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Internal Medicine

Effects of feeding management on disease incidence and blood metabolites in dairy herds in Iwate Prefecture, Japan

Junro KATO[1,2), Tatsuya ODATE[2), Yo-Han KIM[3), Toshihiro ICHIJO[1,3) and Shigeru SATO[1,3)*

[1)United Graduate School of Veterinary Sciences, Gifu University, Gifu 501-1193, Japan
[2)Iwate Prefectural Federation of Agricultural Mutual Aid Association, Oshu, Iwate 023-0023, Japan
[3)Cooperative Department of Veterinary Medicine, Faculty of Agriculture, Iwate University, Morioka, Iwate 020-8550, Japan

ABSTRACT. The objective of the present study was to identify the effect of feeding management on disease incidence and blood metabolite levels in dairy herds in Iwate Prefecture, Japan. A generalized linear model approach was used to identify the risk factors for ketosis and displaced abomasum (DA) in dairy herds (n=30), and metabolic profile test (MPT) results were compared to verify the involvement of the factors. Consequently, the proportion of corn silage (CS) with ≥30% of dry matter (DM) fed to cows during the lactation period was confirmed as the most reliable risk factor for ketosis, while no risk factor was identified for DA. Meanwhile, the incidence rates of ketosis and DA were significantly ($P<0.05$) higher in the herds that were fed CS (n=20) than in those fed a non-CS diet (n=10). When the MPT results of the herds fed with CS containing ≥30% of DM (HCS group, n=4; 76 cows), with CS containing <30% of DM (LCS group, n=14; 285 cows), and a non-CS diet (NCS group, n=12; 236 cows) were compared, the HCS group showed higher beta-hydroxybutyric and lower blood urea nitrogen concentrations for until 49 days after parturition. Overall, feeding cows with CS diets containing over 30% of DM might increase their risk of developing negative energy and protein balances, thereby resulting in increasing incidences of ketosis in the Iwate Prefecture.

KEY WORDS: corn silage feeding, disease incidence, generalized linear model, metabolic profile test, negative energy balance

The incidence of production diseases, such as ketosis, displaced abomasum (DA), and mastitis, is closely associated with the feeding and management of dairy cows. Several risk factors have been identified as being involved in the development of these diseases, such as the barn style and herd size [39], increased parity [34, 46], length of the dry period and previous lactation period [46], and a higher 305-day milk yield during the previous lactation period [34]. The proportion of corn silage (CS) in the diet is also associated with the incidence of DA during the prepartum transition period in Holstein dairy cattle [38]. Furthermore, cows that are fed with only a CS diet are treated more frequently for ketosis [2, 45] and can exhibit an increase in the incidence of DA [2]. However, the effects of CS feeding during the lactation period on the incidence rates of diseases have not yet been completely determined.

CS is a common roughage diet that is used in dairy farms because of its high starch content and high yielding properties. The CS diet is largely composed of ruminally fermentable carbohydrates, and thus, CS diets have different rumen fermentation properties compared to other roughage diets like grass silage. Further, the ruminal pH decreases with increase in the proportion of CS when CS and alfalfa silage are fed together [5]. In addition, a CS diet on its own can induce production of higher concentration of total volatile fatty acids and butyric acid as well as lower acetate-to-propionate ratios and lower concentrations of ammonia nitrogen in the rumen [3, 28, 32]. Overall, CS diets can increase the level of rumen fermentation and improve energy status due to their highly fermentable properties. However, CS diets can increase the concentration of blood non-esterified fatty acids (NEFA) in dairy cows [2] and blood beta-hydroxybutyric (BHB) acid in steers [32]. These reports suggest that failures in the management of CS feeding can lead to disorders owing to the effects of ruminal CS fermentation on metabolic function. However, limited information exists regarding the characteristics of disease incidence in dairy herds that are fed a CS diet.

