Title:

Regional differences in the fatty acid composition, and vitamin and carotenoid concentrations in farm bulk milk in Hokkaido, Japan

Category: Research Articles

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ABSTRACT

In this study, we investigated the regional differences in the composition of farm bulk milk produced at three different dairy areas in Hokkaido, Japan. A field survey was conducted at Central, Tokachi, and North areas of Hokkaido, three or four times a year. At each farm, an interview questionnaire for farm basal data was conducted, and 500 mL of bulk tank milk sample was obtained. Fatty acid composition, and vitamin and carotenoid concentrations in the milk samples were determined. In Central and Tokachi areas, corn silage was used as the main forage. In North area, fresh herbage was the dominant feed in the summer season, and grass hay was the main feed in the winter season. Discriminant analysis revealed that the composition of milk samples differed among the areas and seasons. Milk from Central and Tokachi areas contained a higher ratio of linoleic acid compared with that from North area, but there were only slight differences in the composition of milk between Central and Tokachi areas. The concentrations of carotenoids and α-tocopherol were higher in samples from North area and the ratios of *trans*-vaccenic acid and conjugated linoleic acid were higher in the summer season than in the indoor season.

Key words: feeding management, milk compositions, regional difference, Hokkaido

1 INTRODUCTION

In the Japanese dairy industry, Hokkaido is the most important region accounting for more than half of the milk produced in Japan (Ministry of Agriculture, Forestry, & Fisheries, 2020). Dairy farms in Hokkaido can be divided into three typical feeding management types (Harada, Gonoi & Takahashi, 1983). These types correspond to geographical regions differing in climate, soil, and social environment for dairy farmers (Figure 1). Tokachi and Okhotsk areas are typical upland-cropping dairy areas. Dairy farmers in these areas can use corn silage (CS) as self-making forage owing to the temperate climate. North and Konsen areas are typical grassland dairy areas because the applicable forage in these areas is limited to grass forage owing to low cumulative temperature and short sunshine duration. Central and South areas are typical sub-urban dairy areas where farms for dairy production are small owing to the high price of farmland although CS can be used as forage in these areas.

Feeding management for dairy cows strongly affects the composition of milk. It not only affects the major components of milk, such as milk fat, protein, and lactose, but also the minor components, such as fatty acids (FAs), vitamins, and carotenoids (e.g., Bauman & Griinari, 2003; Chilliard et al., 2007; Palmquist, Beaulieu & Barbano, 1993; Walker, Dunshea & Doyle, 2004). These components strongly influence the physical property and visual aspects of dairy products (Couvreur et al., 2006; Larsen et al., 2014). In addition, feeding management for dairy cows influences the sensory properties of milk and dairy products (Croissant et al., 2007; Martin et al., 2005). Therefore, milk composition could differ among the different regions of Hokkaido, reflecting the differences in the feeding management.

Recently, the interests of consumers in dairy products, especially in Western countries, have changed, not only with regard to taste and functional ingredients beneficial for human health, but also in respect of how and where the milk used in the products is produced, for examples, issues related to the organic nature of product, animal welfare, sustainable development goals (SDGs), and protected denominations of origin (PDO) (Capuano et al., 2013; Elgersma, Tamminga & Ellen, 2006; Trubek & Bowen, 2008). To cater to these demands, the characteristics of milk produced in each region of Hokkaido have been investigated. Wide range on-farm surveys covering standard farmers are essential to determine the differences in the composition of milk resulting from regional differences. However, such on-farm surveys targeting regional differences have been scarce worldwide (Gaspardo et al., 2010; Larsen et al., 2010), as well as in Japan (Yayota et al., 2013), and none has been conducted in Hokkaido. In Europe, a study was conducted to clarify the relationship between the FA composition of milk and farm feeding systems using a dataset of 1,248 farm bulk milk samples (Coppa et al. 2013 and 2015).

