Title: Discrimination of "Grazing milk" using Milk Fatty Acid Profile in the Grassland Dairy Area in Hokkaido

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ABSTRACT

Milk produced by the grazing system, referred to as "grazing milk" contains many components required for human health. The milk fatty acid (FA) profile is strongly associated with the diet on the farms. In the present study, based on the FA profile of farmer's bulk milk, we determined how to discriminate between milk produced on grazing and on a confinement system. A field survey was conducted four times (grazing and confinement season) in the Konsen (29 farms) and Okhotsk (25 farms) area in Hokkaido. Farmer's bulk milk samples and details of feeding management were collected and the FA profile of milk was measured. Milk produced during the grazing season contained less C16:0 and *cis*-9 C16:0, and more C18:0, *cis*-9 C18:1, *trans*-11 C18:1, *cis*-9,12 C18:2, *cis*-9,*trans*-11 C18:2 and *cis*-9,12,15 C18:3 than milk produced during the confinement season. Discrimination analysis using 16 FA revealed that almost all milk samples were discriminated correctly (confinement season: 90% correct and 10% borderline, grazing season: 88% correct, 9% borderline and 3% incorrect). For farmers that were categorized incorrectly and were considered borderline in the grazing season, the dependency on pasture was low compared with that for farmers discriminated correctly. Therefore, to claim "grazing milk", a high dependency on pasture is required for grazing dairy farmers.

Key words: *discrimination, fatty acid profile, grazing milk, grassland dairy area.*

INTRODUCTION

In recent years, grazing management for dairy cows has been re-evaluated in Japan. The evaluation is not only to determine the nutritional value of feed, but also to devise strategies for animal welfare, and to reduce labor and manure handling costs. Furthermore, many studies revealed that milk produced by the grazing system, called “grazing milk” had different characteristics from the milk produced by the confinement system with total mixed ration (TMR) feeding, or conserved forage and concentrate feeding in the barn (Kelly *et al.* 1998; Schroeder *et al.* 2003; Couvreur *et al.* 2007). One of these characteristics, conjugated linoleic acid (CLA: *cis*-9,*trans*-11 C18:2), is as a specific fatty acid (FA) founded in only ruminant fat, and *trans*-vaccenic acid (TVA: *trans*-11 C18:1), which is a precursor of CLA, are presented at higher levels in the milk under a grazing system compared to that in a confinement system. CLA and TVA originate from α-linolenic acid (ALA: *cis*-9,12,15 C18:3) in pasture, ingested ALA is converted to TVA and CLA by isomerization and biohydrogenation of ruminal microbes in the rumen and desaturation in the body tissue or mammary gland (Chilliard *et al.* 2000, 2007; Walker *et al.* 2004). In recent years, research has focused on these FAs because they are functional FAs for human health, and are thought to have anti-atherogenic and anti-cancer effects (Clancy, 2006; Salter *et al.* 2006).

Milk produced by grazing is assumed to be of high added-value because the milk has a scarcity value and contains specific nutrients. However, to be classified as “grazing milk”, there is a need to differentiate between “grazing milk” and other milk. Many studies have examined the effect of grazing feeding on the milk FA profile, and have demonstrated that the FA profile in grazing milk is different from the produced in a confinement system. In particular, the levels of CLA and TVA in milk are doubled by grazing (Kelly *et al.* 1998; Schroeder *et al.* 20003; Kay *et al.* 2005; Croissant *et al.* 2007). Therefore, utilization of the milk FA profile could allow the differentiation to be established between "grazing milk" and other milk. However, these studies were conducted under well-controlled conditions, and few studies have examined this aspect under practical farming conditions (Gaspardo *et al.* 2010; Yayota *et al.* 2013; Benbrook *et al.* 2013; Capuano *et al.* 2014).

Since milk FA is synthesized across multiple complex pathways, many factors influence the difference in milk FA profile. Among these, it is known that changing feed management can strongly modify the milk FA profile (Chilliard *et al.* 2007). As feeding regimens under the practical farming condition are widely spread, the milk FA profile is predicted to vary widely. It is uncertain whether the milk FA profile can be considered an effective factor to differentiate between "grazing milk" and other milk under practical farming conditions. Thus, successful differentiation of the FA profile can establish whether the milk FA profile should be regarded as characteristics of "grazing milk" and other milk.

