Title:

Effects of ear corn silage supplementation on milk production and milk fatty acid profiles in grazing dairy farms

Authors:

Tomohiro MITANI [1], Sadaki ASAKUMA [2], Yuka SHINODA [2], Yasuko UEDA [2], Yasuhiro AOKI [2], Tomoko OSHITA [2]

Institutions:

[1] Field Science Center for Northern Biosphere, Hokkaido University, Sapporo 060-0811, Japan

[2] Hokkaido Agricultural Research Center, NARO, Sapporo, 062-8555, Japan

Corresponding Author:

Tomohiro MITANI

Postal address: Field Science Center for Northern Biosphere, Hokkaido University, Kita 11, Nishi 10, Kita-ku, Sapporo 060-0811, Japan

E-mail: tmitani@fsc.hokudai.ac.jp

ABSTRACT

This study investigated the effects of substituting ear corn silage (ECS) for commercial formula feed on milk production and milk fatty acid profiles in grazing dairy farms during the summer season. A field survey was conducted on five grazing dairy farms in every summer month of 2017, 2018, and 2019. Three of the five farms substituted fresh ECS for the commercial formula feed at a ratio of 2:1 from July of each year (ECS farms). Other farms maintained the same feeding management as before (non-ECS farms). An interview survey was conducted on each farm to calculate feed intake and milk yield per cow. Feed and milk samples were collected in each survey. Milk compositions and milk fatty acid profiles were determined. The substitution of ECS for the commercial formula feed did not affect milk yield or milk composition, but ECS farms maintained low levels of milk urea compared with non-ECS farms (P < 0.01). The ECS substitution also influenced some of the milk fatty acid proportions; C16:0 and C16:1 increased, and *trans*-11 C18:1, *cis*-9,*trans*-11 C18:2, and the sum of polyunsaturated fatty acids decreased, while these fatty acid proportions were maintained in non-ECS farms throughout the summer season (P < 0.05).

Key words:

Ear corn silage, Fatty acid profile, Grazing farm, Milk production

1 INTRODUCTION

Japanese animal production is strongly dependent on imported feed (mainly grain); the feed self-sufficiency ratio for dairy cows is only 30% (Ministry of Agriculture, Forestry and Fisheries, 2020). This method of agriculture in Japan does not seem to be sustainable, because a high dependence on foreign countries reduces flexibility for dairy farmers and increases the environmental impact of dairy production. Therefore, an interest in producing self-sufficient concentrate has begun to grow in Japan. For example, a feed rice (e.g., whole crop silage and soft grain silage) is used in various regions except for Hokkaido, and an ensiling grain and/or ear-leaf of corn (e.g., ear corn silage, high-moisture shelled corn, and corn cob mix) is used mainly in Hokkaido (Ministry of Agriculture, Forestry and Fisheries, 2020). Among these type of feed, the production of ear corn silage (ECS) has been growing in prevalence within TMR centers and contractor organizations in Hokkaido, since ECS has high nutritive value and fermentation qualities (Oshita *et al.*, 2016; Otsu *et al.*, 2012). Milk production from cows fed with ECS supplement in silage-based feeding was equal to that from cows fed with a flaked dry corn supplement (Aoki *et al.*, 2016; Tada, Aoki & Oshita, 2018; Ueda *et al.*, 2014).

In recent years, consumer interest in high-quality food products has risen, which means production systems have become more ethical and have reduced their environmental impact, with regards to animal welfare and geographical origin (Luykx & Van Ruth, 2008). Similar to the trend in EU countries, Japanese consumers' concerns about food safety and security have been increasing, along with the demand for dairy products derived from cows given a self-produced feed. Although the feed self-sufficiency ratio of grazing dairy farmers tends to be high already, farmers can easily improve their self-sufficiency feed ratio by substituting a commercial formula feed into self-produced feeds.

The ruminal starch degradation rate of high-moisture corn feeds (e.g., ECS, high-moisture shelled corn, corn cob mix silage) was higher than that of dry grain (Cooper *et al.*, 2002). The high moisture corn feeds were compatible with alfalfa silage because the ruminal microbial N production was increased by synchronizing the extent and rate of the ruminal degradation of crude protein (CP) in alfalfa silage and those of starch in high-moisture corn feeds (Broderick, Mertens & Simons, 2002). The extent and rate of the ruminal degradation of CP in pastures is as high as that in alfalfa silage, and a low efficiency of N utilization for grazing dairy cows often becomes a nutritive problem (Bargo *et al.*, 2002). Therefore, to substituting a commercial formula feed with ECS for grazing dairy cows can improve not only the feed self-sufficiency ratio, but also the low efficiency of N utilization.

The milk fatty acid (FA) profile is known to be a very important parameter in milk products, due to its strong relation to the melting point of milk fat, which affects the mouth feel of milk products (Couvreur *et al.*, 2006; Larsen *et al.*, 2014). Many studies have investigated the milk FA profile produced by grazing compared with indoor feeding with total mixed rations (Kelly *et al.*, 1998; Schroeder *et al.*, 2003). This is because milk produced by grazing contains a highly functional FA profile for human health, including conjugated linoleic acid (CLA: *cis*-9,*trans*-11 C18:2) and *trans*-vaccenic acid (TVA: *trans*-11 C18:1), and improving ω-6/ω-3 ratio in milk FAs. Furthermore, studies have shown that the milk FA profile is strongly related to the feeding management of dairy cows, as the using milk FA profile was able to discriminate the feeding management on farms (Capuano *et al.*, 2014; Mitani *et al.*, 2016; Vicente *et al.*, 2017). Ueda *et al.* (2014) reported that the γ-lactone concentration in milk increased upon substitution of ECS for flaked dry corn in cows fed silage-based diets. The milk FA profile in the study (Ueda *et al.*, 2014) should also change, because many flavor components in milk are derived from

FAs. However, the effect of ECS supplementation for grazing dairy cows on the milk FA profile has not been investigated.