In this study, we conducted an on-farm survey in three typical dairy areas of Hokkaido, namely Tokachi, North, and Central, to determine regional differences in the composition of farm bulk milk, with respect to the FA composition, and concentrations of vitamins and carotenoids which strongly influenced on the physical property and visual aspects of dairy products. Based on these results, characteristics of milk and different production systems can be described, and the diversity of Hokkaido milk products can be indicated.

2 MATERIALS AND METHODS

2.1 Field survey

The field survey was conducted at three regions in Hokkaido (Tokachi area located around 42°92′N and 143°20′E, North area located around 45°12′N and 142°36′E, and Central area located around 43°06′N and 141°34′E,) during the winter (December) season in 2008, and during the spring (April or May) and summer (August or September) season in 2009. For North area, an additional survey was conducted in the early summer (June) season in 2009. In each season, 47 dairy farmers at 25, 13, and 9 farms in Tokachi, North, and Central areas, respectively, were surveyed. The farmers in Tokachi and Central areas were selected by an introduction of the agricultural cooperative in each area. The all of farmers in North adopted grazing management in the summer season and were selected from the farmers surveyed in the previous study (Mitani et al., 2016). The survey method was similar to one described in our previous study, in which an interview survey was conducted and 500 mL of bulk tank milk was collected (Mitani et al., 2016). The data of milk yield, and the amount and type of supply feeds were obtained from the interview survey. The collected milk samples were brought to a laboratory in cold storage and divided into sub-samples. The sub-sample for analysis of milk composition was immediately sent to the Laboratory of Hokkaido Dairy Milk Recording and Testing Association. The concentrations of milk fat, milk protein, lactose, and solids not fat (SNF) were determined using a Fourier transform infrared device (MilkoScan FT+; Foss Electric, Hillerød, Denmark). The sub-samples used for other analysis were stored at - 80 °C until use.

2.2 Chemical analysis of milk

The milk sample used for FA analysis was thoroughly thawed under tap water, and then warmed in a water bath to dissolve the fat. FAs were extracted using a modified version of the Roese–Gottlieb method (ISO and IDF, 2001), and were methylated using a 2 N solution of $NaOCH_3$ in methanol and a 14% boron trifluoride solution in methanol (Christie, 2001). The FA methyl esters, thus formed, were analyzed using a gas chromatograph equipped with a flame ionization detector (GC-2010; Shimadzu Co., Kyoto, Japan). The FA methyl esters were detected under the conditions described in our previous study (Mitani et al. 2016).

The concentrations of carotenoids and retinol were analyzed using a modified version of a previously described method (Hulshof et al., 2006). The milk fat was extracted using a modified version of the Roese–Gottlieb method (ISO and IDF, 2001), and was saponified using a 5% KOH solution in ethanol. Echinenone was used as an internal standard. The concentrations of carotenoids and retinol in extracted samples were analyzed by high performance liquid chromatography (LaCrome Elite; Hitachi High-Tech Science Corporation, Tokyo, Japan). Carotenoids and retinol were separated on a C18-ODS column (Cadenza CD-C18, 150 × 2 mm; Imtakt, Kyoto, Japan) with a gradient elution method, and detected using a diode array detector (retinol at 325 nm, and lutein and β-carotene at 450 nm).

The concentration of α-tocopherol was determined with a direct saponification method. The milk sample was saponified using 2N KOH solution in methanol, containing ascorbic acid, and α-tocopherol was extracted with hexane. α-tocotrienol was used as an internal standard. The extracted sample was analyzed by high performance liquid chromatography (LaCrome Elite; Hitachi High-Tech Science Corporation). α-Tocopherol was separated on a C18-ODS column (Cadenza CD-C18, 150 × 2 mm; Imtakt), and detected using a diode array detector at 292 nm.

2.3 Statistical analysis

All the farmers in North area conducted grazing management during the summer season. In this area, winter and spring seasons, and early summer and summer seasons were regarded as indoor and grazing seasons, respectively, and results are therefore described for these two seasons. Most of the farmers in Central and Tokachi areas did not conduct grazing management. Therefore, results from these areas were not categorized by seasons.