In the present study, a field survey was conducted four times during the grazing and confinement seasons in the Konsen and Okhotsk areas, which comprise the typical grassland-dairy area in Hokkaido. Thus, the field data collected in the present study were widely spread. The objectives of this study were to characterize the milk FA profile produced under the grazing or confinement systems, and to assess whether it is possible to discriminate between "grazing milk" and other milk using the milk FA profile under practical farming conditions.

MATERIALS AND METHODS

Field surveys were conducted during the summer (Aug.) and autumn (Nov.) seasons in 2007, and during the winter and spring (Mar. and Apr.), and early summer (Jun.) seasons in 2008 in the Konsen (located at 43°17 - 43°23' N latitude and 145°0' - 145°12' E longitude, 5.4℃ of mean air temperature and 1135 mm of mean rainfall) and Okhotsk (located at 44°28 - 45°19' N latitude and 142°6' - 143°7' E longitude, 5.5℃ of mean air temperature and 1078 mm of mean rainfall) areas in Hokkaido. In total, 54 farmers were researched during each season, comprising 29 farms in Konsen and 25 farms in Okhotsk. All farmers in early summer and summer seasons conducted grazing on pasture. Three farmers in spring and autumn seasons, one in autumn and two in spring, conducted grazing on pasture, and all of others conducted confinement feeding. Thereby, 3 data in confinement season were regarded as in grazing season. Five-hundred milliliters of bulk-tank milk were collected from each farm, and feeding regimens, delivered milk yield and the number of milking cows were determined simultaneously using visiting questionnaires. All collected milk samples were immediately placed into a cold storage box. Next, milk samples were transferred to 5-mL polypropylene tubes and a plastic vessel for the analysis of milk components (fat, protein, lactose and urea-N). The 5-mL tubes were frozen and stored at -80℃ until FA analysis. Milk samples were immediately sent to the Laboratory of Hokkaido Dairy Milk

Recording and Testing Association and the milk fat, protein, lactose and urea-N concentrations were determined using a Fourier Transform Infrared device (MilkoScan FT+; Foss Electric, Hillerød, Denmark).

Milk yield per cow was calculated by dividing the amount of delivered milk by the number of cows milked. Concentrate intake was considered as an amount of supply. Forage intake was calculated by dividing the total digestible nutrient (TDN) content (Standard Tables of Food Composition in Japan 2001) of each feed by the TDN intake of each feed; where the forage TDN intake was calculated by subtracting the TDN requirement calculated by milk yield and milk fat content (Japanese Feeding Standard for Dairy Cattle 2006) to the concentrate TDN intake. In the grazing season, the pasture intake was calculated by dividing the TDN concentration (Standard Tables of Food Composition in Japan 2001) by the pasture TDN intake; where the pasture TDN intake was calculated by subtracting the total forage TDN intake with the sum of the TDN intake of other forage. In this study, the proportion of each feed to total intake was used for analysis because the intake of each feed was strongly correlated with the milk yield and fat content (Japanese Feeding Standard for Dairy Cattle 2006).

Milk FA extraction was performed using a direct methylation method modified based on the method described by Loor *et al.* (2005). The frozen milk was thawed slowly with tap water. One-hundred-fifty microliters of thawed milk was dispensed into a grass tube, and then lyophilized. The lyophilized milk was directly methylated by adding 1-mL of 2N-$NaOCH_3$ solution at room temperature for 20 min, followed by 1-mL of 14% boron trifluoride-methanol solution at room temperature for 20 min (Christie *et al.* 2001). Fatty acid methyl esters were recovered in 1-mL of hexane. Tricosanoate was used as the internal standard. The FA methyl esters were analyzed using a gas chromatograph equipped with a flame ionization detector (GC-2010; Shimadzu, Kyoto, Japan). Methyl esters in each sample were separated on a 50 m × 0.25 mm internal diameter fused silica capillary column (ULBON HR-SS-10; Shinwa Chemical Industries Ltd., Kyoto, Japan). Methyl ester analysis was performed as split analysis (a 1-μL injection at a 75:1 split ratio), the injector and detector temperatures were both maintained at 250°C. The initial oven temperature was 160°C, which was increased by 1.5°C/min to 220°C, and was held at 220°C for 10min. Helium was used as the carrier gas. The gas flow rate at the injector was kept constant at 1.5-mL/min. The detection of each FA methyl ester was evaluated according to retention time compared with the standard mix (Supelco 37-Component FAME Mix, Sigma-Aldrich Japan K.K., Tokyo, Japan and fatty acid methyl ester mix, supelco) and self-methylated CLA.