Therefore, a substitution of ECS for the commercial formula feed for grazing dairy cows is predicted to improve the ruminal environment of cows and change the milk FA profile. In the present study, a field survey was conducted for grazing dairy farms in Hokkaido for three years, and the effects of ECS substitution for the commercial formula feed on milk production and milk FA profiles were investigated.

2 MATERIALS AND METHODS

2.1 Research farms and feeding management

The field survey was conducted on five grazing dairy farms (Farm A, B, C, D, and E) in Tokachi, Hokkaido during 2017, 2018, and 2019. These farms belong to a producer group that regulates the use of non-transgenic feed and conduct grazing practices during the grazing season. The grazing season survey was conducted every month from July (end of June in 2019) to October of each year (except for September of 2018), and the indoor feeding survey was conducted in December of each year. All five farms conducted grazing practices during the summer. Among them, only Farm A adopted day-time grazing, and the others adopted one-day grazing. No farm changed its feeding management during the grazing season (Table 1).

Three of the five farms started to substitute ECS feeding for commercial formula feed feeding after an initial survey in July of each year (ECS farms: Farms A and B in 2017, 2018, and 2019, and Farm C only in 2018 and 2019). Other farms maintained the feeding management system same as before the start of study (Non-ECS farms: Farms D and E in 2017, 2018, and 2019, and Farm C only in 2017). The substitution ratio of commercial formula feed to ECS for ECS farms was 1 kg to 2 kg, weighed fresh (about

1 kg:1.2 kg in DM basis), with the moisture and total digestible nutrients (TDN) content of each feed taken into consideration. Upper limits of 8 kg per day, and 4 kg per feeding were set for ECS. The ECS used in this study was harvested with a corn crusher, and prepared into a roll bale with a roll baler in the prior year, and then conserved until use. The ECS used in 2017 was harvested by a contractor organization in the Tokachi prefecture of Hokkaido (Obihiro, Hokkaido), and the ECS used in 2018 and 2019 was harvested at the Hokkaido Agricultural Research Center (Sapporo, Hokkaido). The ECS bales were carried to each farm at the end of June of each year.

2.2 Survey method and sample analysis

An interview survey was conducted at each farm, and supply feeds including pasture and milk samples in the bulk tank were collected at the same time. Interview parameters included the number of lactating cows, daily milk production (shipping milk amount), types and amounts of the supply feeds, grazing methods, impression of using ECS and others. Each supply feed was collected at the first survey in each year. Pasture samples were gathered by hand-plucking on the pastures every month. Other forage samples were collected whenever the production batch changed.

The collected feed samples were brought to the Research Center of the Tokachi Federation of Agricultural Cooperatives (Obihiro, Hokkaido); analyses were preformed to determine the chemical compositions of pasture, roll baled grass silage, and corn silage by a near infrared analysis (NIRS XDS Analyzer; Methrohm AG, Herisau, Switzerland). The chemical compositions and fermented qualities of ECS (not in 2017) were analyzed according to official methods. Milk samples (500 mL) were collected from the top of the bulk tank using a stainless dipper after stirring. The milk samples were brought to a laboratory in cold storage and divided into sub-samples. The sub-sample for milk FA analysis was stored at -80°C until use. The sub-sample for milk composition analysis was dispensed into a dedicated tube and immediately sent to the Laboratory of Hokkaido Dairy Milk Recording and Testing Association. Then, milk fat, milk protein, lactose, solids not fat, and milk urea N (MUN) concentrations were analyzed using a Fourier transform infrared device (MilkoScan FT+; Foss Electric, Hillerød, Denmark).

Daily milk yield per cow was calculated by dividing the amount of milk shipped by the number of lactating cows. The intake of the concentrate and conserved forage was considered as the amount of supply from the interview survey. The amount of concentrate including ECS was confirmed as the amount of a shovel at the first time of survey. The intake of roll baled grass silage was calculated by dividing a supplying (number of used rolls) by numbers of cows. Pasture intake was calculated using the TDN requirement, TDN intake of other feeds, and TDN contents (National Agriculture and Food Research Organization, 2017; Mitani et al., 2016). The TDN contents used in the present study were estimated from the above chemical analysis (Table 2).

The milk sample for milk FA analysis was thoroughly thawed with tap water, and then warmed in a water bath to solve the fat. Milk FAs were extracted using a modified version of the Roese-Gottlieb method (ISO and IDF, 2001), and methylated by a modified method based on ISO and IDF (2002). The FA methyl esters were analyzed using a gas chromatograph equipped with a flame ionization detector (GC-2010; Shimadzu Co., Kyoto, Japan). The analysis was conducted in split mode with the following conditions: injection, 1μl; injector temperature, 250°C; split ratio, 40:1; carrier gas, helium; linear velocity, 30 cm/sec. The FA methyl esters were separated on a fused silica capillary column (SP-2560 100 m × 0.25 mm internal diameter, Sigma-Aldrich Japan K.K., Tokyo, Japan) with a temperature-rising condition (initial oven temperature at 60°C for 1 min, increased by 40 °C/min to 160 °C, held at 160 °C for 18 min, increased by 0.8 °C/min to 220 °C, and held at 220 °C for total time of 110 min). Each FA methyl ester was identified according to retention time compared with a standard mix (Supelco 37-Component FAME Mix: Sigma-Aldrich Japan K.K., Tokyo, and GLC-603 FAME mix: Nu-Chek-Prep, Inc., MA, USA) and self-methylated CLA.

2.3. Statistical analysis

Statistical analysis was conducted using JMP Pro 14.3 (SAS Institute Inc., Cary, NC, USA).The milk yield, milk composition, and FA profile data were analyzed with a 2-way ANOVA model using the Fit Model Platform in JMP. The model included ECS feeding (ECS farms or non-ECS farms), month of sampling (July, August, September, October, and December), interactions between those as fixed effects, and farm (Farm A, B, C, D, and E) as a random effect. If the possibility of difference was less than 0.05 or 0.10, the result was regarded as significant or tendency, respectively. The results are shown as least square means and standard errors of means. In addition, the results of the FA profile were analyzed with a factor analysis using the Multivariate Methods Platform of JMP. The factor analysis was conducted using 20 FAs, estimated by the maximum likelihood method, and rotated using the varimax rotation method for two components.