The objective of the present study is to identify the effects of feeding management on disease incidence and to evaluate whether

*Correspondence to: Sato, S.: sshigeru@iwate-u.ac.jp

CS feeding affects the disease incidence in dairy herds in Iwate Prefecture, Japan. In order to accomplish this, first, a generalized linear model approach was undertaken to identify the risk factors related to production diseases. Subsequently, the disease incidence was compared between herds that were fed CS and those fed non-CS (NCS) diets; furthermore, the results of metabolic profile tests (MPT) of the herds being fed the following diets were compared to determine the relationship between CS feeding and disease occurrence: CS with ≥30% of dry matter (DM), CS with <30% of DM, and an NCS diet.

MATERIALS AND METHODS

The animal care and experimental procedures were performed according to the Iwate University's guidelines for animal research.

Herd characteristics and disease incidence data

Disease incidence data associated with 30 dairy herds recorded between July 2017 to June 2018 in Iwate Prefecture of Japan were analyzed. It was possible only to extract the data of these herds accurately from the veterinary medical records, and data associated with herds with less than 25 dairy cows and herds with incomplete data were excluded from the present study. The included herds were used to compare the data of herds fed CS diets (CS herd, n=20) to those of herds fed NCS diets (NCS herd, n=10). Data on the occurrence of diseases, such as ketosis, DA, milk fever, mastitis, and metritis, recorded during the past year were obtained from veterinary medical records. The diagnosis of each of the studied diseases was based on the following clinical signs: ketosis was diagnosed when cows were observed to lose their appetites, especially when most of the grain was left and only small amounts of hay were eaten, accompanied with an acetone smell or with the detection of ketones in their urine [30]; milk fever was diagnosed when the cows suffered from muscular weakness, cold extremities, and recumbency and were highly responsive to intravenous calcium administration [46]; mastitis was detected during milking by the presence of clots in the milk or by inflammation of the udders [29]; and finally, metritis was diagnosed when cows developed fever with a fetid, watery, uterine discharge during the early postpartum period [40]. The disease incidence for each herd was calculated using the following formula:

Disease incidence (%)=(number of disease occurrences during the past year / total number of dairy cows) ×100

Data associated with 19 herds that were registered in the Japan Dairy Herd Recordings were extracted for a generalized linear model approach while the other unregistered 11 herds were excluded. The distribution of NCS (n=5) and CS (n=14) diets across the farms and the feeding characteristics are described in Table 1, and the herd information and milk production and composition data that were recorded from July 2017 to June 2018 are described in Table 2. The items presented in Table 1 were subjected to statistical analyses as categorical explanatory variables, and those outlined in Table 2, as continuous explanatory variables.

Table 1. The number of dairy herds divided by farm and feeding characteristics in the present study

| Herds[a] | Barn style[b] | | Feeding style[c] | | Lead feeding[d] | | CS feeding | | | | | |
| | | | | | | | ≥20% DM[e] | | ≥30% DM[f] | | During dry period | |
	TS	FS	SF	TMR	No	Yes	No	Yes	No	Yes	No	Yes
NCS group	5	0	4	1	0	5						
CS group	12	2	5	9	3	11	2	12	5	9	2	12

a) NCS group: Herds fed non-corn silage diets (n=5), CS group: Herds fed corn silage (CS) diets (n=14). b) TS: Tie stall, FS: Free stall. c) SF: Separate feeding, TMR: Total mixed ration. d) Increasing concentrates during the last 2 to 3 weeks prior to parturition. e) The proportion of CS in the diets: ≥20% of dry matter (DM). f) The proportion of CS in the diets: ≥30% of DM.