Results from the summer season in 2007 and early summer season in 2008 were regarded as the grazing season. Results from the autumn season in 2007, and winter and spring season in 2008 were regarded as the confinement season, except for 3 data in autumn, and winter and spring season which were regarded as the grazing season. Statistical analysis was conducted using the statistical softwares JMP 9.02 (SAS Institute Inc., Cary, USA) and SIMCA 13.0.3 (Umetrics AB. Umeå, Sweden). First, data were analyzed with the general linear model using the Fit Model Platform in JMP. The model included area, seasons, and interaction as fixed effect. If the possibility of difference was less than 0.05 ($P < 0.05$), the result was regarded as significant. Second, data were re-analyzed with the one-way ANOVA model including seasons as fixed effect using the Fit Model Platform in JMP because most of area and interaction effects were not significant. If the possibility of difference was less than 0.05 ($P < 0.05$), the result was regarded as significant.

Data from the discrimination analysis were analyzed using the orthogonal partial least squares discrimination analysis (OPLS-DA) model using standardized data in SIMCA. In OPLS-DA of SIMCA, a magnitude and reliability value was calculated as a predictive component. As the calculation was conducted using a standardized value, a magnitude value of each was regarded as a standardized partial regression coefficient, which indicated a force of impact. A positive magnitude value indicated that the FA was influenced strongly in the confinement season. Conversely, a negative magnitude value indicated that the FA was influenced strongly in the grazing season. A reliability value indicated a reliability of a magnitude value of each FA in the discrimination model. After that, the probability of a sample belonging to either category was also calculated. If a sample was included in the 95% confidence interval, it was estimated to be correctly discriminated in each category. If a sample was included in the 90-95% confidence interval, the sample was estimated discriminated on borderline.

RESULTS

In the present study, because of the limited differences (data not shown) in the results between the Konsen and Okhotsk areas in each season, the results from both areas in each season were merged. The mean proportion of feed intake to total intake and milk production are shown in Table 1. The mean proportion of total forage intake in both the grazing and confinement seasons was approximately 60% of the total DM intake, which ranged from 32% to 84% of total intake, all of which were grass forage. The proportion of total forage intake in the grazing season was slightly higher compared with that in the confinement season ($P < 0.01$). In the confinement season, 65% of grass forage was dried grass, which was hay or rolled grass silage, and another was grass silage. In the grazing season, 75% of grass forage was from grazing pasture, and consisted of 17 to 100% grass forage. The mean proportion of concentrate intake was approximately 40% of the total intake in both grazing and confinement seasons. Seventy-five percent of the concentrate intake was from commercial formula feed or solely grain feed (corn and barley), and ranged from 0 to 100% of the concentrate intake. Others of the concentrate intake were mainly from beet pulp pellets. The data described in the present study were collected from many dairy farms that used a variety of feeding management systems in the grassland dairy area of Hokkaido. Table 1

The mean milk yield was higher during the grazing season than that in the confinement season ($P < 0.01$), because some of farmers took to a management closed to a seasonal breeding. The mean milk fat concentration was lower during the grazing season than that during the confinement season ($P < 0.01$). The mean milk protein content was lower during the grazing season than that during the confinement season ($P < 0.05$). The mean concentrations of lactose and solid not fat concentration were similar in both the grazing and confinement season. The mean concentration of milk urea nitrogen was higher during the grazing season than that during the confinement season ($P < 0.01$).

