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Author(s)	Mitani, Tomohiro; Asakuma, Sadaki; Shinoda, Yuka; Ueda, Yasuko; Aoki, Yasuhiro; Oshita, Tomoko
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1 **Title:**

2 Effects of ear corn silage supplementation on milk production and milk fatty acid profiles
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4

5 **Authors:**

6 Tomohiro MITANI¹, Sadaki ASAKUMA², Yuka SHINODA², Yasuko UEDA², Yasuhiro
7 AOKI², Tomoko OSHITA²

8

9 **Institutions:**

10 ¹ Field Science Center for Northern Biosphere, Hokkaido University, Sapporo 060-0811,
11 Japan

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

13

14 **Corresponding Author:**

15 Tomohiro MITANI

16 Postal address: Field Science Center for Northern Biosphere, Hokkaido University, Kita
17 11, Nishi 10, Kita-ku, Sapporo 060-0811, Japan

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

19

20

1 **ABSTRACT**

2 This study investigated the effects of substituting ear corn silage (ECS) for commercial
3 formula feed on milk production and milk fatty acid profiles in grazing dairy farms during
4 the summer season. A field survey was conducted on five grazing dairy farms in every
5 summer month of 2017, 2018, and 2019. Three of the five farms substituted fresh ECS
6 for the commercial formula feed at a ratio of 2:1 from July of each year (ECS farms).
7 Other farms maintained the same feeding management as before (non-ECS farms). An
8 interview survey was conducted on each farm to calculate feed intake and milk yield per
9 cow. Feed and milk samples were collected in each survey. Milk compositions and milk
10 fatty acid profiles were determined. The substitution of ECS for the commercial formula
11 feed did not affect milk yield or milk composition, but ECS farms maintained low levels
12 of milk urea compared with non-ECS farms ($P < 0.01$). The ECS substitution also
13 influenced some of the milk fatty acid proportions; C16:0 and C16:1 increased, and *trans*-
14 11 C18:1, *cis*-9,*trans*-11 C18:2, and the sum of polyunsaturated fatty acids decreased,
15 while these fatty acid proportions were maintained in non-ECS farms throughout the
16 summer season ($P < 0.05$).

17

18 Key words:

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

20

1 INTRODUCTION

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

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

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

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

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

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

8

9 **2 MATERIALS AND METHODS**

10 **2.1 Research farms and feeding management**

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

20 Three of the five farms started to substitute ECS feeding for commercial formula
21 feed feeding after an initial survey in July of each year (ECS farms: Farms A and B in
22 2017, 2018, and 2019, and Farm C only in 2018 and 2019). Other farms maintained the
23 feeding management system same as before the start of study (Non-ECS farms: Farms D
24 and E in 2017, 2018, and 2019, and Farm C only in 2017). The substitution ratio of
25 commercial formula feed to ECS for ECS farms was 1 kg to 2 kg, weighed fresh (about

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

9

10 2.2 Survey method and sample analysis

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

18 The collected feed samples were brought to the Research Center of the Tokachi
19 Federation of Agricultural Cooperatives (Obihiro, Hokkaido); analyses were performed
20 to determine the chemical compositions of pasture, roll baled grass silage, and corn silage
21 by a near infrared analysis (NIRS XDS Analyzer; Methrohm AG, Herisau, Switzerland).
22 The chemical compositions and fermented qualities of ECS (not in 2017) were analyzed
23 according to official methods. Milk samples (500 mL) were collected from the top of the
24 bulk tank using a stainless dipper after stirring. The milk samples were brought to a
25 laboratory in cold storage and divided into sub-samples. The sub-sample for milk FA

1 analysis was stored at -80°C until use. The sub-sample for milk composition analysis was
2 dispensed into a dedicated tube and immediately sent to the Laboratory of Hokkaido
3 Dairy Milk Recording and Testing Association. Then, milk fat, milk protein, lactose,
4 solids not fat, and milk urea N (MUN) concentrations were analyzed using a Fourier
5 transform infrared device (MilkoScan FT+; Foss Electric, Hillerød, Denmark).