3 RESULTS AND DISCUSSION

3.1 Feed intake and chemical composition

The average feed intake of each farm is shown in Table 1. During the summer season, over 50% of the total intake was from pasture for farms conducting one-day grazing, and for Farm A, which conducted day-time grazing, pasture accounted for about 30% of the total intake. Farms D and E, the non-ECS farms, were highly dependent on pasture, which made up over 70% of the total intake. The proportions of formula feed in ECS farms were decreased with the supply of ECS, as expected, but the proportions of other feeds were not affected by the supply of ECS. As ECS was substituted for a formula feed, the self-sufficiency rate of grazing dairy farms increased by 2 to 12 points.

The chemical compositions of the pasture and ECS are shown in Table 2 (other feeds in Table S1). Qualities of pasture in every farm were comparatively good, containing high CP, low neutral detergent fiber (NDF), and high TDN, because all farms researched in the present study conducted rotational grazing and maintained pastures at a low sward height. The average CP and NDF contents of pasture differed among farms and ranged from 19.2% to 24.5% of dry matter (DM) and from 48.4% to 54.3% of DM, respectively. The difference in pasture chemical compositions among farms was caused by botanical differences, because the interval of rotation and stocking intensity differed among the farms. As the summer seasons progressed, the CP contents of the pastures increased, and water-soluble carbohydrate (WSC) contents decreased. The changes in chemical composition of the pastures were similar as changes in the common cool season grass (Wilkinson *et al.*, 2014). The chemical compositions of pastures also changed year to year. However, the difference of chemical compositions of pasture during years was less than that during seasons or farms. The trend of change in chemical composition of pastures during season were similar in each year (data not shown).

Chemical compositions of the ECS differed among harvested years, although they were within the ranges for ECS reported in the Hokkaido region (Oshita *et al.*, 2016). This could be caused by differences in harvest conditions, such as region and climate. The ECS used in 2018 was of good quality, with low moisture and high starch content, but the ECS used in 2017 was not as good, as the starch content was low. The ECS used in 2019 was of intermediate quality, between those of 2017 and 2018. However, the fermentation quality of each ECS used in the present study was excellent, having low pH, ammonia-N, and organic acids (except for lactic acid).

3.2 Milk production

The results of milk production are shown in Table 3. There was no difference in milk yield per cow between the ECS and non-ECS farms, although milk yield per cow for each farm decreased with seasonal progress ($P < 0.01$). The decrease in milk yield was caused by the progress of the lactation stage and the declining nutritive value of the pasture as the season progressed, but the results suggest that the substitution of ECS for concentrate feed in the grazing season does not influence milk yield. Milk composition parameters including milk fat, milk protein, lactose, and solids not fat content also changed with seasonal progress ($P < 0.01$) but did not differ between the ECS and non-ECS farms. In a study comparing the supply of ECS and flaked dry corn for lactating cows fed silage-based diets (Tada *et al.*, 2018; Ueda *et al.*, 2014), there was no difference in milk yield or milk composition.

The interaction effect between the ECS supply and month effect for milk urea nitrogen (MUN) content was significant ($P < 0.01$). Although MUN content for non-ECS farms increased seasons progressed, the changes in MUN content for ECS farms were small, and those for ECS farms were lower than those for non-ECS farms during months of ECS supply ($P < 0.05$ in August, September, and October). Low efficiency of N utilization for grazing dairy cows is often a problem, which results from nutritional characteristics of the pasture, a substantially high ruminal degradation rate and extent of CP, and a relatively low degradation rate of carbohydrates (Bargo *et al.*, 2002). Milk urea nitrogen concentration is an indicator of the ruminal degradation balance between CP and carbohydrates. When the ruminal degradation of CP is excessive, much NH_3-N is produced in the rumen and is absorbed by the rumen wall. NH_3-N is converted into urea in the liver and excreted into urine, milk, and saliva, thus increasing the MUN concentration. In spring, the MUN concentration does not increase significantly because of the high WSC content in spring pastures, but the MUN concentration after summer is likely to increase, resulting from a decrease in WSC in pastures (Bargo *et al.*, 2002; Wilkinson *et al.*, 2014). In the present study, MUN concentrations for non-ECS farms rose after August, but those of ECS farms were comparatively maintained at low levels. This was because CP content of total intake for ECS farms lowered with substituting commercial formula feed (21% of CP) to ECS (about 8.5% of CP), not for non-ECS farms. In addition, the ruminal degradation rate of starch in the ECS was very fast compared with that in flaked dry corn (Tada *et al.*, 2018). Therefore, as a result of low MUN in ECS farms, NH_3-N capture by ruminal microbes proceeds via synchronization of CP degradation of the pasture and carbohydrate degradation of ECS in the rumen.

3.3 Milk fatty acid profile

The average milk FA profile is shown in Table 4 (other FA profiles in Table S2). Proportions of *de novo* FA, including C16 (even carbon number FA: C4-C16), were higher in the ECS farms than in the non-ECS farms throughout the grazing season, including July, in which ECS was not supplied ($P < 0.05$). In contrast, the *trans*-10 C18:1, TVA, and CLA proportions were lower ($P < 0.05$), and the C18:0 and C20:0 proportions tended to be lower ($P < 0.10$) in ECS farms than in non-ECS farms. The interaction effects between ECS supply and month effect in C16:0, TVA, CLA, the sum of poly unsaturated FA, and a mixed FA proportion were significant ($P < 0.05$), and that in the sum of mono-unsaturated FA proportion was tendency ($P = 0.09$). Proportions of C16:0 and a mixed FA (C16:0 + C16:1) for ECS farms increased after August (when ECS was supplied), but those for non-ECS farms did not change much during the grazing season. In contrast, TVA, CLA, and poly unsaturated FA proportion for ECS farms decreased after August (supplying ECS), but those for non-ECS farms maintained high levels throughout the grazing season. The differences in most FA between the ECS and non-ECS farms resulted from a basic feeding management of each farm, which was grazing management, amounts and types of concentrate and conserved forage before the start of study, because the differences in most FA between ECS and non-ECS farms were continuous from July, when ECS was not supplied for all farms. However, the substitution of ECS for the commercial formula feed in the grazing season should affect the C16:0, TVA, and CLA proportions.