The milk FA profile identified in the present study, as well as the feeding management in each season ranged widely (Figure 1). The concentration of most of the FAs was significantly different between the grazing and confinement seasons ($P < 0.05$), excepted for the proportions of C8:0, C12:0, and C17:0. Although the proportions of C10:0, C14:0, *cis*-9-C14:1, C15:0, and C20:0 in milk differed significantly between the grazing and confinement seasons ($P < 0.05$), the absolute difference measured between the grazing and confinement seasons was low, and each distribution was duplicated. The proportions of C16:0 and *cis*-9-C16:1 in milk produced during the grazing season were lower than that in milk during the confinement season ($P < 0.01$), and the distribution of each was clear shape as a normal distribution. The proportions of C18:0, *cis*-9-C18:1, *cis*-9,12-C18:2 and ALA in milk produced during the grazing season were higher than that produced during the confinement season ($P < 0.01$), and each distribution was also clear shape as a normal distribution. The proportion of TVA and CLA in milk produced during the grazing season was also higher than that produced during the confinement season ($P < 0.01$), but the distribution in the grazing season was more extensive compared with that in the confinement season. Figure 1

The result of the discrimination analysis based on 16 FAs is shown in Table 2. The high R^2 value and low RSD in the model indicated that discrimination using the milk FA profile in the present study was precise. Incidentally, when the discrimination was conducted using the milk fat, protein, lactose and milk urea concentrations in addition to the milk FA profile, a lower R^2 value and higher RSD of the model was found, compared with the model using the milk FA profile only (data not shown). The predictive component, both of the magnitude and reliability values, indicated that the C16:0 content had the strongest power. Milk high in C16:0 was classified as milk produced during the confinement season. Many of the fatty acids with 18 carbon atoms which are C18:0, cis-9-C18:1, TVA, and CLA, also had high magnitude and reliability values, although the predictive power of these FAs was less than that of C16:0. Since the predictive components of those FAs with 18 carbon atoms were negative, milk high in 18-carbon FAs was classified as milk produced in the grazing season. The magnitude value of CLA and TVA content, which are signature FAs of grazing system, was low compared with that of C16:0 content, but reliability of those FAs was enough high. As a result, the percentage of correctly classified milk was 88.9% (192/216). Table 3

The mean of feed intake in each category of discrimination analysis is shown in Table 3. In the confinement season, the percentage of correct discrimination (CON/CON) was 89.5% (94/105), 11 milk samples were classified as borderline (BORDER/CON), and no samples were classified as the grazing season. Although the proportion of beet pulp tended to be high in CON/CON compared with BORDER/CON, the difference in the proportion of each feed intake between CON/CON and BORDER/CON was low. In the grazing season, the percentage of correct discrimination (GRA/GRA) was 88.2% (98/111), 10 milk samples were classified as borderline (BORDER/GRA), and 3 milk samples were classified as the confinement season (CON/GRA). The difference in the proportion of feed intake among GRA/GRA, BORDER/GRA and CON/GRA in the grazing season was low, except in the pasture intake. The proportion of pasture intake in the total intake and the total forage intake was 1.4-fold higher in GRA/GRA compared with that in BORDER/GRA and CON/GRA ($P < 0.01$).

DISCUSSION

Because the data were collected from practical farming conditions using various feeding management systems, the proportion of each feed intake ranged widely in each season in the present study. Furthermore, the milk yield and compositions also ranged widely in each season. Changes in milk components occurred during the transition from the confinement to the grazing season in this study, which was mainly milk fat depression and urea elevation, was observed well (Polan *et al.* 1986; Bargo *et al.* 2002). The milk fat depression during the grazing season was expected to be caused by low fiber intake (Sutton 1989), and high intake of long-chain and *trans*-FAs (Barber *et al.* 1997; Bauman & Griinari, 2003). The urea nitrogen elevation in milk produced during the grazing season is caused by high degradable protein intake from pasture and a mismatch of protein and easily digestible carbohydrate intake (Bargo *et al.* 2002).

The milk FA profile was also widely distributed, and most FAs were significantly different between the grazing and confinement season. Many studies have compared the milk FA profile between the grazing and confinement systems offered a TMR (Schroeder *et al.* 2003; Kay *et al.* 2005; Couveur *et al.* 2007; Croissant *et al.* 2007). In the present study, although the absolute value and statistical significance differed in the grazing and confinement seasons, the difference in milk FAs showed a similar trend to that observed in previous studies. However, very few studies have compared the distribution of each FA in the grazing and confinement systems. From the results of the present study, the shape of distribution in the difference of each FA indicated that each FA could be clearly categorized into four groups. The FA groups played a key role in the discrimination between milk produced during the confinement or grazing season.