Table 2. Herd information and milk production and composition of dairy herds examined in the present study

| | NCS group[a] | | | CS group[b] | | | P-value |
	Median	25th percentile	75th percentile	Median	25th percentile	75th percentile	
Herd information							
Number of rearing dairy cows	62	38	72	44	34	60	0.49
Mean age	4.2	3.9	4.2	4.0	3.8	4.3	0.89
Mean parity	2.0	2.0	2.4	2.2	2.0	2.3	0.82
Mean calving interval, day	453	436	494	441	427	455	0.38
Mean dry period, day	60	58	64	57	53	59	0.46
Milk production and composition							
305-d Milk yield, ×10^3 kg	8.9	8.7	8.9	9.3	9.0	9.6	0.19
Standardized milk yield, kg/day	31.0	30.0	31.3	33.0	32.0	34.7	0.25
Milk fat, %	3.8	3.7	3.9	3.9	3.8	4.0	0.15
Milk protein, %	3.3	3.2	3.4	3.3	3.2	3.4	0.81

a) NCS group: Herds fed non-corn silage diets (n=5). b) CS group: Herds fed corn silage diets (n=14).

Metabolic profile tests

For the MPT data analysis, the data of 14 dairy herds that underwent the MPT from April 2010 to December 2015 in Iwate Prefecture of Japan were used. A total number of 30 MPTs were performed and the results associated with multiple practices conducted on the same farm were considered as different groups because the management and test cows differed depending on the implementation period. These were the only data that were arranged accurately in the present study, and they were associated with 236 Holstein-Friesian dairy cows from nine NCS-fed herds (NCS group, n=12) and 361 Holstein-Friesian dairy cows from five CS-fed herds, comprising of 76 cows fed CS diets with ≥30% of DM (HCS group, n=4) and 285 cows fed CS diets with <30% of DM (LCS group, n=14). The cows were divided based on the days in milk (DIM), as reported previously [24]. The number of cows in the NCS, LCS, and HCS groups in the dry, early (0–49 DIM), peak (50–109 DIM), middle (110–219 DIM), and late (220–365 DIM) lactation stages were 37, 53, and 12; 35, 36, and 15; 43, 50, and 17; 49, 68, and 17; and 72, 78, and 15, respectively.

To conduct the MPTs, 2–3 hr after morning feeding, blood samples were collected from the jugular vein into vacuum tubes with serum separation agents (SST^{TM}; BD, Tokyo, Japan), granular dipotassium ethylenediaminetetraacetic acid (K_2EDTA) (EDTA; TERUMO, Tokyo, Japan), and sodium fluoride (NaF; TERUMO), following which, the body condition score (BCS) was examined. All the procedures were performed carefully without causing the animals pain. The plasma samples from the EDTA and NaF tubes were separated by centrifugation at 2,207 ×g for 10 min at 4°C, and the concentrations of ammonia (NH_3) and lactic acid (LA) were analyzed, respectively. The SST^{TM} tubes were incubated at 37°C, and the serum was separated by centrifugation at 2,207 ×g for 15 min. Subsequently, within 1 hr of sampling, the plasma and serum components were analyzed using an automatic biochemical analyzer (Accute, TOSHIBA, Tokyo, Japan). The MPT components that were analyzed were as follows: glucose (Glu, HK-G6PDH), NEFA (ACS-ACOD), BHB (enzymatic cycling), acetoacetic acid (AcAc, enzymatic colorimetric), total cholesterol (T-Chol, enzymatic colorimetric), calcium (Ca, O-CPC), albumin (Alb, BCG), blood urea nitrogen (BUN, Urease-GLDH), aspartate aminotransferase (AST, IFCC), gamma glutamyl transferase (GGT, IFCC), NH_3 (enzymatic colorimetric), LA (enzymatic colorimetric), surface BCS [14], milk yield, milk fat, and milk protein. The data on milk production and composition were extracted from the most recent data from the Dairy Herd Recordings. Additionally, the total digestible nutrient (TDN) and crude protein (CP) sufficiency rates were calculated to meet the requirements for dairy cows in each lactation stage outlined by the Nutrient Requirements of Dairy Cattle guidelines (NRC) as reported previously [24].