Statistical analysis was conducted using the JMP Pro 14.3.0 (SAS Institute Inc., Cary, NC, USA) and SIMCA 13.0.3 (Umetrics AB., Umeå, Sweden) software. Data were analyzed with the one-way analysis of variance model using the Fit Model Platform in JMP. The regional differences in milk composition were analyzed using the orthogonal partial least squares discrimination analysis model in SIMCA. The analyses were conducted using standardized data and the full cross-validation method (leave one out). Predictive and orthogonal parameters in the model were estimated by the auto-fitting procedure in SIMCA.

3 RESULTS

3.1 Farm basal data and feeding management

The mean values and the range of farm basal data and feeding management are shown in Table 1. The farm characteristics were distinct for each area. In Tokachi area, the herd size was big and milk yield per cow and per lactation was high. In Central area, the herd size was not large but milk yield per cow and per lactation was high as in the farms of Tokachi area. In North area, the herd size was small and milk yield per cow and per lactation was not high. Milk yield per cow and per lactation in Central and Tokachi areas was 1.2-times higher than that in North area.

The supply of primary and secondary forage also differed among the regions (Table 2). In Central and Tokachi areas, CS was used as the main forage, but the supply of fresh herbage was less. Although farmers conducted similar feeding management in Central and Tokachi areas, those in Central area supplied relatively more hay, and those in Tokachi area supplied relatively more grass silage (GS). Forage in North area was limited to grass forage, such as a GS, hay, and fresh herbage. Fresh herbage was used as the main forage during the grazing season in North area. The supply of concentrate in Central and Tokachi areas was higher than that in North area, which mostly ranged between 5 and 8 kg/day. Half of the farmers in North area supplied less than 5 kg/day of concentrate.

3.2 Regional differences in milk composition

The milk composition differed significantly among regions and seasons, except for the concentration of milk protein, the ratio of C10:0 FA, and retinol concentration (Table 3). For visualization of the regional differences in milk composition, a discriminant analysis was conducted using 23 parameters, which included 4 of major milk composition, 15 of FAs, and 4 of vitamins and carotenoids (Figure 2). The discriminant model included three predictive parameters (P1, P2, and P3) and seven orthogonal parameters by auto-fitting. The cumulative R^2 value was 0.578 (0.269, 0.197, and 0.112 for P1, P2, and P3, respectively), and the cumulative Q^2 value was 0.488 (0.259, 0.171, 0.058 for P1, P2, and P3, respectively). The results showed that the model significantly indicated the regional differences in milk composition. The model also correctly distinguished each area using milk composition. The percentage of correct discrimination was high, being 96% in Tokachi (72/75), 88.5% in North-indoor (23/26), and 88.5% in North-grazing (23/26); however, the percentage in Central area was low, being 55.6% (15/27).

Predictive parameter 1 separated Tokachi and Central areas (positive), and North area (negative). Milk composition clearly differed between Tokachi and Central area, and North area. Predictive parameter 1 correlated positively with the ratio of *cis*-9,12 C18:2 (linoleic acid: LA) and lactose concentration, and negatively with *β*-carotene, lutein, and *α*-tocopherol concentrations, and ratios of *trans*-11 C18:1 (*trans*-vaccenic acid: TVA), *cis*-9, *trans*-11 C18:2 (conjugated linoleic acid: CLA), and cis-9,12,15 C18:3 (α-linolenic acid: ALA).

Predictive parameter 2 separated the indoor (positive) and grazing seasons (negative) in North area. Milk composition clearly differed between the indoor and grazing seasons in North area. Predictive parameter 2 correlated positively with milk fat concentration, the ratios of C16:0, C16:1, C17:0, and C20:0, and negatively with the ratio of C18:0, *cis*-9 C18:1 (Oleic acid: OA), TVA, LA, and CLA.