To visually investigate the effect of the substitution of ECS on the concentrate, a factor analysis was conducted using 20 milk FAs (Figure 1). In the present model using two factors, 60.4% of the total variance was accounted for. Factor 1 was positively related to proportions of short to mid-chain FAs among *de novo* FAs, and negatively to those of *cis*-9 C18:1, C20:0, *trans*-10 C18:1, and C18:0 (Figure 1-A). The analysis showed that factor 1 was assumed to be a factor related to *de novo* synthesis, because the factor was related negatively to *trans*-10 C18:1, which strongly inhibits *de novo* synthesis in the mammary gland (Barber *et al.*, 1997; Bauman & Griinari, 2003), and positively related to many of the *de novo* FAs. Factor 2 was positively related to the proportions of TVA, CLA, cis-9,12,15 C18:3, and negatively related to proportions of C16:0 and C16:1. Mitani *et al.* (2016) demonstrated that farm milks produced by grazing or indoor feeding could be discriminated using milk FA profiles. In the study by Mitani *et al.* (2016), FAs of C16:0 and C16:1 were the marked FAs during the indoor feeding period, and those of TVA and CLA were the marked FAs during the grazing period. Therefore, factor 2 was assumed to be a factor related to the dependency on pasture intake.

The results of the factor analysis indicated that the milk FA profile is an indicator of characteristics in each farm, because plots of each farm during the grazing (July to October) and indoor feeding (December) periods closely distributed (Figure 1-B). The plots of all farms distributed on the upper side during the grazing season (positive in factor 2), and the lower side in the indoor feeding period (negative in factor 2). For the grazing season, the plots of farms D and E were distributed more on the upper side than those of the other farms. These results also indicate that factor 2 is related to a dependency on pasture intake. Most plots of farm D were in the first quadrant, and those of farm E were in the second quadrant. Therefore, a feeding factor affected milk FAs related to factor 1. However, it could not be clarified which aspects of feeding management affected factor 1 in the present study.

The plots of ECS farms after supplying the ECS moved to the lower side and closed to those in the indoor feeding period. The movement of plots for ECS farms is a direct effect of the substitution of ECS for the concentrate, because pasture intake in ECS farms did not decrease, even when ECS was supplied. The values of nutritive characteristics of ECS fall between those of whole crop corn silage and corn grain, because ECS contains ear and leaf in addition to grain. Therefore, the movement of plots for ECS farms resulted from the nutritive characteristics of ECS as forage, compared with those of grain feed, contained in the formula feed.

In conclusion, it was made clear in this study that a substitution of ECS for the commercial formula feed in grazing dairy farms during the grazing season does not decrease pasture intake, then does not also affect milk yield and milk composition. The substitution of ECS lowered MUN concentrations in grazing dairy farms; a high MUN concentration indicates low efficiency of N utilization and is often a nutritive problem during the summer grazing season. In addition, the substitution of ECS changed the milk FA profile of milk produced by grazing dairy farms, which closed to those in indoor feeding period.

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References

Aoki, Y., Oshita, T., Ueda, Y., Nemoto, E. & Aoki, M. (2016). Effect of feeding of ear corn silage, high moisture shelled corn or dry shelled corn on lactation performance and metabolic profile of dairy cows. *Japanese Journal of Grassland Science*, *62*, 146-151.

Barber, M. C., Clegg, R. A., Travers, M. T. & Vernon, R. G. (1997). Lipid metabolism in the lactating mammary gland. *Biochimica et Biophysica Acta*, *1347* (2-3), 101-126. https://doi.org/Doi 10.1016/S0005-2760(97)00079-9

Bargo, F., Muller, L. D., Delahoy, J. E. & Cassidy, T. W. (2002). Performance of high producing dairy cows with three different feeding systems combining pasture and total mixed rations. *Journal of Dairy Science*, *85* (11), 2948-2963. https://doi.org/10.3168/jds.S0022-0302(02)74381-6

Bauman, D. E. & Griinari, J. M. (2003). Nutritional regulation of milk fat synthesis. *Annual Review of Nutrition*, *23*, 203-227.

https://doi.org/10.1146/annurev.nutr.23.011702.073408

Broderick, G. A., Mertens, D. R. & Simons, R. (2002). Efficacy of carbohydrate sources for milk production by cows fed diets based on alfalfa silage. *Journal of Dairy Science*, *85* (7), 1767-1776. https://doi.org/10.3168/jds.S0022-0302(02)74251-3

Capuano, E., van der Veer, G., Boerrigter-Eenling, R., Elgersma, A., Rademaker, J., Sterian, A. & van Ruth, S. M. (2014). Verification of fresh grass feeding, pasture grazing and organic farming by cows farm milk fatty acid profile. *Food Chemistry*, *164*, 234-241. https://doi.org/10.1016/j.foodchem.2014.05.011

Cooper, R. J., Milton, C. T., Klopfenstein, T. J., Scott, T. L., Wilson, C. B. & Mass, R. A. (2002). Effect of corn processing on starch digestion and bacterial crude protein flow in finishing cattle. *Journal of Animal Science*, *80* (3), 797-804. https://doi.org/10.2527/2002.803797x

Couvreur, S., Hurtaud, C., Lopez, C., Delaby, L. & Peyraud, J. L. (2006). The linear relationship between the proportion of fresh grass in the cow diet, milk fatty acid composition, and butter properties. *Journal of Dairy Science*, *89* (6), 1956-1969.

ISO (International Organization for Standardization), IDF (International Dairy Federation). (2001). *Milk and milk products - Extraction methods for lipids and liposoluble compounds: ISO 14156: 2001, IDF 172:2001*. Geneva and Brussels, Switzerland: ISO and IDF.