The FAs of C10:0, C14:0, *cis*-9-C14:1, C15:0, and C20:0 in milk were categorized as Group 1, in which the absolute difference between the grazing and confinement seasons was low and each distribution was duplicated. The FAs of C16:0 and *cis*-9-C16:1 in milk was categorized as Group 2, in which each distribution was clear shape of a normal distribution, and the mean value in the grazing season was significantly lower than that in the confinement season. The FAs of C18:0, *cis*-9-C18:1, *cis*-9,12-C18:2, and ALA were categorized as Group 3, in which each distribution was clear shape of a normal distribution, and the mean value in the grazing season was significantly higher than that in the confinement season. The FAs of TVA and CLA were categorized as Group 4, in which the mean value in the grazing season was significantly higher than that in the confinement season, but the distribution in the grazing season was more extensive compared with that in the confinement season.

Milk FAs categorized into Group 1 did not have an important role in the discrimination analysis, because the distributions of each FA in the grazing and confinement seasons were duplicated and the difference was small. Milk FAs categorized into Groups 2 and 3 should have an important role in the discrimination analysis, because these FAs were distributed in two clear normal distributions in each season. Milk FAs categorized into Group 4 were contained double in milk produced during the grazing season, and these are expected to have the most important role in the discrimination analysis. However, the TVA and CLA contents were less important compared with the C16:0 content. The low power of TVA and CLA in the discrimination analysis was probably because the TVA and CLA contents were widely distributed in the grazing season, while these were narrowly distributed in the confinement season. In the comparison of milk FA profile between the grazing and confinement seasons, the TVA, CLA, and ALA contents have previously been the focus of study, because these FAs were functional FAs and were doubled during the grazing season. However, the results of the present study indicate that the difference in milk FAs produced under the grazing and confinement systems was due to the difference in the dominant FAs, which was represented by the C16:0, C18:0, and *cis*-9-C18:1 content. In Dutch study which verified discriminations of fresh grass feeding, pasture grazing, and organic farming by PLS-DA using FA profile (Capuano *et al.* 2014), a similar result was reported, which C16:0 was the most important role for classification of fresh grass feeding toward other feeding systems. The difference in the absolute value in these FAs would influence the physical properties of milk fat. The butter produced from milk under grazing conditions was found to melt easily at room temperature compared with that under the confinement system (Couvreur *et al.* 2006).

Many studies (e.g. Chilliard *et al.* 2000, 2007) have shown that FAs in milk originated from two pathways, one is produced by *de-novo* synthesis in the mammary grand, and the other is derived from FAs originating from ingested feed. Milk FA containing less than 16 carbon atoms originates from acetate and butyrate produced in the rumen, and synthesized using fatty acid synthase and acetyl-CoA carboxylase in the mammary grand, while half of the C16 is derived from feed. Most of the FAs in Groups 1 and 2 were synthesized by the *de-novo* pathway. Milk FAs containing more than 18 carbon atoms originates from FAs of an ingested feed. However, the FA profile in milk differs from the FA profile in ingested feed, since FAs originating in feed are converted by biohydrogenation and isomerization in the rumen, and desaturation with $\Delta 9$-desaturase in the adipose tissue and mammary grand. All of the FAs in Groups 3 and 4 were synthesized by the pre-formed pathway, that is, they were derived from ingested feed.

The difference in the milk FA profile between that produced in the grazing and confinement seasons could be attributed to the difference in ingested feed, which led to a reduction in milk fat depression during the grazing season (Chilliard *et al.* 2000, 2007; Bauman & Griinari, 2003). In the past, it was thought that the high level of roughage intake provided by hay or rolled grass silage (semi-dry) promoted acetate production in the rumen, and the high levels of substrate for milk fat flowed into the mammary grand would promote *de-novo* synthesis of milk fat in the mammary grand. As a result, the C16 content in milk increased with feed containing high levels of roughage (Sutton 1989). In contrast, a low fiber intake in the grazing pasture would result in a low flow of substrate into the mammary grand, which would therefore, promote low milk fat content. However, this theory could not explain the nutritional milk fat depression (Bauman & Griinari, 2003).