Predictive parameter 3 separated Central (positive) and Tokachi (negative) areas. However, the separation was insufficient, and half of the milk samples in Central area were included in a similar range of milk from Tokachi area. Predictive parameter 3 correlated positively with the ratio of C12:0 and C14:0, and negatively with the ratio of C16:1, ALA, C20:0, and retinol concentration.

4 DISCUSSIONS

In the present study, we analyzed, for the first time, the regional differences in milk composition in Hokkaido. Hokkaido is very interesting island with regard to the rich diversity in the feeding management of cows. The rich diversity in each region was originated from the difference in the available forage. The differences in the supplied forage among the regions of Hokkaido have not changed much from the 1980s (Harada et al., 1983). Therefore, the differences in feeding management among the regions in Hokkaido essentially resulted from the differences in regional characteristics, such as climate and social environment.

There have been few studies investigating the regional differences in milk composition, with regard to FA composition, and vitamin and carotenoid concentrations (Gaspardo et al., 2010; Larsen et al., 2010; Yayota et al., 2013). The results of the discriminant analysis (Figure 2) indicate that the milk derived from farms with specific feeding management in each area could be distinguished using the milk composition. This also indicates that the distinct milk composition in each area represents characteristics for selected farms located in each area. Gaspardo et al. (2010) showed that the origin of bulk milk from Italy or Slovenia could be discriminated using milk the FA composition. Larsen et al. (2010) reported that the FA composition, and vitamin and carotenoid concentrations in farm bulk milk differed among geographical areas and seasons in Sweden.

The milk FA composition in Tokachi and Central areas showed a higher ratio of LA (positive P1 in Figure 2). This was because farmers in these areas supplied more CS and grain feeds than those in North area. A high LA intake in Tokachi and Central areas was easily expected because grain feeds, including CS, contain higher amount of LA compared with grass forage (Walker et al., 2004). Not only the high LA intake, the high grain intake, including CS, promotes an uptake of LA from the duodenum. In general, high grain intake tends to depress the rumination function and reduces the ruminal pH. Under these circumstances, the function of biohydrogenation by rumen microbes reduces and the amount of LA escaping from the rumen increases (Chilliard et al., 2007; Dewhurst et al., 2006).

The milk composition differed slightly between Tokachi and Central areas because of similar feeding management in these areas. The slightly higher ratios of C12:0 and C14:0 in Central area compared with those in Tokachi area would have resulted from the difference in secondary forage such as hay and low moisture GS in Central area and GS in Tokachi area. This would probably be caused by a high intake of rough forage, such as hay and low moisture GS, which induces not only the rumination behavior in cows but also increases the production of acetate in the rumen, a substrate in the *de novo* synthesis of FAs in the mammary glands (Chilliard et al., 2000).

The samples from North area were high in TVA, CLA, and ALA (negative P1 in Figure 2). This was because of the high intake of grass forage in this area. The main FA in grass forage is ALA and that in grain feeds is LA (Walker et al., 2004). The ingested ALA is converted to TVA and CLA upon biohydrogenation and isomerization by rumen microbes, and these FAs are then absorbed from the duodenum and excreted into the milk after transformation (Chilliard et al., 2007). Therefore, a high ratio of these FAs in milk samples from North areas was a natural consequence of feeding.

Because FAs, including ALA in GS or hay, decreased during ensiling or drying (Dewhurst et al., 2006; Kalač & Samková, 2010; Villeneuve et al., 2013), the ratio of TVA, CLA, and ALA in milk was higher in the grazing season than in the indoor season. The milk FA composition between grazing and indoor feeding has been compared in several studies (e.g., Croissant et al., 2007; Kay et al., 2005; Kelly et al., 1998; Schroeder et al., 2003). In a previous study on milk FA composition of dairy farmers in grassland dairy areas of Hokkaido (Mitani et al., 2016), a high ratio of CLA, TVA, and OA during the grazing season and a high ratio of C16:0 during the indoor season was confirmed. Our results are in agreement with those of the previous study.

