final day of the DL (0) treatment and weekly during the grazing treatment (days 7, 14, 21, and 28 of grazing). After sampling, the blood and milk samples used for BC analysis were protected from light by wrapping in aluminum foil immediately. The blood samples were centrifuged at $1,000 \times g$ for 20 min at 4°C and the serum samples were stored at −20°C until serum BC concentration analysis. The milk samples were separated into two portions for milk fat and BC concentration analyses. The milk fat concentrations were assessed using Fourier-transform infrared spectroscopy (MilkoScan FT120; Foss Japan Ltd., Tokyo, Japan). The portion for BC analysis was stored at −20°C until use.

4. BC analyses

All solvents and reagents were high-performance liquid chromatography (HPLC) grade. All extractions were performed at room temperature under dim light. The serum BC concentrations were measured using reversed-phase HPLC as described previously (Haga et

Table 1. Ingredients and mean dry matter intake (DMI) of total mixed rations (TMR) or partial mixed rations (PMR), expected DMI of pasture, and total nutrient composition of the feed in experiments (Exp. 1 and 2)

	Exp. 1			Exp. 2	
	DL[1]	4hG[2]	7hG	DL	8hG
Ingredients of TMR or PMR (% DM)					
Glass silage	12.6	7.5	5.2	8.2	5.4
Corn silage	25.8	20.8	14.2	24.3	16.5
Alfalfa hay	6.3	1.1	0.7	4.9	3.2
Mixed concentrates	32.3	39.4	31.5	41.3	19.6
Beet pulp	13.3	18.7	21.3	11.7	11.4
Corn flake	2.9	8.8	26.0	0.0	34.9
Soybean meal	6.1	2.9	0.0	8.4	7.4
Salt	0.8	0.9	1.1	1.1	1.5
DMI (kg/d)					
TMR or PMR[3]	22.0	17.4	14.2	25.0	17.7
Pasture (expected DMI)	0.0	4.0	8.0	0.0	8.0
Total nutrient composition (% DM)					
TDN[4]	72.6	72.6	72.3	73.5	74.6
CP[5]	15.3	15.2	15.2	16.8	16.5

[1] DL = drylot feeding before grazing (TMR feeding)
[2] 4hG, 7hG, and 8hG = daily grazing time of 4, 7, and 8 h (PMR and pasture feeding). The condition in 8hG (Exp. 2) was set as similar to that in 7hG (Exp. 1).
[3] represents average during experimental period
[4] TDN = total digestible nutrients
[5] CP = crude protein

al. 2014). Briefly, each sample was combined with purified water, ethanol, and n-hexane (Merck and Co., Inc., NJ, USA). The phases were separated by centrifugation (200 × g for 10 min at 20°C); the hexane layer was transferred and dried under reduced pressure in a centrifugal evaporator (SpeedVac; Thermo Fisher Scientific Inc., MA, USA) at 43°C. The dried residues were resuspended in methanol-chloroform (7:3, vol/vol). Then, the suspension was injected into a HPLC system (1500; Nippon Bunkou Co., Tokyo, Japan), a 250 × 4.6 mm C8 column (Luna; Phenomenex Inc., CA, USA), with the column temperature controlled at 25°C. The mobile phase was methanol-chloroform (9:1, vol/vol) and the flow rate was 1 mL/min. The absorbance was optimized at 450 nm. The retention time of BC was about 7.55 min. Peak areas were recorded and integrated using ChromNav software (Nippon Bunkou Co.). The concentration of BC was quantified against external standard curves (31.25-1000 µg/dL, $R^2 = 0.99$) using pure BC (Wako, Osaka, Japan).