Statistical analysis

All analyses were performed using EZR (Saitama Medical Centre, Jichi Medical University, Saitama, Japan) [22]. Significant differences in disease incidence between the CS and NCS groups were determined using the Mann-Whitney *U* test, and the Kruskal-Wallis test and *post hoc* Bonferroni test were used to evaluate differences in the MPT data among the three groups.

The generalized linear model approach in order to analyze the relationship between herd data and disease incidence was performed in three steps. First, in order to convert the continuous variables to categorical dependent variables, among the 19 herds, groups with disease incidence rates that were ≤the 25th percentile of the total 30 herds were defined as the low incidence group, and those with rates ≥the 75th percentile of the total, as the high incidence group. Second, all the single explanatory variables described in Table 1 and Table 2 were screened using the Fisher's exact test. Only variables that were at *P*≤0.15 were considered for further analysis. Finally, a backward stepwise selection (*P*<0.05 for retention) of variables were performed in the logistic regression analysis. An adequate fit for the model was estimated by the likelihood ratio test (Pr>[Chi]). Correlations between explanatory variables were evaluated using the Spearman's correlation coefficient, and the selection of the variables was performed according to a previous report [46].

All data are median values. Differences were considered significant at *P*<0.05.

RESULTS

Risk factors related to disease incidence

While analyzing the risk factors for ketosis, as the first step, three herds from the NCS herd were assigned to the low incidence (the percentage of disease incidence=0) group, and four and six herds from the CS herd were assigned into the low and high incidence (the percentage of disease incidence ≥4.1) groups, respectively. In the second step of the analysis, three variables were identified to be related to the ketosis incidence in the regression model: the proportion of CS in the diets with ≥30% of DM (*P*=0.10), mean age of the animals (*P*=0.09), and mean parity (*P*=0.02). Because strong correlations were observed between the mean age and mean parity (r=0.85, *P*<0.01), the mean parity was selected for further analysis. In the final step of the analysis, the logistic regression model that included the proportion of CS in the diets with ≥30% of DM (Odds ratio (OR)=30.5, 95% confidence interval (95% CI)=1.3–693, *P*=0.03) and mean parity (OR=0.1, 95% CI=0.00002–311, *P*=0.55) was produced (P r>[Chi]=0.03), and the proportion of CS in the diets with ≥30% of DM was identified as a significant risk factor for developing clinical ketosis (OR=30.0, 95% CI=1.5–612, *P*=0.03).

The risk factor analysis for DA was performed in the same manner. Consequently, four herds from the NCS herd were assigned to the low incidence (the percentage of disease incidence=0) group, and four and three herds from the CS herd were assigned to the low and high DA incidence (the percentage of disease incidence ≥2.9) groups, respectively. Furthermore, the proportion of CS in the diets with ≥20% of DM (*P*=0.15), CS feeding during dry (*P*=0.15) and lactation periods (*P*=0.09), feeding style (*P*=0.06), and number of rearing dairy cows (*P*=0.11) were associated with DA incidence. Because strong correlations were observed among the CS feeding categories (r>0.78, *P*<0.05), CS feeding during the lactation period was selected for further analysis. However,
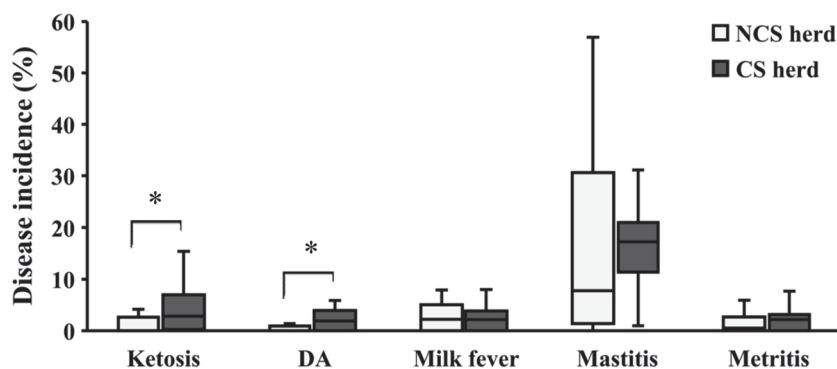
Fig. 1. Box plots representing the disease incidence in the dairy herds fed non-corn silage (CS) (NCS herd, n=10) or CS (CS herd, n=20) diets. Median and quartiles are displayed in the box. Upper and lower bars represent maximum and minimum values, respectively. *Significant differences ($P<0.05$) between the NCS and CS herds. DA; displaced abomasum.