The content of fat-soluble antioxidants, such as carotenoids and α-tocopherol, is higher in grasses than in grains, such as corn (Noziere et al., 2006). The content of carotenoids and α-tocopherol in grass forage decreases in the order fresh herbage > GS > hay because these compounds were easily broken down upon exposure to sunlight (Agabriel et al., 2007; Noziere et al., 2006). Therefore, the high carotenoid and α-tocopherol content in samples from North area, especially during the grazing season, was a natural consequence of higher intake of grass forage and grazing in the selected farms in North areas than in Tokachi and Central areas.

To summarize, milk samples from the upland cropping and sub-urban dairy areas had a white aspect with low carotenoid content, and contained melty fat with a high ratio of LA. Milk produced during the grazing season in farms adopting grazing management in grassland dairy areas had a strong orange aspect with high carotenoid content, and contained melty fat with a high ratio of CLA and ALA, and that produced during the indoor season in these areas had an orange aspect, and contained firm fat with a high ratio of saturated FAs.

In conclusion, the characteristics of milk in each area originated strongly from farm feeding management, especially of the main supply forage, which was defined as climate and social environment for dairy farmers in each area. The present study is the first to unravel the characteristics of farm bulk milk produced in each of the dairy areas of Hokkaido. However, the results presented here also indicate that milk composition varies widely within each area. Therefore, factors, such as feeding management in each farm, influencing the distribution of milk composition within each area need to be investigated in a future study.

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Conflict of interest

Authors declare no conflict of interests for this article.

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Figure legends

Figure 1. Map of the dairy areas and farming types in Hokkaido, Japan.

Figure 2. Results of the orthogonal partial least squares discrimination analysis based on milk composition and area (-season) of Hokkaido, Japan. Upper panels indicate predictive parameters 1 and 2 (P1 and P2). Lower panels indicate P1 and P3. Left and right panels indicate the eigen values and eigenvectors, respectively.