We extracted the BC in milk as reported previously (Jensen & Nielsen 1996). Each sample was combined with 200 g/L ascorbic acid solution, methanol, ethanol, and 60% KOH solution. Samples were vortex-mixed and placed in a water bath. Saponification was performed at 70°C for 20 min. After cooling of the tubes on ice, n-hexane was added. The tubes were vigorously vortex-mixed and the phases were separated by centrifugation (400 × g for 5 min at 20°C). The top, organic layer was carefully transferred and dried under reduced pressure in SpeedVac. The dried residues were resuspended in methanol-chloroform (1:1, vol/vol). We used the same HPLC system for measurement of BC in the serum, but the column temperature was maintained at 40°C. The retention time of BC was about 6.50 min. The concentration of BC was quantified against external standard curves (0.78-50 µg/dL, $R^2 = 0.99$).

5. Calculations and statistical analysis

The milk BC concentrations were related to the milk fat content and expressed as micrograms per gram of milk fat.

In Exp. 1, the data of milk yield, milk fat concentration, and serum and milk BC concentrations were analyzed statistically using the MIXED model procedure of SAS software (Version Add-In 7.1 for Microsoft Office; SAS Institute Japan Ltd., Tokyo, Japan), with treatment (4hG and 7hG), day (days -5, 0, 4, 7, 14, and 21 of grazing), and interaction between treatment and day as fixed effects, and animal as a random effect. Upon determining a significance of $P < 0.05$ for the interaction, differences among LSMEANS

were analyzed using Tukey-Kramer tests, with statistical significance at $P < 0.05$. Linear regression analysis was used to assess the relationship between the serum and milk BC concentrations in the 4hG and 7hG groups. Differences of correlation coefficients of the regression lines between the 4hG and 7hG groups were determined by testing the t-value that was calculated using a sum of squared deviation and a standard deviation of the serum BC concentrations, with statistical significance at $P < 0.05$ by TDIST in Excel software (Microsoft Cop., WA, USA).

In Exp. 2, the data (milk yield, milk fat concentration, and serum and milk BC concentrations) were analyzed statistically using the MIXED model procedure, with day (days 0, 7, 14, 21, and 28 of grazing) as a fixed effect and animal as a random effect. Upon determining significance of $P < 0.05$ for the day, differences among LSMEANS were analyzed using Tukey-Kramer tests, with statistical significance at $P < 0.05$. We set the concentrations of serum and milk BC in the DL treatment as the reference and calculated their rates of increase. The increase rates of serum and milk BC concentrations were analyzed statistically using the MIXED model procedure and day, sample (serum and milk), and interaction between day and sample as fixed effects and animal as a random effect. Upon finding a significance of $P < 0.05$ for the interaction, the differences among LSMEANS were analyzed using Tukey-Kramer tests, with statistical significance at $P < 0.05$. Given a serum flow through the mammary gland of 400 L/kg of milk produced (Linzell 1974), it is possible to estimate the efficiency of micronutrient transfer from serum to milk (Calderon et al. 2007). The result of the estimation might more clearly demonstrate BC transfer from serum to milk via mammary gland by considering not only the serum and milk BC concentrations but also the milk yield and the total amount of BC flow through the mammary gland. Using our data of serum and milk BC concentrations and milk yield, we calculated the estimated mammary extraction rate of BC as follows:

$$\text{Extraction rate (\%)} = N_1 \times 100 \, / \, N_2 \times N_3$$

where N_1 is the total amount of BC in milk (milk BC concentration × milk yield), N_2 is the amount of BC flow into the mammary gland (serum BC concentration × 400 [L]) for the production of 1 kg milk, and N_3 is the milk yield. The estimated mammary extraction rate of BC was analyzed statistically using the MIXED model procedure, with day (days 0, 7, 14, 21, and 28 of grazing) as a fixed effect and animal as a random effect. Upon determining significance of $P < 0.05$ for the day, the differences

among LSMEANS were analyzed using Tukey-Kramer tests, with statistical significance at $P < 0.05$.