the logistic regression model (Pr>[Chi]=0.10) did not demonstrate that the following data were adequate fits: CS feeding during lactation periods (OR=1.5 × 108, 95% CI=0.0-infimum, $P=1.00$), feeding style (OR=7.5, 95% CI=0.1–329, $P=0.30$), and number of rearing dairy cows (OR=0.1, 95% CI=0.9–1.1, $P=0.86$).

In the analysis of risk factors for milk fever, mastitis, and metritis, all the variables analyzed in the present study were not selected in the second step of analysis and failed to fit the logistic regression model.

Disease incidence

The incidence rates of each disease in the CS and NCS herds are presented in Fig. 1. The incidence rates of ketosis and DA were significantly (both $P<0.05$) higher in the CS herd compared to in the NCS herd. However, the incidence of other diseases did not significantly differ between the CS and NCS herds.

MPT data

The blood Glu concentration in the HCS group was significantly ($P<0.05$) higher than that of the NCS group during the dry period, and it was significantly ($P<0.05$) higher than those of the NCS and the LCS groups during the middle and late lactation stages, respectively (Fig. 2). The BHB concentration in the HCS group was significantly higher than that of the NCS group during the early, middle, and late lactation stages ($P<0.01$, $P<0.01$, and $P<0.05$, respectively) and was significantly ($P<0.05$) higher than that of the LCS group during the middle stage (Fig. 2). The BHB concentration was also significantly ($P<0.01$) higher in the LCS group compared to in the NCS group during the early lactation stage. The AcAc concentration in the HCS group was significantly ($P<0.05$) higher and tended ($P=0.06$) to be higher compared to that of the NCS group during the middle and early lactation stages, respectively (Fig. 2).

Further, the Alb concentration was significantly lower in the LCS group compared to in the NCS and HCS groups ($P<0.01$ and $P<0.05$, respectively) during the peak lactation stage (Fig. 3). Additionally, the BUN concentration was significantly ($P<0.05$) lower in the HCS group than in the NCS group during the early and middle stages and was significantly ($P<0.01$) lower in the LCS group than in the NCS group during the late stage (Fig. 3).

Furthermore, the AST concentration was significantly ($P<0.01$) higher in the HCS group than in the LCS group during the middle lactation stage and was significantly lower in the LCS group than in the NCS group during the early, middle, and late lactation stages ($P<0.05$, $P<0.01$, and $P<0.01$, respectively) (Fig. 4). The GGT activity was significantly ($P<0.05$) higher in the HCS group than in the LCS group during the peak stage (Fig. 4). The NH_3 concentrations in the HCS and LCS groups were significantly (both $P<0.01$) higher than those in the NCS group during the peak and late lactation stages, and the LA concentration was significantly ($P<0.01$) higher in the HCS group than in the NCS group during the late stage (Fig. 4).

Lastly, the percentage of milk protein was significantly ($P<0.05$) lower in the HCS group than in the LCS group during the middle lactation stage (Fig. 5). However, no significant differences were observed among the groups in the NEFA levels, T-Chol and Ca concentrations, BCS scores, milk yield, and the percentage of milk fat. Additionally, the TDN and CP sufficiency rates were not different among the groups during each lactation stage (Fig. 6).