和文抄録：

表題：

北海道における酪農家バルク乳中の脂肪酸組成、ビタミンおよびカロテノイド含量の地域間の違い

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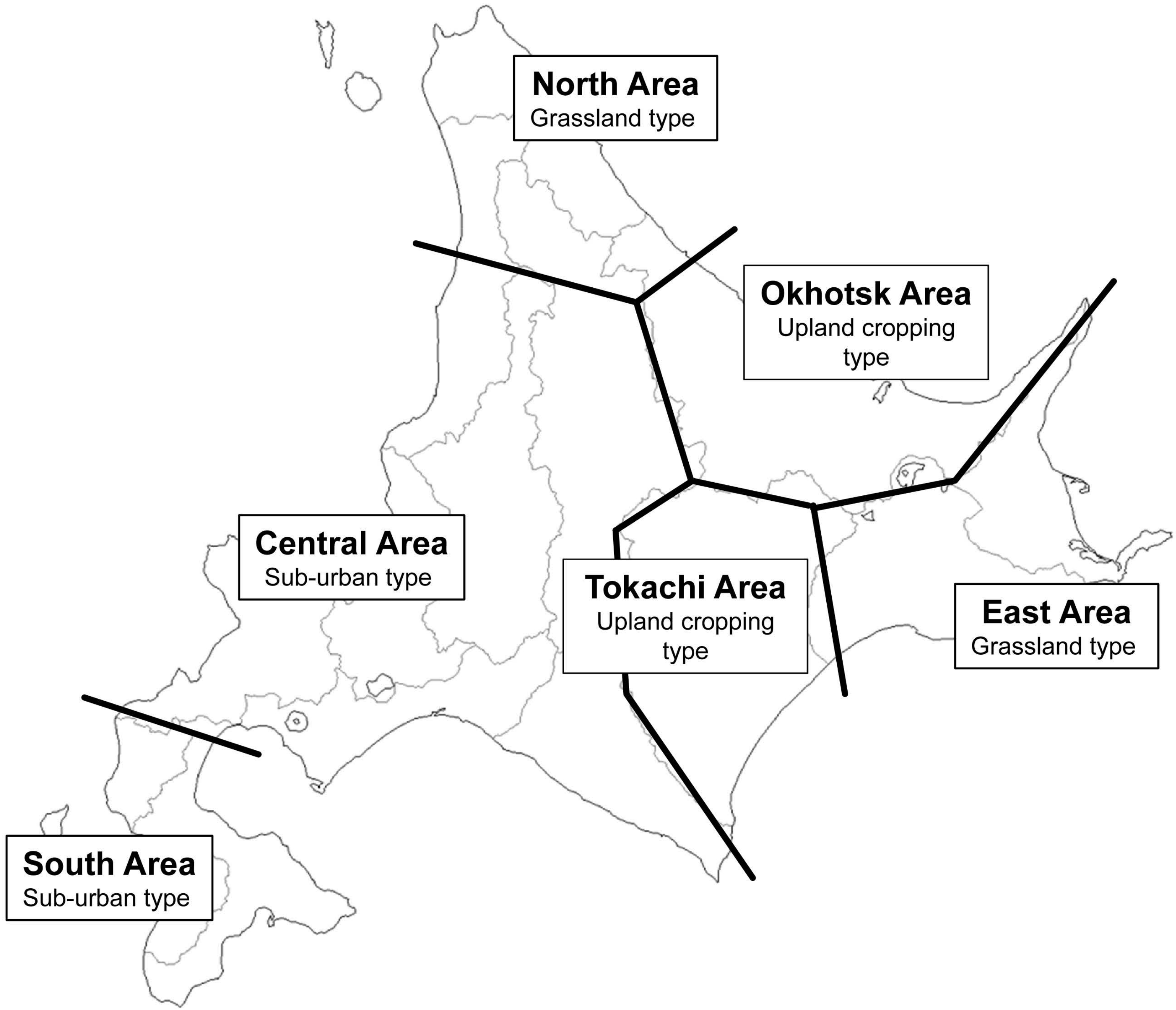
抄録本文:

本研究では、北海道における酪農家バルク乳成分の地域間の差異を調査した。北海道の道央、十勝および道北地域において年 3 回もしくは 4 回、現地調査を実施した。それぞれの農家で農家基礎情報についてのアンケート調査を実施し、500 mL のバルクタンク乳サンプルを採取した。各乳サンプル中の脂肪酸組成、ビタミンおよびカロテノイド含量を測定した。道央および十勝地域ではコーンサイレージが主な粗飼料であり、道北地域では夏期に放牧草が、冬期には乾牧草が主な粗飼料であった。乳成分 23 パラメータを用いた判別分析の結果、各地域および季節を正確に判別可能であった。道央および十勝地域の乳はこれらの地域間の差異は小さかったが、道北地域と比較して乳中リノール酸割合が高かった。道北地域の乳はカロテノイドおよび α-トコフェロール含量が高

く、舎飼い期の牛乳と比較して夏期の牛乳はトランス-バクセン酸および共役リノール酸割合が高かった。


North Area
Grassland type
Okhotsk Area
Upland cropping type
Central Area
Sub-urban type
Tokachi Area
Upland cropping type
East Area
Grassland type
South Area
Sub-urban type