Results

1. Exp. 1

The mean milk yields (27.7 vs. 30.1 kg, SE = 0.5) and milk fat concentrations (3.99% vs. 3.76%, SE = 0.10) were not significantly different between the 4hG and 7hG treatments, respectively. The changes in mean serum and

milk BC concentrations are shown in Fig. 1A. During DL treatment, cows were fed TMR without vitamin supplementation to stabilize serum BC concentrations (baseline = 121 μg/dL). The serum and milk BC concentrations in the 4hG and 7hG treatments increased significantly ($P < 0.01$) during the grazing period. The serum BC concentration in the 7hG group was significantly higher ($P < 0.05$) than that in the 4hG group at day 14 of grazing, while the milk BC concentrations were not significantly different between the 4hG and 7hG
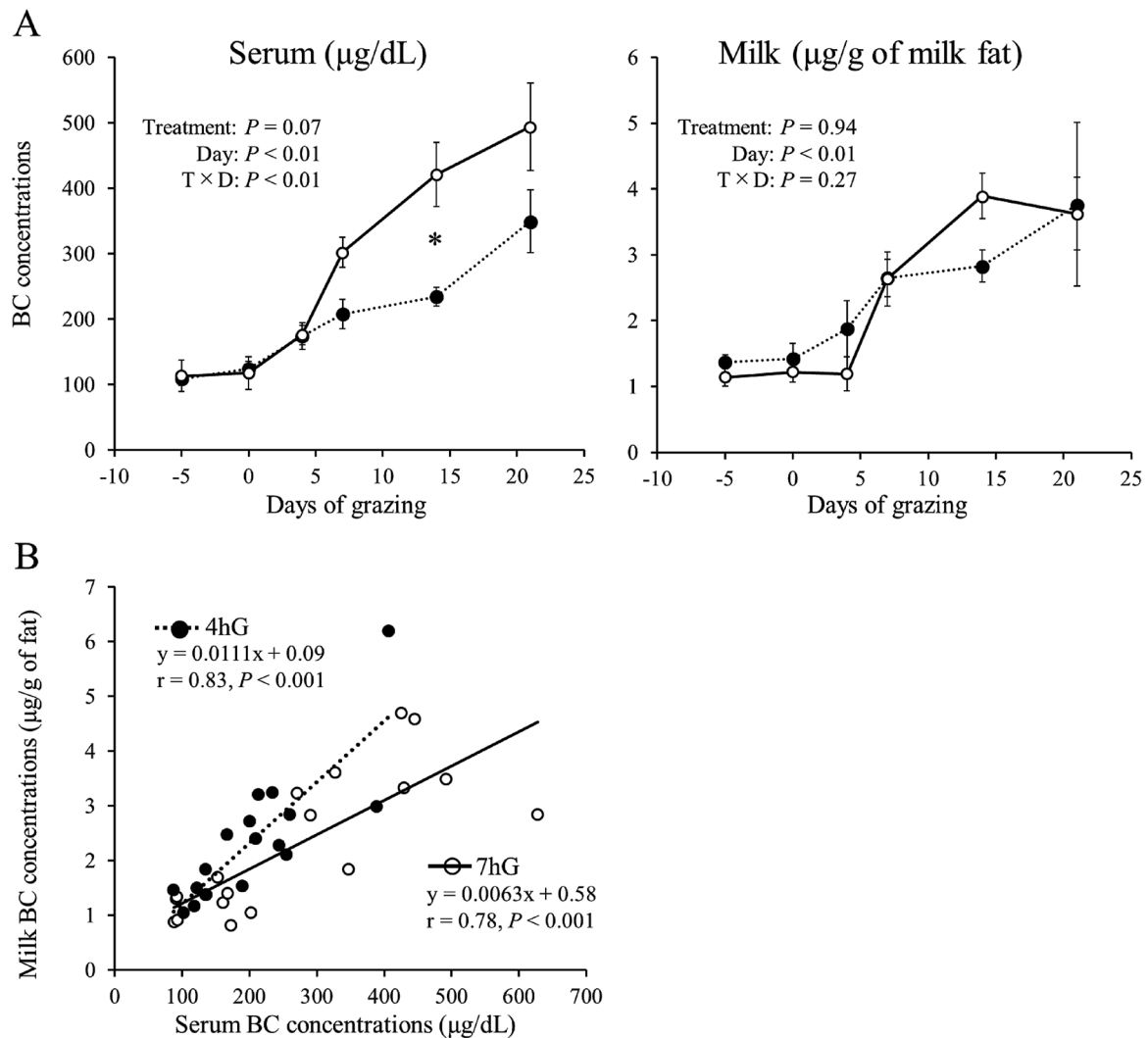
Fig. 1. Serum and milk β-carotene (BC) concentrations in dairy cows following a shift from drylot (DL) feeding to time-limited grazing in experiment 1