Recently, in the reviews of Barber *et al.* (1997) and Bauman and Griinari (2003), it was reported that high flow of long-chain FA and *trans*-FA into the mammary grand interfered the *de-novo* synthesis of milk fat in the mammary grand. Since half of the milk FAs originate from *de-novo* synthesis in the mammary grand, a decrease in the ability of *de-novo* synthesis occurred due to milk fat depression (Kalač & Samková, 2010). Fresh grass contained a high amount of FAs, most of which is ALA, compared with hay or grass silage following which a substantial loss of FAs occurred during drying or ensiling (Dewhurst *et al.* 2006; Kalač & Samková, 2010). It is expected that in cows fed with high amount of fresh grass, the amount of *trans*-FAs absorbed from the small intestine should increase because higher amount of *trans*-FAs is produced by an incomplete biohydrogenation and isomerization of ALA in the rumen and pass into the duodenum (Chilliard *et al.* 2007). Therefore, the characteristics of the milk FA profile during the grazing season, which was low in Group 2 FAs and high in Groups 3 and 4 FAs, resulted from reduced *de-novo* synthesis in the mammary grand due to a high long-chain FA and ALA intake from pasture.

The present study indicated that, using a combination of 16 milk FAs, 90% of the milk samples in each grazing and confinement season were correctly classified into each season. Although the previous studies reported similar results that, using milk FA profile, the difference between grazing and confinement feeding was correctly classified (Gaspardo *et al.* 2010; Capuano *et al.* 2014), there were very few studies investigating the detailed feeding management in each farm. The results of the present study indicated that 10% of the milk samples were not classified correctly or were borderline in each season. In the confinement season, farmers that were borderline tended to supply less beet pulp compared with farmers that were discriminated correctly. The decrease in beet pulp intake substituted with high-moisture corn decreased acetate production in the rumen (Voelker & Allen, 2003). Thus, the low beet pulp intake could attribute a decrease in Group 2 FAs and an increase in Group 3 FAs in milk. However, since other nutritional and environmental factors would influence the milk FA profile, it was unlikely that only a low beet pulp intake attributed a decrease in Group 2 FAs and an increase in Group 3 FAs in milk. Further discussion is beyond the scope of the present study.

On the other hand, the nutritional factor that was discriminated incorrectly in the grazing season was clear. Compared with farmers discriminated correctly, a dependency on pasture was significantly lower for farmers that were borderline or incorrectly discriminated, with milk containing low levels of Group 3 and 4 FAs and high Group 2 FAs. Therefore, the results of the present study indicate that, to claim "grazing milk", grazing dairy farmers needed to achieve a high pasture intake. Many studies have demonstrated that several supplementary forage and concentrates decreased pasture intake (McGilloway & Mayne, 1996; Peyraud & Delaby, 2001; Bargo *et al.* 2003). To achieve a high dependency on pasture, farmers need to avoid supplying excess supplementary feed, nevertheless an adequate pasture allowance was a precondition.

In the present study, although the TVA and CLA contents of Group 4 FAs were not the most effective FA for discrimination analysis, the TVA and CLA content of Group 4 FAs ranged widely in the grazing season. In the study by Courveur *et al.* (2006), a linear increase in pasture intake led to higher TVA and CLA contents than the other FA contents. Although the TVA and CLA contents in milk were correlated with pasture intake in the present study, other factors of pasture, such as species or quality would also have an influence on FA contents (Dewhurst *et al.* 2001). Therefore, although the TVA and CLA contents were less important in FAs for the naive discrimination between the grazing and confinement seasons, those FAs would be useful to calculate the dependency on pasture intake. Further information is required to determine the relationship between the grazing and milk TVA and CLA contents under practical farming conditions.

In conclusion, using the milk FA profile, milk produced during the grazing or confinement season could be discriminated, and the percentage of correct discrimination was 90%. Among the 16 FAs in the milk measured in the present study, C16:0 contents, but not the TVA or CLA content, had the strongest power of discrimination. The result of the present study indicates that the characteristic FA profile in "grazing milk" was determined by differences in dominant FAs, such as C16:0, C18:0 and *cis*-9-C18:1. Although the farmers conducted grazing, some of the milk produced by farmers in low dependency pastures was classified as milk produced during the confinement season. To claim milk as "grazing milk", a high dependency on pasture is required by grazing dairy farmers.