DISCUSSION

Whole-crop corn is high in starch and is easily fermented to silage that remains stable for long periods [27, 48]. In addition, corn yields are higher compared to those of other roughage [6]. Therefore, corn silage is one of the most important forages used to feed dairy cows in the Iwate Prefecture of Japan, similar to in Europe and North America [5, 28]. However, several studies have documented the development of disorders that affect cows that are solely fed CS diets. For example, Thomas *et al*. [45] reported
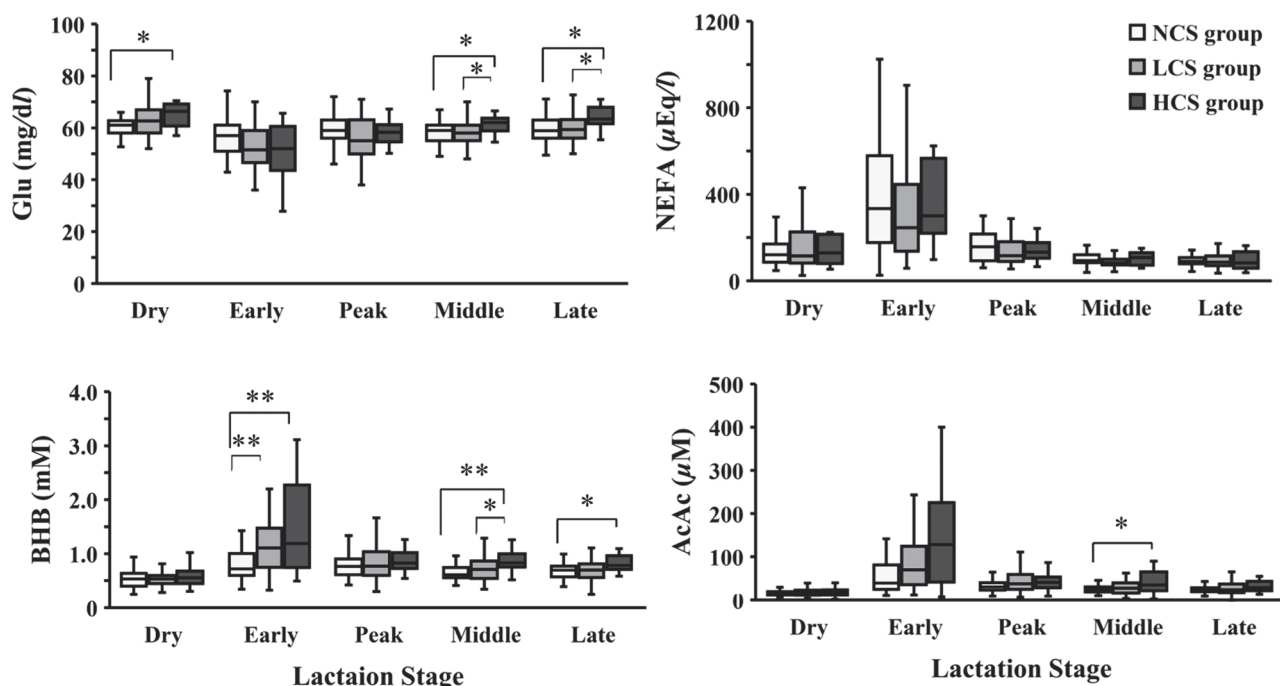
Fig. 2. Box plots representing the blood glucose (Glu), non-esterified fatty acid (NEFA), beta-hydroxybutyric acid (BHB), and acetoacetic