One treatment group grazed for 4 h per day (4hG, *n* = 3, dashed line and filled circle), and the other grazed 7 h per day (7hG, *n* = 3, solid line and open circle). A) Changes in the serum and milk BC concentrations at day -5, 0, 4, 7, 14, and 21 of grazing. All data are expressed as means ± standard error. Asterisk denotes a significant difference in the serum concentration of BC cows in the 4hG and 7hG groups (*P* < 0.05, Tukey-Kramer test). B) Relationship between the serum and milk BC concentrations. The regression lines using the milk BC concentration as the outcome variable (y) and the serum BC concentration as the predictor variable (x) are shown. Simple regression analysis revealed a strong correlation between the serum and milk BC concentrations in both 4hG (r = 0.83, *P* < 0.001) and 7hG (r = 0.78, *P* < 0.001) groups. In addition, statistically significant differences of correlation coefficients of the regression lines from 4hG (0.0111) and 7hG (0.0063) groups were determined (*P* < 0.05).

treatments. For assessment of the relationship between the serum and milk BC concentrations, the regression lines using the milk BC concentration as the outcome variable (y) and the serum BC concentration as the predictor variable (x) are shown in Fig. 1B. Simple regression analysis revealed the strong correlation between serum and milk BC concentrations in both 4hG (r = 0.83, $P < 0.001$) and 7hG (r = 0.78, $P < 0.001$) treatments. However, it is particularly interesting that the regression coefficients of the regression lines were not synchronized between the 4hG and 7hG groups. In fact, the correlation coefficient of the regression line from the 7hG group (0.0063) was significantly lower than that from 4hG group (0.0111, $P < 0.05$).

2. Exp. 2

No significant difference was found in the mean milk yields and fat concentrations during the DL and 8hG treatments for 28 days (Table 2). The changes in the serum and milk BC concentrations are presented in Table 2. During DL feeding, the baseline BC level in serum was 126 μg/dL. The serum and milk BC concentrations significantly increased depending upon the grazing period ($P < 0.05$) until day 21, but no significant difference was found between those values at days 21 and 28. The rates of BC concentration increase are shown in Fig. 2. The rates of serum BC increase were significantly higher ($P < 0.05$) than those of milk BC at days 14, 21, and 28, respectively. In fact, the serum and milk values at days 28 were about 6.0 and 4.3 times higher than those at DL treatment, respectively. During the first 3 weeks of grazing, the estimated mammary extraction rate of BC (Table 2) significantly decreased, and the values at days

14 and 21 were significantly lower ($P < 0.05$) than those at DL feeding. The extraction rate recovered from day 21 to day 28, at which point the values were not significantly different from those at DL feeding.
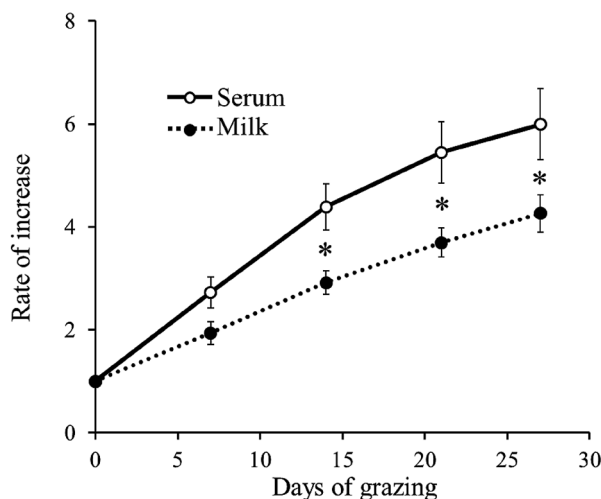
Fig. 2. Changes in the rate of increase of β-carotene (BC) concentrations in dairy cows following a shift from drylot (DL) feeding to time-limited grazing in experiment 2