ISO (International Organization for Standardization), IDF (International Dairy Federation). (2002). *Milk fat - Preparation of Fatty acid methyl esters: ISO 15884: 2002, IDF 182:2002*. Geneva and Brussels, Switzerland: ISO and IDF.

Kelly, M. L., Kolver, E. S., Bauman, D. E., Van Amburgh, M. E. & Muller, L. D. (1998). Effect of intake of pasture on concentrations of conjugated linoleic acid in milk of lactating cows. *Journal of Dairy Science*, *81* (6), 1630-1636.

https://doi.org/10.3168/jds.S0022-0302(98)75730-3

Larsen, M. K., Andersen, K. K., Kaufmann, N. & Wiking, L. (2014). Seasonal variation in the composition and melting behavior of milk fat. *Journal of Dairy Science*, *97* (8), 4703-4712. https://doi.org/10.3168/jds.2013-7858

Luykx, D. M. A. M. & Van Ruth, S. M. (2008). An overview of analytical methods for determining the geographical origin of food products. *Food Chemistry*, *107* (2), 897-911. https://doi.org/10.1016/j.foodchem.2007.09.038

Ministry of Agriculture, Forestry and Fisheries. 2020. The situation over the livestock farming, statistics data, press release [homepage on the Internet]. Ministry of Agriculture, Forestry and Fisheries, Tokyo, Japan; [cited 2020 2 April]. Available from URL: https://www.maff.go.jp/j/chikusan/kikaku/lin/l_hosin/

Mitani, T., Kobayashi, K., Ueda, K. & Kondo, S. (2016). Discrimination of "grazing milk" using milk fatty acid profile in the grassland dairy area in Hokkaido. *Animal Science Journal*, *87* (2), 233-241.

National Agriculture and Food Research Organization. (2017). *Japanese Feeding Standard for Dairy Cattle*. Tokyo, Japan: Japan Livestock Industry Association.

Oshita, T., Nemoto, E., Aoki, Y., Ueda, Y. & Aoki, M. (2016). Comparison of chemical composition, nutritive value and production yield between high moisture shelled corn and ear corn silage. *Japanese Journal of Grassland Science*, *62*, 140-145.

Otsu, E., Oshita, T., Namekawa, H., Takada, M., Takahashi, S. & Nishiura, A. (2012). Construction of Ear-corn Silage Harvesting and Ensiling System. *Japanese Journal of Grassland Science*, *58* (2), 95-101.

Schroeder, G. F., Delahoy, J. E., Vidaurreta, I., Bargo, F., Gagliostro, G. A. & Muller, L. D. (2003). Milk fatty acid composition of cows fed a total mixed ration or pasture plus concentrates replacing corn with fat. *Journal of Dairy Science*, *86* (10), 3237-

3248. https://doi.org/10.3168/jds.S0022-0302(03)73927-7

Tada, S., Aoki, Y. & Oshita, T. (2018). Ruminal degradability, feeding behavior and production performance in dairy cows fed ear corn silage. *Nihon Chikusan Gakkaiho*, *89* (4), 431-437.

Ueda, Y., Oshita, T., Aoki, Y., Nemoto, E., Aoki, M. & Nishiura, A. (2014). Effect of ear-corn silage on milk production and volatile compounds of milk in lactating cows. *Nihon Chikusan Gakkaiho*, *85* (3), 301-307.

Vicente, F., Santiago, C., Jimenez-Calderon, J. D. & Martinez-Fernandez, A. (2017). Capacity of milk composition to identify the feeding system used to feed dairy cows. *Journal of Dairy Research*, *84* (3), 254-263. https://doi.org/10.1017/S0022029917000383

Wilkinson, J. M., Allen, J. D., Tunnicliffe, R., Smith, M. & Garnsworthy, P. C. (2014). Variation in composition of pre-grazed pasture herbage in the United Kingdom, 2006–2012. *Animal Feed Science and Technology*, *196*, 139-144. https://doi.org/10.1016/j.anifeedsci.2014.07.001

Figure legends

Figure 1. Results of factor analysis of fatty acid profiles for farms supplied with ear corn silage (ECS) or without ECS from July to December for three years (2017, 2018, and 2019)

Figure A (left side): factor loading score, figure B-1 (right side): average of loading plots of each farm, figure B-2: loading plots of each sample

Figure symbols were, Farm A: circle (○), Farm B: square (□), Farm C: diamond (◇), Farm D: triangle (△), Farm E: cross (×), Grazing without ECS: opened, Grazing with ECS: gray, Indoor feeding period: blackened

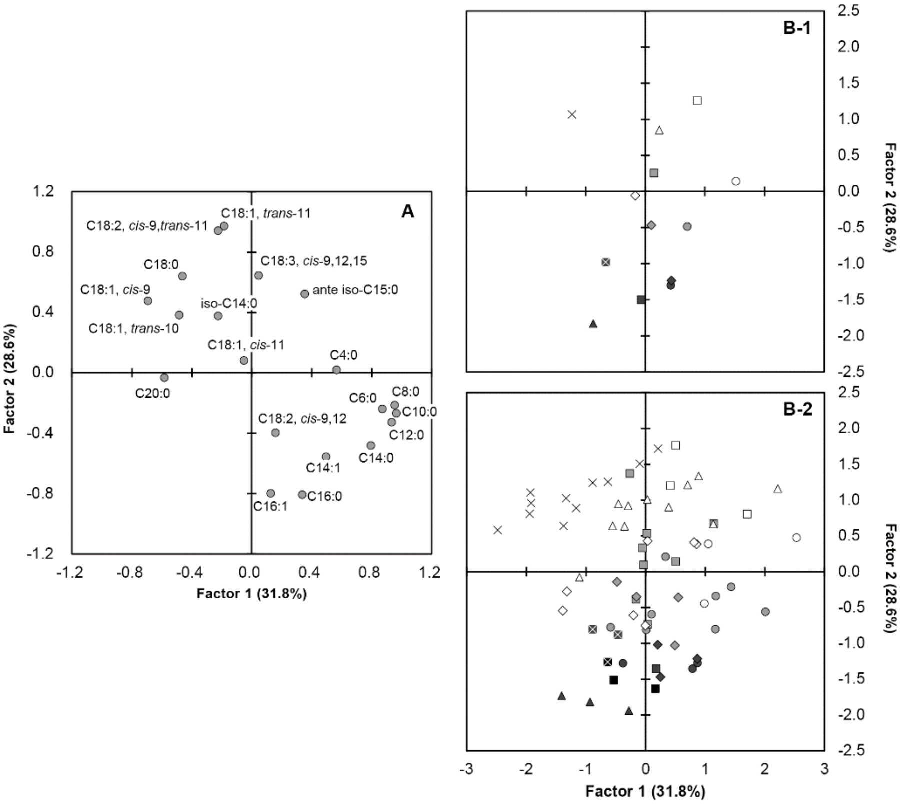
A
C18:1, trans-11
C18:2, cis-9,trans-11
C18:0
C18:3, cis-9,12,15
ante iso-C15:0
C18:1, cis-9
iso-C14:0
C18:1, trans-10
C18:1, cis-11
C4:0
C20:0
C6:0
C8:0
C10:0
C18:2, cis-9,12
C12:0
C14:0
C14:1
C16:0
C16:1
Factor 1 (31.8%)
Factor 2 (28.6%)
B-1
B-2