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

15 The milk sample for milk FA analysis was thoroughly thawed with tap water,
16 and then warmed in a water bath to solve the fat. Milk FAs were extracted using a
17 modified version of the Roese-Gottlieb method (ISO and IDF, 2001), and methylated by
18 a modified method based on ISO and IDF (2002). The FA methyl esters were analyzed
19 using a gas chromatograph equipped with a flame ionization detector (GC-2010;
20 Shimadzu Co., Kyoto, Japan). The analysis was conducted in split mode with the
21 following conditions: injection, 1 µl; injector temperature, 250°C; split ratio, 40:1; carrier
22 gas, helium; linear velocity, 30 cm/sec. The FA methyl esters were separated on a fused
23 silica capillary column (SP-2560 100 m × 0.25 mm internal diameter, Sigma-Aldrich
24 Japan K.K., Tokyo, Japan) with a temperature-rising condition (initial oven temperature
25 at 60°C for 1 min, increased by 40 °C/min to 160 °C, held at 160 °C for 18 min, increased

1 by 0.8 °C/min to 220 °C, and held at 220 °C for total time of 110 min). Each FA methyl
2 ester was identified according to retention time compared with a standard mix (Supelco
3 37-Component FAME Mix: Sigma-Aldrich Japan K.K., Tokyo, and GLC-603 FAME
4 mix: Nu-Chek-Prep, Inc., MA, USA) and self-methylated CLA.

6 **2.3. Statistical analysis**

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

19 **3 RESULTS AND DISCUSSION**

20 **3.1 Feed intake and chemical composition**

21 The average feed intake of each farm is shown in Table 1. During the summer season,
22 over 50% of the total intake was from pasture for farms conducting one-day grazing, and
23 for Farm A, which conducted day-time grazing, pasture accounted for about 30% of the
24 total intake. Farms D and E, the non-ECS farms, were highly dependent on pasture, which
25 made up over 70% of the total intake. The proportions of formula feed in ECS farms were

1 decreased with the supply of ECS, as expected, but the proportions of other feeds were
2 not affected by the supply of ECS. As ECS was substituted for a formula feed, the self-
3 sufficiency rate of grazing dairy farms increased by 2 to 12 points.

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

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

1 ammonia-N, and organic acids (except for lactic acid).

2

3 **3.2 Milk production**

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

15 The interaction effect between the ECS supply and month effect for milk urea
16 nitrogen (MUN) content was significant ($P < 0.01$). Although MUN content for non-ECS
17 farms increased seasons progressed, the changes in MUN content for ECS farms were
18 small, and those for ECS farms were lower than those for non-ECS farms during months
19 of ECS supply ($P < 0.05$ in August, September, and October). Low efficiency of N
20 utilization for grazing dairy cows is often a problem, which results from nutritional
21 characteristics of the pasture, a substantially high ruminal degradation rate and extent of
22 CP, and a relatively low degradation rate of carbohydrates (Bargo *et al.*, 2002). Milk urea
23 nitrogen concentration is an indicator of the ruminal degradation balance between CP and
24 carbohydrates. When the ruminal degradation of CP is excessive, much $\text{NH}_3\text{-N}$ is
25 produced in the rumen and is absorbed by the rumen wall. $\text{NH}_3\text{-N}$ is converted into urea

1 in the liver and excreted into urine, milk, and saliva, thus increasing the MUN
2 concentration. In spring, the MUN concentration does not increase significantly because
3 of the high WSC content in spring pastures, but the MUN concentration after summer is
4 likely to increase, resulting from a decrease in WSC in pastures (Bargo *et al.*, 2002;
5 Wilkinson *et al.*, 2014). In the present study, MUN concentrations for non-ECS farms
6 rose after August, but those of ECS farms were comparatively maintained at low levels.
7 This was because CP content of total intake for ECS farms lowered with substituting
8 commercial formula feed (21% of CP) to ECS (about 8.5% of CP), not for non-ECS farms.
9 In addition, the ruminal degradation rate of starch in the ECS was very fast compared
10 with that in flaked dry corn (Tada *et al.*, 2018). Therefore, as a result of low MUN in ECS
11 farms, NH₃-N capture by ruminal microbes proceeds via synchronization of CP
12 degradation of the pasture and carbohydrate degradation of ECS in the rumen.