All data are expressed as means ± standard error ($n = 8$). The rates of the serum (solid line and open circle) and milk (dashed line and filled circle) BC concentrations increased in dairy cows following a shift from DL feeding (day 0) to time-limited grazing (8hG) for day 28. We set each value of DL feeding as the reference. Asterisk denotes a significant difference between the rates of BC increase in the serum and milk ($P < 0.05$, Tukey-Kramer test).

Table 2. Time-course changes in milk production, serum and milk β-carotene (BC) concentrations, and estimated mammary extraction rate of BC in cows in experiment 2

			Days of grazing			
	DL[1](0)	7	14	21	28	SE[2]
Milk production						
Milk yield (kg/d)	37.70	36.70	37.40	35.00	35.20	0.60
Milk fat (%)	3.09	3.52	3.34	3.36	3.60	0.11
BC						
Serum conc. (μg/dL)	126.10[d]	345.30[c]	557.70[b]	686.30[ab]	739.20[a]	44.00
Milk conc. (μg/g of milk fat)	0.82[d]	1.58[c]	2.41[b]	3.07[a]	3.50[a]	0.20
Mammary extraction rate[3] (10^{-3} %)	5.21[a]	4.32[ab]	3.95[b]	3.96[b]	4.44[ab]	0.19

[1] DL = drylot feeding
[2] SE = standard error
[3] The calculation of the estimated mammary extraction rate is described in Materials and methods.
Values are means representing eight cows weekly; those without common letters in the same row are significantly different ($P < 0.05$, Tukey-Kramer test).

Discussion

The present results suggested that the serum BC concentration increase strongly reflects the daily grazing time, but, in case of more than 7 h daily grazing time, the milk BC concentration increase is slower to respond than that of serum during the period following a shift from DL feeding to time-limited grazing.

Nozière (2006b) reviewed that the blood BC concentrations of dairy cows fed a diet based on concentrate, maize silage, and grass hay were 174, 126, and 141 μg/dL, respectively. We regarded the serum BC concentration of cows receiving DL treatment as a proper baseline level for reference in the present study.

In Exp. 1, we calculated the estimated DMI of pasture by animal performance using the estimation method (Baker 1982). These values were 0.0, 4.0, and 8.7 kg/day in the DL, 4hG, and 7hG treatment groups, respectively, and similar to the values we expected. This estimation was also supported by previous reports describing that the daily grazing time with supplemental herbage affected the DMI of pasture (Kristensen et al. 2007; Pérez-Ramírez et al. 2008). Ueda (2003) reported that the mean BC content in perennial ryegrass and white clover mixed pasture was approximately 353 mg/kg DM in the spring in Japan. According to these data, the estimated amount of BC intake from pasture in the 4hG and 7hG treatments was approximately 1.4 and 3.1 g/day, respectively.