Figure 1

Table 1. Averages of feed intake for farms supplied with ear corn silage (ECS) or without ECS (non-ECS) from July to December for three years (2017, 2018, and 2019)

	Farm†															
	A			B			C				D			E		
	Jul.	Aug. - Oct.	Dec.	Jul.	Aug. - Oct.	Dec.	Jul.	Aug. - Oct.	Aug. - Oct.	Dec.	Jul.	Aug. - Oct.	Dec.	Jul.	Aug. - Oct.	Dec.
ECS or non-ECS	Non-ECS	ECS		Non-ECS	ECS		Non-ECS	Non-ECS (2017)	ECS		Non-ECS	Non-ECS		Non-ECS	Non-ECS	
Feed intake, % of total intake as dry matter basis																
Pasture	33	25	-	60	59	-	54	61	50	-	71	71	-	81	79	-
Baled grass silage	38	35	48	8	10	62	10	6	12	29	11	9	67	0	0	42
Corn Silage	0	0	0	0	0	0	7	0	2	34	0	0	0	0	0	38
Commercial formula feed	17	15	29	15	3	22	12	14	8	17	6	6	12	0	0	0
Ear corn silage	0	12	8	0	15	0	0	0	12	0	0	0	0	0	0	0
Others‡	12	12	15	16	13	16	18	20	16	20	12	13	22	19	21	20
Self sufficiency rate	83	85	71	85	97	78	88	86	92	83	94	94	88	100	100	100

† Grazing period was from July to October and indoor feeding period was in December.

Farms A, B, and C (2018 and 2019) were supplied ECS from August to October (a part of December) and Farm C (2017), Farms D and E were not supplied ECS.

‡ Feed of others was included sugar beet pulp, wheat, and rice bran.

Table 2 . Averages of chemical compositions and feremention score of pasture and ear corn silage (see detail to Table S1)

Feed	DM,%	Chemical compositions, % of DM					TDN, %	Fermentation score						
		CP	NDF	NFC	Starch	WSC		pH	Ammonia-N,%	Lactic acid, %	Acetic acid, %	Propionic acid, %	Butyric acid, %	V score
Pasture														
Farm														
Farm A	19.5	21.6	53.9	18.9	-	7.5	69.9							
Farm B	17.7	24.5	48.4	20.3	-	8.0	72.6							
Farm C	19.2	19.2	54.3	19.5	-	9.3	69.7							
Farm D	18.2	21.2	51.0	21.3	-	9.4	71.5							
Farm E	18.9	22.5	50.9	20.0	-	8.7	70.9							
Month														
July	19.1	20.5	53.1	19.7	-	10.0	71.2							
August	20.1	21.7	54.6	17.2	-	7.9	70.7							
September	17.4	22.4	50.4	21.3	-	7.2	70.5							
October	17.7	22.9	48.3	22.1	-	8.8	71.2							
Year														
2017	16.6	22.4	54.2	18.3	-	7.5	72.1							
2018	20.0	21.9	52.7	18.0	-	8.6	71.2							
2019	19.6	21.1	49.9	22.0	-	9.2	69.9							
Ear corn silage														
2017	55.4	9.2	27.0	58.8	50.4	-	80.4	-	-	-	-	-	-	-
2018	58.4	8.2	17.6	70.3	63.1	-	83.7	4.03	0.04	1.71	0.66	0.00	0.00	98.0
2019	47.2	8.3	21.4	64.9	57.8	-	82.9	3.90	0.08	2.50	1.17	0.00	0.00	94.5

DM: dry matter, CP: crude protein, NDF: neutral detergent fiber, NFC: non-fibrouns carbohydrate, WSC: water soluble carbohydrate, TDN: total digestible nutrients

Table 3. Averges of milk yield and milk composition for farms supplied with ear corn silage (ECS) or without ECS (non-ECS) from July to December for three years (2017, 2018, and

	Month†											Possibility (P =)‡		
	Jul.		Aug.		Sep.		Oct.		Dec.					
	ECS	Non-ECS	ECS	Non-ECS	ECS	Non-ECS	ECS	Non-ECS	ECS	Non-ECS	SEM	ECS	Month	Int.
Milk yield, kg/day/cow	26.2	26.1	25.4	24.1	21.4	22.9	22.9	22.8	22.0	19.3	0.8	0.59	<.01	0.17
Milk compositions, %														
Milk fat	3.69	3.76	3.84	3.80	3.89	3.95	4.06	3.95	4.13	4.11	0.05	0.79	<.01	0.38
Milk protein	3.24	3.30	3.24	3.31	3.31	3.38	3.36	3.46	3.25	3.29	0.03	0.11	<.01	0.94
Lactose	4.40	4.44	4.34	4.34	4.29	4.29	4.32	4.29	4.35	4.37	0.02	0.87	<.01	0.42
Solids not fat	8.65	8.73	8.58	8.64	8.62	8.67	8.70	8.76	8.61	8.65	0.04	0.23	<.01	0.98
Milk urea nitrogen, mg/dL	13.9	14.6	14.9	16.7	15.5	17.3	13.9	17.4	9.3	6.5	0.8	0.23	<.01	<.01

† Grazing period was from July to October and indoor feeding period was in December.
Farms A, B, and C (2018 and 2019) were supplied ECS from August to October (a part of December) and Farm C (2017), Farms D and E were not supplied ECS.