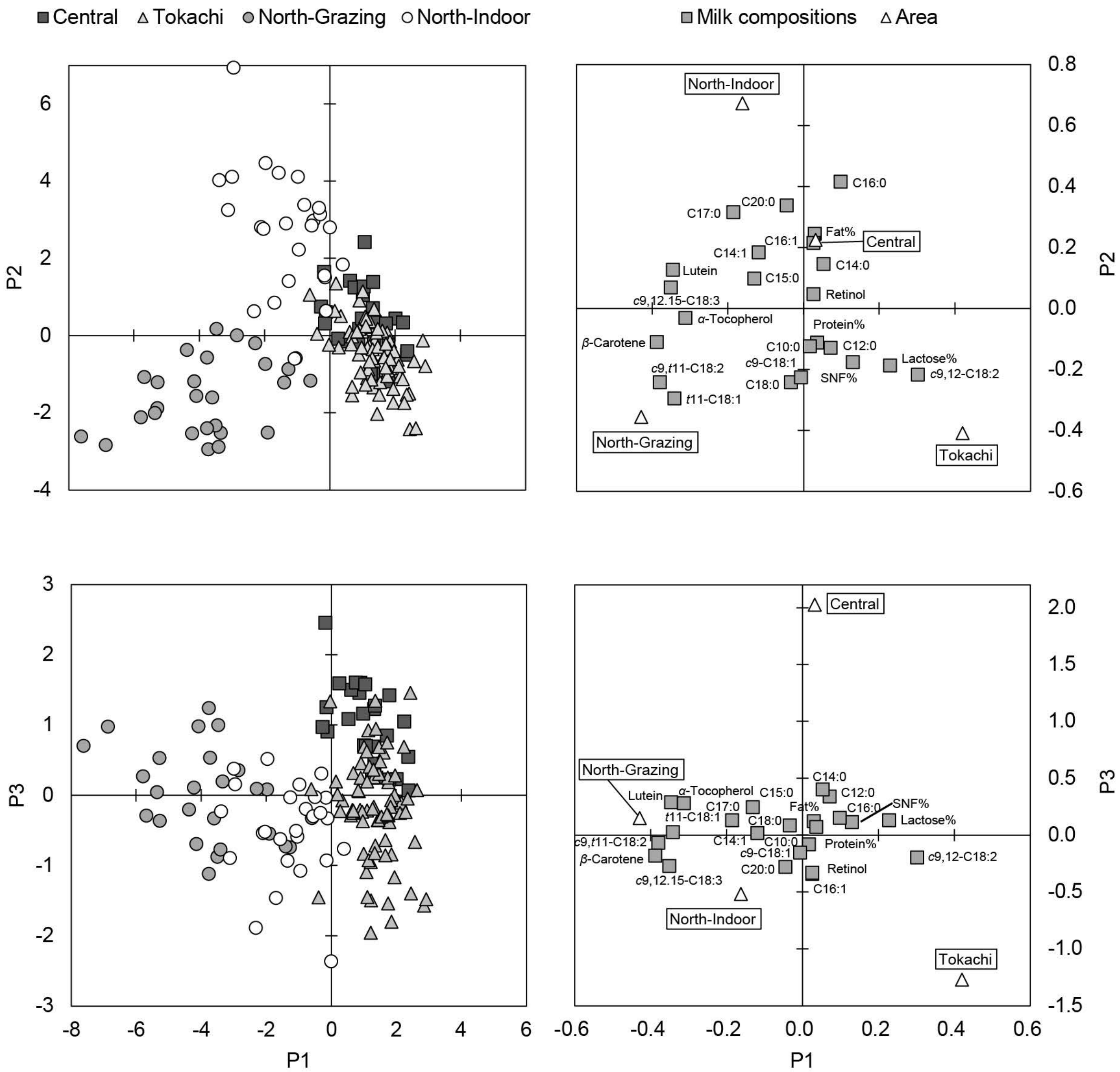

Central
Tokachi
North-Grazing
North-Indoor
Milk compositions
Area
North-Indoor
North-Grazing
Central
Tokachi
C16:0
C20:0
C17:0
C16:1
Fat%
C14:1
C14:0
Lutein
C15:0
Retinol
c9,12.15-C18:3
α-Tocopherol
Protein%
β-Carotene
C10:0
C12:0
c9-C18:1
Lactose%
c9,t11-C18:2
c9,12-C18:2
C18:0
SNF%
t11-C18:1
Protein%
C16:1
C20:0
P1
P2
P3