Interestingly, a difference was identified between the 4hG and 7hG groups regarding the relationship between serum and milk BC concentrations during the shift from DL feeding to grazing for 3 weeks. Calderon et al. (2007) also reported that despite the linear increase in plasma BC concentrations in response to dietary BC levels following a shift from a hay diet (low level of BC, 15 mg/kg of DM) to grass silage and alfalfa protein concentrate (high level of BC, 98 and 247 mg/kg of DM, respectively), the milk secretion of BC did not increase. They noted a saturation of BC secreted from blood into milk when the serum BC concentration exceeded 500 μg/dL. However, even in the 7hG treatment, the mean serum BC concentration was lower than 500 μg/dL. Furthermore, the milk BC concentration in Holstein dairy cows fed BC-rich forage or pasture was observed to be higher (more than 5 μg/g of milk fat) than that in the 7hG group cows (Nozière *et al.* 2006b; our unpublished data). These results indicated a low probability of saturation of milk fat globules and limited transfer of BC into milk under the serum BC concentration in Exp. 1. Therefore, the increase in milk BC might be temporally delayed compared with that in serum BC under the 7hG treatment.

This may have caused the difference in the relationship between the serum and milk BC concentrations in the 4hG and 7hG treatments. The determining factor of the milk BC concentration is assumed to be not only the blood BC concentration itself but also the speed of blood BC concentration increase during a shift from DL feeding to time-limited grazing. Further studies using more cows and increased serum BC concentrations over 500 μg/dL with more than 7 h of daily grazing, were necessary to confirm this speculation.

Therefore, we conducted Exp. 2 to investigate time-course changes and whether a difference exists in the rates of increase of serum and milk BC with time-limited grazing 8 h daily for 4 weeks. The milk BC concentrations increased even if the serum BC concentrations exceeded 500 μg/dL. However, the rate of BC increase in milk was lower than that in serum during days 14-28. To compare these results, we also sampled from a supplemental grazing experiment that was expected to entail lower DMI of pasture (less than 4 kg/days) for 6 weeks. In this experiment, the serum BC concentration increased more slowly. Specifically, 6 weeks were required to reach the mean serum concentration at day 21 in Exp. 2. The results revealed that the mean milk BC concentration in the supplemental experiment was higher, even though the serum BC concentrations were equal (supplemental vs. Exp. 2, 5.32 vs. 3.07 μg/g of milk fat and 655 vs. 686 μg/dL of serum, respectively). In fact, no delay in the rate of milk BC increase compared with that of serum was observed under the same serum concentrations as those shown in Exp. 2. These results also suggested that one important determining factor of the milk BC concentration might be the speed of serum BC concentration increase during the shift from DL feeding to time-limited grazing.

In addition, the efficiency of BC transfer from serum to milk was estimated by flow through the mammary gland. Nozière et al. (2006a) and Calderon et al. (2007) proposed that the mean rates of these transfer efficiencies in mid-lactation cows were 0.007% and 0.008%, respectively. In this study, we also confirmed the approximate value (mean 0.0052%) in the DL period, but in the 8hG period, the rate decreased by 24% compared with that of the DL period and reached a nadir during weeks 2-3. Beta carotene is called provitamin A because the body can convert it into retinol. However, in this grazing period, the serum retinol concentration did not change (data not shown) and Mitani (2011) reported that the milk retinol concentration did not change during the transition period from barn feeding to grazing that increase the milk BC concentration. These results indicated that the activity of conversion BC into retinol

might not be related this decrease of efficiency of BC transfer from serum to milk. We were unable to clarify the mechanism underlying this phenomenon of delayed rate of milk BC increase compared with that of serum BC and the decreased mammary extraction rate of BC. However, the high pasture intake following a shift from DL feeding to time-limited grazing might engender a rapid increase in the serum BC concentrations and a delay of transfer into the mammary glands and milk. Additional studies, adding the information about grassland (changes in the BC contents of the pasture), are necessary to ascertain a more useful grass-based feeding system for improvement of the nutritional value of milk and dairy product quality.

Conclusions

We hypothesized that the rate of serum BC concentration increase is dependently defined by the daily grazing time, but the rate of milk BC concentration increase does not reflect that of serum concentration following a shift from drylot feeding to time-limited grazing. The present results suggest that the serum BC concentration strongly reflects the daily grazing time (4 and 7 h per day), but, in case of more than 7 h of daily grazing time, the milk BC status is slower to respond during the period following a shift from DL feeding to time-limited grazing. The relationship between the serum and milk BC concentrations may time-course change in dairy cows following a shift from drylot feeding to time-limited grazing.