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表題: 北海道の草地型酪農地域における乳中脂肪酸組成による放牧牛乳の判別

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抄録:放牧飼養で生産された牛乳"放牧牛乳"はヒトの健康へ寄与する成分含量が高く、その関心は日本でも高まっている。乳中の脂肪酸(FA)組成は飼養管理の影響を強く受けるため、乳中 FA 組成を基に放牧牛乳とその他の牛乳を判別できる可能性がある。そこで、本研究では北海道の代表的な草地型酪農地域における酪農家バルク乳の FA 組成を用い、放牧酪農家の放牧時期と舎飼時期の牛乳を判別可能か検討した。調査は、北海道の根釧地域 29 戸および道北地域 25 戸、計 54 戸の酪農家を対象に年 4 回実施した。各農家のバルクタンクから約 500ml の生乳サンプルを採取し、同時に給与飼料および乳生産量について聞き取りを行った。乳は一般乳成分および乳中 FA 組成を測定した。放牧時期の牛乳は舎飼時期の牛乳と比較して C16:0 および *cis*-9 C16:0 割合が低く、C18:0、*cis*-9 C18:1、*trans*-11 C18:1、*cis*-9,12 C18:2、*cis*-9,*trans*-11 C18:2 および *cis*-9,12,15 C18:3 割合が高かった。16FA を用いた判別分析の結果、約9割のサンプルは正しく判別できた(舎飼時期: 正解 90%、ボーダーライン 10%、放牧時期: 正解 88%、ボーダーライン 9%、不正解 3%)。放牧時期に判別分析で偽判別された農家は、正しく判別された農家と比較して、併給飼料給与量が多く、放牧草への依存度が低かった。したがって、放牧牛乳を名乗るためには、より高い放牧依存度が必要とされる。

Table 1 The mean and range of feed intake and milk production produced during the grazing and confinement seasons in the grassland dairy area in Hokkaido

	Grazing (N= 111)				Confinement (N = 105)				Confinement
	Min	Max	Mean	SD	Min	Max	Mean	SD	vs. Grazing
Feed intake, % of total dry matter intake									
Forage									
Pasture	8	81	48	17	-	-	-	-	-
Hay + Rolled grass silage	0	56	12	13	0	75	38	26	**
Grass silage	0	41	4	10	0	81	20	24	**
Total	36	84	65	11	32	81	58	10	**
Concentrate									
Commercial feed + Grain feed	0	59	26	11	0	68	32	10	**
Beet pulp	0	32	9	6	0	28	10	6	NS
Others	0	13	0	1	0	8	0	1	NS
Total	16	64	35	11	19	68	42	10	**
Milk yield, kg/cow/day	13.9	32.5	24.9	3.8	12.7	31.0	21.6	3.8	**
Milk fat, %	3.36	4.36	3.80	0.18	3.62	5.32	4.22	0.26	**
Milk protein, %	2.88	3.49	3.21	0.12	2.76	3.60	3.25	0.14	*
Lactose, %	4.21	4.59	4.39	0.07	4.20	4.52	4.38	0.07	NS
Solid not fat, %	8.19	9.01	8.60	0.16	8.25	9.01	8.62	0.15	NS
Milk urea nitrogen, mg/dL	6.7	27.8	16.8	4.3	6.6	20.1	13.1	2.5	**

NS not significant, ** $P < 0.01$, * $P < 0.05$

Table 2 Results of discrimination analysis based on 16 fatty acids (FAs) of milk produced during the grazing and confinement seasons in the grassland dairy area in Hokkaido

	Predictive component	
	Magnitude †	Reliability ‡
Fatty acid, % of total FA		
C8:0	0.02	0.15
C10:0	-0.03	-0.15
C12:0	-0.02	-0.09
C14:0	0.14	0.41
C14:1, *cis*-9	0.07	0.41
C15:0	0.04	0.32
C16:0	0.72	0.91
C16:1, *cis*-9	0.15	0.86
C17:0	0.01	0.12
C18:0	-0.36	-0.76
C18:1, *cis*-9	-0.34	-0.58
C18:1, *trans*-11 (TVA)	-0.36	-0.88
C18:2, *cis-9,12*	-0.09	-0.48
C18:2, *cis*-9,*trans*-11 (CLA)	-0.20	-0.85
C18:3, *cis*-9,12,15 (ALA)	-0.09	-0.64
C20:0	0.01	0.22
R^2 of the model	0.78	
RSD of the model	0.24	
% of correctly discrimination	#REF!	