acid (AcAc) levels in the herds fed non-corn silage (NCS group, n=12), CS with <30% of dry matter (DM) (LCS group, n=14), and CS with ≥30% of DM (HCS group, n=4) diets. The number of cows in the NCS, LCS, and HCS groups in the dry, early (0–49 days in milk [DIM]), peak (50-109 DIM), middle (110-219 DIM), and late (220-365 DIM) lactation stages were 37, 53, and 12; 35, 36, and 15; 43, 50, and 17; 49, 68, and 17; and 72, 78, and 15, respectively. Median and quartiles are displayed in the box. Upper and lower bars represent maximum and minimum values, respectively. **, *Significant differences (*P*<0.01 and *P*<0.05, respectively) between the two groups.
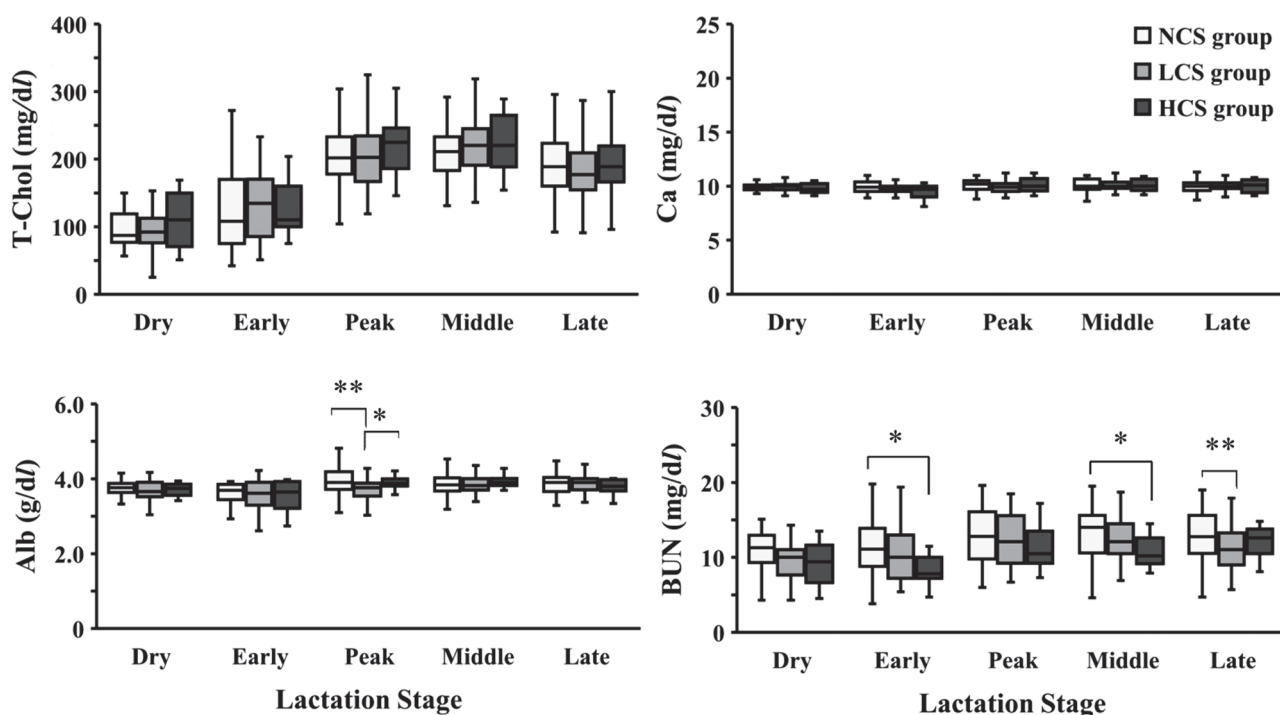