Acknowledgments

References

Aarts, H.F.M. et al. (2000) Nitrogen (N) management in the 'De Marke' dairy farming system. *Nutr Cycl Agroecosys,* **56,** 231-240.

Baker, R.D. (1982) Estimating herbage intake from animal performance. *In* Herbage Intake Handbook. ed. Leaver, J. D., The British Grassland Society, Berkshire, UK, 77-93.

Butler, G. et al. (2008) Fatty acid and fat-soluble antioxidant concentrations in milk from high- and low-input conventional and organic systems: seasonal variation. *J Sci Food Agric,* **88,** 1431-1441.

Calderon, F. et al. (2007) Variations in carotenoids, vitamins A and E, and color in cow's plasma and milk following a shift from hay diet to diets containing increasing levels of carotenoids and vitamin E. *J Dairy Sci,* **90,** 5651-5664.

Haga, S. et al. (2014) Increase in plasma total antioxidant capacity of grazing Japanese Black heifers and cows in forestland in Japan. *Anim Sci J,* **85,** 135-142.

Havemose, M.S. et al. (2004) Influence of feeding different types of roughage on the oxidative stability of milk. *Int Dairy J,* **14,** 563-570.

Jensen, S.K. & Nielsen, K.N. (1996) Tocopherols, retinol, β-carotene and fatty acids in fat globule membrane and fat globule core in cows' milk. *J Dairy Sci,* **63,** 565-574.

Kennedy, E. et al. (2009) Effect of restricted access time to pasture on dairy cow milk production, grazing behavior, and dry matter intake. *J Dairy Sci,* **92,** 168-176.

Kristensen, T. et al. (2007) Effect of time at pasture combined with restricted indoor feeding on production and behaviour in dairy cows. *Animal,* **1,** 439-448.

Linzell, J.L. (1974) Mammary blood flow and methods of identifying and measuring precursors of milk. *In* Lactation Vol. 1. eds. Larson, B. L. & Smith, V. R., Academic Press, New York, USA.

Mitani, T. et al. (2011) Changes in milk compositions from cows during transition period from barn feeding to grazing. *Animal science and agriculture Hokkaido,* **53,** 29-34 [In Japanese].

National Agriculture and Food Research Organization, NARO. (2006) *Japanese feeding standard for dairy cattle.* Japan Livestock Industry Association, Tokyo, Japan [In Japanese].

Nozière, P. et al. (2006a) Variations of carotenoids, fat soluble micronutrients and color in cows' plasma and milk following changes in forage and feeding level. *J Dairy Sci,* **89,** 2634-2648.

Nozière, P. et al. (2006b) Carotenoids for ruminants: From forages to dairy products. *Anim Feed Sci Technol,* **131,** 418-450.

Pérez-Ramírez, E. et al. (2008) Herbage intake and behavioral adaptation of grazing dairy cows by restricting time at pasture under two feeding regimes. *Animal,* **2,** 1384-1392.

Pérez-Ramírez, E. et al. (2009) Restricting daily time at pasture at low and high pasture allowance: Effects on pasture intake and behavioral adaptation of lactating dairy cows. *J Dairy Sci,* **92,** 3331-3340.

Sairanen, A. et al. (2006) Comparison of part-time grazing and indoor silage feeding on milk production. *Agr Food Sci,* **15,** 280-292.

Ueda, K. (2003) The estimated feed component of grass species type mixed white clover pasture. *Hokkaido Prefectural Konsen Agricultural Experiment Station Research Communications,* **12,** 5-6 [In Japanese].

Yang, A. et al. (1992) Carotenoid and retinol concentrations in serum, adipose tissue and liver and carotenoids transport in sheep, goats and cattle. *Aust J Agric Res,* **43,** 1809-1817.