‡ ECS: ECS vs. non-ECS, Month: July, August, September, October, vs. December, Int.: interaction between ECS and Month

Table 4. Averges of fatty acid metyl ester (FAME) concentration for farms supplied with ear corn silage (ECS) or without ECS (non-ECS) from July to December for three years (2017, 2018, and 2019)

	Month†											Possibility (P =)‡		
	Jul.		Aug.		Sep.		Oct.		Dec.					
	ECS	Non-ECS	ECS	Non-ECS	ECS	Non-ECS	ECS	Non-ECS	ECS	Non-ECS	SEM	ECS	Month	Int.
FAME concentration, % of total FAME														
C4:0	2.40	2.29	2.40	2.24	2.38	2.24	2.34	2.18	2.27	2.24	0.05	0.05	0.32	0.71
C6:0	1.75	1.60	1.71	1.53	1.67	1.51	1.70	1.50	1.71	1.60	0.04	<.01	0.20	0.84
C8:0	1.13	1.03	1.08	0.95	1.06	0.94	1.09	0.96	1.09	1.01	0.03	0.01	0.11	0.88
C10:0	2.61	2.39	2.45	2.15	2.37	2.08	2.52	2.20	2.54	2.36	0.08	0.03	0.02	0.90
C12:0	3.05	2.84	2.91	2.55	2.83	2.50	3.03	2.66	3.07	2.87	0.10	0.05	0.02	0.87
C14:0	11.0	10.1	10.7	9.8	10.5	9.6	10.9	9.8	11.2	10.9	0.2	<.01	<.01	0.34
C14:1	0.96	0.83	0.99	0.88	1.03	0.91	1.06	0.91	1.12	1.07	0.03	0.02	<.01	0.69
iso-C14:0	0.12	0.13	0.12	0.14	0.12	0.13	0.11	0.12	0.11	0.11	0.01	0.09	<.01	0.71
ante iso-C15:0	0.55	0.58	0.51	0.54	0.51	0.51	0.48	0.50	0.47	0.48	0.01	0.27	<.01	0.73
C16:0	28.0	24.5	28.3	25.1	28.3	25.4	29.5	25.2	32.5	32.4	0.7	<.01	<.01	0.03
C16:1	1.69	1.57	1.75	1.61	1.83	1.72	1.88	1.69	2.14	2.05	0.05	0.03	<.01	0.85
C18:0	11.1	12.4	11.2	12.0	11.4	12.1	10.8	11.3	9.3	10.0	0.3	0.08	<.01	0.73
C18:1, *trans*-10	0.32	0.37	0.29	0.40	0.29	0.35	0.28	0.36	0.26	0.31	0.02	0.02	0.02	0.46
C18:1, *trans*-11	2.58	2.87	2.27	3.04	2.14	2.57	1.80	2.82	1.09	1.05	0.14	0.01	<.01	<.01
C18:1, *cis*-9	20.0	21.6	20.8	22.2	21.8	23.6	20.6	22.2	19.6	19.3	0.6	0.10	<.01	0.31
C18:1, *cis*-11	0.41	0.41	0.41	0.41	0.39	0.40	0.41	0.41	0.39	0.37	0.02	0.96	0.31	0.93
C18:2, *cis*-9,12	1.43	1.38	1.33	1.29	1.29	1.27	1.29	1.26	1.61	1.50	0.06	0.57	<.01	0.95
C18:2, *cis*-9,*trans*-11	1.17	1.24	1.07	1.42	1.14	1.29	0.88	1.39	0.62	0.55	0.06	0.03	<.01	<.01
C18:3, *cis*-9,12,15	0.69	0.67	0.65	0.65	0.62	0.55	0.59	0.63	0.46	0.39	0.03	0.58	<.01	0.36
C20:0	0.16	0.19	0.17	0.19	0.17	0.17	0.16	0.18	0.17	0.20	0.01	0.10	0.56	0.83
Sum of FAME														
Mono unsaturated	27.2	29.1	27.7	30.2	28.6	31.0	27.2	30.0	25.6	25.4	0.6	0.03	<.01	0.09
Poly unsaturated	3.77	3.99	3.53	4.06	3.47	3.74	3.25	3.99	3.11	3.01	0.10	0.02	<.01	<.01
De Novo (< C16)	22.9	21.1	22.2	20.1	21.9	19.8	22.7	20.2	23.1	22.1	0.5	<.01	<.01	0.57
Mixed (C16)	29.7	26.1	30.0	26.7	30.1	27.2	31.4	26.9	34.6	34.5	0.7	<.01	<.01	0.04
Pre-Formed (C16 <)	39.9	43.7	40.2	44.3	41.0	44.7	38.8	43.1	35.2	35.8	1.0	0.02	<.01	0.28

† Grazing period was from Jul. to Oct. and indoor feeding period was in Dec.

Farm A, B, and C (2018 and 2019) were supplied ECS from Aug. to Oct. (a part of Dec.) and Farm C (2017), D and E were not supplied ECS.