ALA, α-linolenic acid; CLA, conjugated linoleic acid; TVA, *trans*-vaccenic acid; RSD, residual standard diviation

† Positive and negative magnitude values indicated that those FAs were influenced strongly in the confinement and grazing season,

‡ Reliability value indicated a reliability of a magnitude value of each FA in the dicrimination model

Table 3 Average feed intake in each category of discrimination analysis based on 16 fatty acids of milk produce during the grazing and confinement seasons in the grassland dairy area in Hokkaido

Actual	Grazing (GRA)				Confinement (CON)			
Discriminate to	CON	- line	GRA	SEM	CON	- line	GRA	SEM
N	3	10	98		94	11	0	
Forage, % of total intake								
Pasture	34.4	35.4	50.2	6.2	-	-	-	
Hay + Rolled grass silage	11.3	19.9	11.3	5.1	37.2	40.4	-	6.0
Grass silage	13.8	5.9	3.8	3.6	20.3	18.3	-	5.4
Total Forage	59.5	61.2	65.2	4.1	57.6	58.7	-	2.2
Pasture, % of total Forage	57.8	57.8	76.9	7.7				
Concentrate, % of total intake								
Commercial feed + Grain feed	26.6	28.1	26.0	4.1	32.2	34.9	-	2.3
Beet pulp	13.9	10.7	8.6	2.3	10.1	6.4	-	1.4
Others	0.0	0.0	0.2	0.5	0.1	0.0	-	0.2
Total Concentrate	40.5	38.8	34.8	4.1	42.4	41.3	-	2.2
Total supplement, % of total intake	65.6	64.6	49.8	6.2	-	-	-	


Confinement (CON)
Grazing (GRA)
Frequency, N
Mean†
CON 0.62
GRA 0.58
< 0.2 0.3 0.4 0.5 0.6 0.7 0.8 0.9 0.9 <
C8:0, % of total FA
Mean
CON 0.89
GRA 0.88
< 0.7 0.8 0.9 1.0 1.1 1.1 <
C17:0, % of total FA
Mean*
CON 1.96
GRA 2.03
< 1.6 1.8 2.0 2.2 2.4 2.4 < 2.6 <
C10:0, % of total FA
Mean**
CON 10.9
GRA 12.9
< 8 9 10 11 12 13 14 15 16 16 <
C18:0, % of total FA
Mean
CON 2.84
GRA 2.90
< 2.2 2.4 2.6 2.8 3.0 3.2 3.4 3.6 3.6 <
C12:0, % of total FA
Mean**
CON 20.5
GRA 22.8
17 18 19 20 21 22 23 24 25 26 27
C18:1, cis-9, % of total FA
Mean**
CON 11.5
GRA 10.9
< 9.0 9.4 9.8 10.2 10.6 11.0 11.4 11.8 12.2 12.6 13.0 <
C14:0, % of total FA
Mean**
CON 1.39
GRA 3.12
< 1.0 1.5 2.0 2.5 3.0 3.5 4.0 4.5 5.0 5.5 5.5 <
C18:1, trans-11, % of total FA
Mean**
CON 1.17
GRA 1.00
< 0.6 0.8 1.0 1.2 1.4 1.6 1.6 <
C14:1, cis-9, % of total FA
Mean**
CON 1.45
GRA 1.66
< 1.0 1.2 1.4 1.6 1.8 2 2.2 2.2 <
C18:2, cis-9,12, % of total FA
Mean**
CON 1.20
GRA 1.15
0.9 1.0 1.1 1.2 1.3 1.4 1.5 1.6
C15:0, % of total FA
Mean**
CON 0.54
GRA 1.08
< 0.4 0.6 0.8 1.0 1.2 1.4 1.6 1.8 2.0 2.2 <
C18:2, cis-9 trans-11, % of total FA
Mean**
CON 36.1
GRA 29.3
< 25 27 29 31 33 35 37 39 41 41 <
C16:0, % of total FA
Mean**
CON 0.44
GRA 0.60
< 0.3 0.4 0.5 0.6 0.7 0.8 0.9 0.9 <
C18:3, cis-9,12,15, % of total FA
Mean**
CON 1.70
GRA 1.37
< 1.2 1.3 1.4 1.5 1.6 1.7 1.8 1.9 2.0 2.0 <
C16:1, cis-9, % of total FA
Mean**
CON 0.19
GRA 0.18
< 0.15 0.16 0.17 0.18 0.19 0.20 0.21 0.22 0.22 <
C20:0, % of total FA