Figure 2

Table 1. Mean and range of management parameters for dairy farms in three areas of Hokkaido, Japan

	Area in Hokkaido		
	Central	Tokachi	North
	Mean ± SD	Mean ± SD	Mean ± SD
Number of farms	9	25	13
Herd size, lactating cows/farm	71 ± 52	105 ± 57	52 ± 17
Farming area, ha/farm	52 ± 31	45 ± 21	63 ± 15
Milk yield, kg/cow/day	26.5 ± 4.1	27.9 ± 3.1	23.0 ± 4.8
Milk production, kg/lactation	8067 ± 724	8570 ± 1271	6631 ± 2816

Table 2. Number of primary and secondary forage intake and concentrate supply level for farms in three areas (-seasons) of Hokkaido, Japan

	Area (-season)							
	Central		Tokachi		North-Indoor		North-Grazing	
Number	27		75		26		26	
Supply forage, number of farms	Primary	Secondary	Primary	Secondary	Primary	Secondary	Primary	Secondary
Grass silage (GS)	5	2	15	40	10	-	2	2
Corn silage	11	9	50	22	-	-	-	-
Hay + Low moisture GS	11	10	10	9	16	5	-	19
Pasture	-	-	-	-	-	-	24	2
Concentrate supply level, number of farms								
Low (< 5 kg/day)	4		11		14		12	
Midium (5 - 8 kg/day)	20		36		10		14	
High (> 8 kg/day)	3		28		2		-	

Table 3. Mean of milk compositions, milk fatty acid (FA) profile, and vitamin and carotenoid concentration for three areas (-season) of Hokkaido, Japan

	Area (-season)				Adusted	
	Central	Tokachi	North-Indoor	North-Grazing	SEM †	*P* value
Milk compositions, %						
Milk fat	4.03	3.93	4.06	3.85	0.04	< 0.01
Milk protein	3.25	3.26	3.21	3.26	0.02	0.32
Lactose	4.50	4.51	4.42	4.44	0.01	< 0.01
Solid not fat	8.75	8.77	8.62	8.70	0.03	< 0.01
FA profile, % of total FA methyl esters						
C10:0	2.16	2.19	2.03	2.21	0.06	0.19
C12:0	3.36	3.21	2.98	3.20	0.06	< 0.01
C14:0	12.2	11.8	12.0	11.5	0.1	< 0.01
C14:1	1.25	1.22	1.38	1.30	0.03	< 0.01
C15:0	1.17	1.13	1.18	1.20	0.02	0.06
C16:0	33.7	32.2	35.6	29.2	0.4	< 0.01
C16:1	1.55	1.59	1.78	1.48	0.05	< 0.01
C17:0	0.60	0.58	0.66	0.62	0.01	< 0.01
C18:0	11.0	11.2	10.2	11.8	0.2	< 0.01
C18:1, *cis*-9	21.1	21.8	20.7	22.5	0.3	< 0.01
C18:1, *trans*-11	1.49	1.60	1.54	3.28	0.09	< 0.01
C18:2, *cis*-9,12	2.22	2.56	1.58	1.57	0.10	< 0.01
C18:2, *cis*-9,*trans*-11	0.54	0.58	0.66	1.28	0.03	< 0.01
C18:3, *cis*-9,12,15	0.34	0.37	0.55	0.63	0.02	< 0.01
C20:0	0.17	0.17	0.19	0.16	0.003	< 0.01
Vitamin and Carotenoid, μg/dL						
α-Tocopherol	107	92	118	150	5	< 0.01
Retinol	36.6	39.5	42.6	38.4	2.0	0.24
β-Carotene	6.6	7.7	12.0	19.2	0.6	< 0.01
Lutein	0.49	0.32	0.68	0.83	0.04	< 0.01

† Standard error of the mean corrected replication (n = 31.4)