13

14 **3.3 Milk fatty acid profile**

15 The average milk FA profile is shown in Table 4 (other FA profiles in Table S2).
16 Proportions of *de novo* FA, including C16 (even carbon number FA: C4-C16), were
17 higher in the ECS farms than in the non-ECS farms throughout the grazing season,
18 including July, in which ECS was not supplied ($P < 0.05$). In contrast, the *trans*-10 C18:1,
19 TVA, and CLA proportions were lower ($P < 0.05$), and the C18:0 and C20:0 proportions
20 tended to be lower ($P < 0.10$) in ECS farms than in non-ECS farms. The interaction effects
21 between ECS supply and month effect in C16:0, TVA, CLA, the sum of poly unsaturated
22 FA, and a mixed FA proportion were significant ($P < 0.05$), and that in the sum of mono-
23 unsaturated FA proportion was tendency ($P = 0.09$). Proportions of C16:0 and a mixed
24 FA (C16:0 + C16:1) for ECS farms increased after August (when ECS was supplied), but
25 those for non-ECS farms did not change much during the grazing season. In contrast,

1 TVA, CLA, and poly unsaturated FA proportion for ECS farms decreased after August
2 (supplying ECS), but those for non-ECS farms maintained high levels throughout the
3 grazing season. The differences in most FA between the ECS and non-ECS farms resulted
4 from a basic feeding management of each farm, which was grazing management, amounts
5 and types of concentrate and conserved forage before the start of study, because the
6 differences in most FA between ECS and non-ECS farms were continuous from July,
7 when ECS was not supplied for all farms. However, the substitution of ECS for the
8 commercial formula feed in the grazing season should affect the C16:0, TVA, and CLA
9 proportions.

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

25 The results of the factor analysis indicated that the milk FA profile is an indicator

1 of characteristics in each farm, because plots of each farm during the grazing (July to
2 October) and indoor feeding (December) periods closely distributed (Figure 1-B). The
3 plots of all farms distributed on the upper side during the grazing season (positive in factor
4 2), and the lower side in the indoor feeding period (negative in factor 2). For the grazing
5 season, the plots of farms D and E were distributed more on the upper side than those of
6 the other farms. These results also indicate that factor 2 is related to a dependency on
7 pasture intake. Most plots of farm D were in the first quadrant, and those of farm E were
8 in the second quadrant. Therefore, a feeding factor affected milk FAs related to factor 1.
9 However, it could not be clarified which aspects of feeding management affected factor
10 1 in the present study.

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

19

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

1 by grazing dairy farms, which closed to those in indoor feeding period.