‡ ECS: ECS vs. Non-ECS, Month: Jul., Aug., Sep., Oct., vs. Dec., Int.: interaction of ECS and Month

Table S1. Averages of chemical compositions and feremention score of supplied feed

Feed	DM,%	Chemical compositions, % of DM†									TDN, %	Fermentation Score							
		CP	NDF	ADF	ADL	NFC	Starch	WSC	EE	Ash		pH	Ammonia-N,%	Ammonia/Total N	Lactic acid, %	Acetic acid, %	Propionic acid, %	Butyric acid, %	V score
Baled grass silage																			
Farm A	75.1	12.0	68.5	39.4	4.7	14.5	-	-	2.2	6.3	57.6								
Farm B	66.3	9.2	70.9	40.5	3.9	13.2	-	-	2.9	6.6	59.7								
Farm C	64.4	13.4	65.2	37.5	3.7	15.2	-	-	2.8	7.2	61.8								
Farm D	70.7	10.1	68.8	40.3	4.4	14.4	-	-	2.3	7.0	56.9								
Farm E	62.2	13.2	64.9	37.2	3.7	15.5	-	-	2.9	6.9	60.2								
Corn silage																			
Farm C	30.2	8.9	42.6	24.8	2.6	41.9	25.7	-	3.3	4.9	71.2	3.80	0.06	4.60	7.64	2.68	0.05	0.00	96.0
Farm E	33.0	8.0	39.1	21.8	2.1	46.7	28.7	-	2.9	4.9	72.9	3.80	0.06	5.00	7.40	1.53	0.00	0.00	98.0
Concentrate																			
Formula feed A	86.8	21.1	16.3	7.3	1.0	54.3	40.1	-	3.6	6.2	87.5								
Formula feed B	85.9	8.9	12.0	4.0	0.6	74.2	68.6	-	3.9	1.9	92.0								
Sugar beet pulp	88.0	11.2	41.4	19.4	3.2	41.7	5.0	-	0.7	11.0	62.7								
Wheat	85.5	16.0	13.8	3.9	1.8	68.8	41.7	-	1.9	2.1	82.7								
Rice bran	86.5	15.9	26.9	11.1	7.0	26.7	18.7	-	21.7	11.2	88.1								

DM: dry matter, CP: crude protein, NDF: neutral detergent fiber, ADF: acid detergent fiber, ADL: acid detergent lignin, NFC: non-fibrouns carbohydrate, WSC: water soluble carbohydrate, EE: ether extract, TDN: total digestible nutrients

Table S2. Averges of fatty acid metyl ester (FAME) concentration for farms supplied with ear corn silage (ECS) or without ECS (non-ECS) from July to December for three years (2017, 2018, and 2019)

	Month†													
	Jul.		Aug.		Sep.		Oct.		Dec.			Possibility (P =)‡		
	ECS	Non-ECS	ECS	Non-ECS	ECS	Non-ECS	ECS	Non-ECS	ECS	Non-ECS	SEM	ECS	Month	Int.
FAME concentration, % of total FAME														
C5:0	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.00	0.26	0.67	0.85
C7:0	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.00	0.43	0.14	0.49
C9:0	0.02	0.02	0.02	0.01	0.02	0.01	0.02	0.02	0.02	0.02	0.00	0.17	0.05	0.39
C11:0	0.33	0.29	0.32	0.28	0.32	0.29	0.33	0.30	0.34	0.32	0.01	0.04	0.15	0.83
C15:0	1.07	1.03	1.03	1.02	1.06	1.01	1.04	1.04	1.04	1.06	0.02	0.70	0.80	0.72
C17:0	0.54	0.56	0.52	0.51	0.55	0.52	0.53	0.50	0.54	0.56	0.02	0.73	0.25	0.46
t6-C18:1	0.32	0.35	0.29	0.38	0.29	0.35	0.27	0.35	0.25	0.29	0.01	<.01	<.01	0.18
t9-C18:1	0.23	0.25	0.22	0.28	0.22	0.26	0.20	0.27	0.18	0.21	0.01	<.01	<.01	0.17
c6-C18:1	0.40	0.40	0.38	0.42	0.39	0.40	0.38	0.44	0.35	0.40	0.02	0.16	0.25	0.30
n6-C18:3	0.03	0.03	0.03	0.03	0.02	0.02	0.02	0.04	0.03	0.03	0.00	0.74	0.32	0.19
C19:0	0.15	0.16	0.14	0.16	0.14	0.15	0.13	0.15	0.11	0.13	0.01	0.16	<.01	0.86
C21:0	0.03	0.03	0.03	0.06	0.03	0.05	0.03	0.03	0.03	0.03	0.01	0.24	0.53	0.58
C23:0	0.03	0.05	0.03	0.04	0.03	0.04	0.03	0.04	0.04	0.03	0.00	0.22	0.54	0.20
C20:2	0.04	0.05	0.04	0.05	0.04	0.06	0.04	0.05	0.04	0.04	0.01	0.11	0.93	0.69
C22:0	0.07	0.08	0.06	0.08	0.06	0.08	0.06	0.08	0.06	0.07	0.01	0.06	0.46	0.62
n6-C20:3	0.07	0.08	0.06	0.08	0.06	0.07	0.06	0.07	0.06	0.07	0.00	0.08	0.17	0.51
n3-20:5	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.05	0.05	0.00	0.71	<.01	0.88
C24:0	0.05	0.05	0.04	0.04	0.04	0.04	0.04	0.04	0.04	0.04	0.00	0.52	0.31	0.91
n3-C22:5	0.09	0.08	0.09	0.09	0.09	0.08	0.09	0.09	0.07	0.07	0.00	0.55	<.01	0.13
Others	5.07	6.16	5.21	6.07	4.69	5.60	4.89	7.01	4.78	4.77	0.41	0.12	0.04	0.13
Sum of FAME														
Odd-Chain	2.21	2.15	2.13	2.10	2.18	2.09	2.15	2.09	2.15	2.17	0.04	0.46	0.39	0.72

† Grazing period was from July to October and indoor feeding period was in December.

Farms A, B, and C (2018 and 2019) were supplied ECS from August to October (a part of December) and Farm C (2017), Farms D and E were not supplied ECS.

‡ ECS: ECS vs. non-ECS, Month: July, August, September, October, vs. December, Int.: interaction between ECS and Month