2

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11

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1 **Figure legends**

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

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

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

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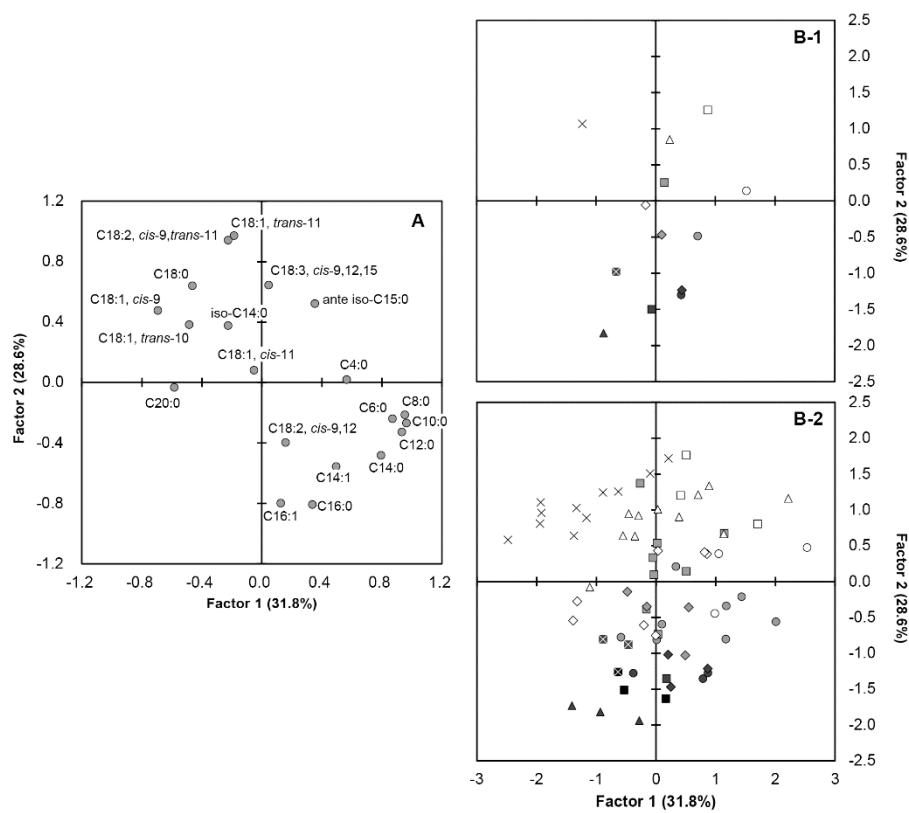


Figure 1

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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)

ECS or non-ECS	Farm ¹														
	A			B			C				D			E	
	Jul	Aug	Dec	Jul	Aug	Dec	Jul	Aug	Aug	Dec	Jul	Aug	Dec	Jul	Aug
	Oct	Oct	Oct	Oct	Oct	Oct	Oct	Oct	Oct	Oct	Oct	Oct	Oct	Oct	Oct
	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															
Forage	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
Corn Silage	0	0	0	0	0	0	7	0	2	34	0	0	0	0	38
Commercial formula feed	17	15	29	15	3	22	12	14	8	17	6	6	12	0	0
Ear corn silage	0	12	8	0	15	0	0	0	12	0	0	0	0	0	0
Others ²	12	12	15	16	13	16	18	20	16	20	12	13	22	19	21
Self-sufficiency rate	83	85	71	85	97	78	88	86	92	83	94	94	88	100	100

¹ 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.

² Feed of others was included sugar beet pulp, wheat, and rice bran.

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Table 2 . Averages of chemical compositions and fermentation 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-fibrous carbohydrate, WSC: water soluble carbohydrate, TDN: total digestible nutrients

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Table 3. Averages 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 ¹										Possibility ($P =$) ²			
	Jul.		Aug.		Sep.		Oct.		Dec.		SEM	ECS	Month	Int.
	ECS	Non-ECS	ECS	Non-ECS	ECS	Non-ECS	ECS	Non-ECS	ECS	Non-ECS				
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

¹ 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.

² ECS: ECS vs. non-ECS, Month: July, August, September, October, vs. December, Int.: interaction between ECS and Month

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Table 4. Averages of fatty acid methyl 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.		SEM	ECS	Month	Int.
	ECS	Non-ECS	ECS	Non-ECS	ECS	Non-ECS	ECS	Non-ECS	ECS	Non-ECS				
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, <i>trans</i> -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, <i>trans</i> -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, <i>cis</i> -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, <i>cis</i> -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, <i>cis</i> -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, <i>cis</i> -9, <i>trans</i> -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, <i>cis</i> -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 fermentation score of supplied feed

Feed	DM,%	Chemical compositions, % of DM ¹									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	-	2.17	11.2	88.1								

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

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Table S2. Averages of fatty acid methyl 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.		SEM	ECS	Month	Int.
	ECS	Non-ECS	ECS	Non-ECS	ECS	Non-ECS	ECS	Non-ECS	ECS	Non-ECS				
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