Fig. 3. Box plots representing the blood total cholesterol (T-Chol), calcium (Ca), albumin (Alb), and urea nitrogen (BUN) concentrations in the herds fed non-corn silage (NCS group, n=12), CS with <30% of dry matter (DM) (LCS group, n=14), and CS with ≥30% of DM (HCS group, n=4) diets. The number of cows in the three groups are the same as in Fig. 2. Median and quartiles are displayed in the box. Upper and lower bars represent maximum and minimum values, respectively. **, *Significant differences (*P*<0.01 and *P*<0.05, respectively) between the two groups.
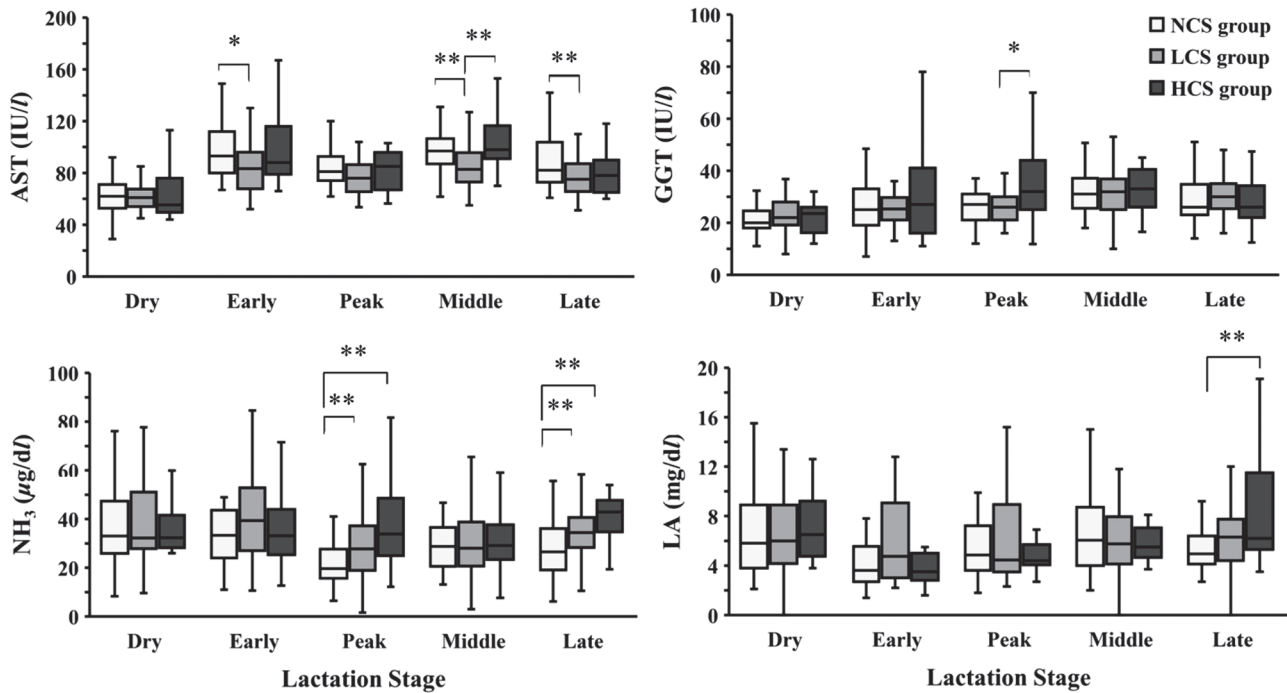
Fig. 4. Box plots representing the blood aspartate aminotransferase (AST), gamma glutamyl transferase (GGT), ammonia (NH_3), and lactic acid (LA) levels in the herds fed non-corn silage (NCS group, n=12), CS with <30% of dry matter (DM) (LCS group, n=14), and CS with ≥30% of DM (HCS group, n=4) diets. For the AST and GGT levels, the number of cows in the three groups are the same as in Fig. 2. For the NH_3 and LA levels, the number of cows were 35, 49, and 10; 33, 30, and 15; 42, 46, and 17; 46, 63, and 17; and 68, 75, and 14, respectively. Median and quartiles are displayed in the box. Upper and lower bars represent maximum and minimum values, respectively. **, *Significant differences ($P<0.01$ and $P<0.05$, respectively) between the two groups.
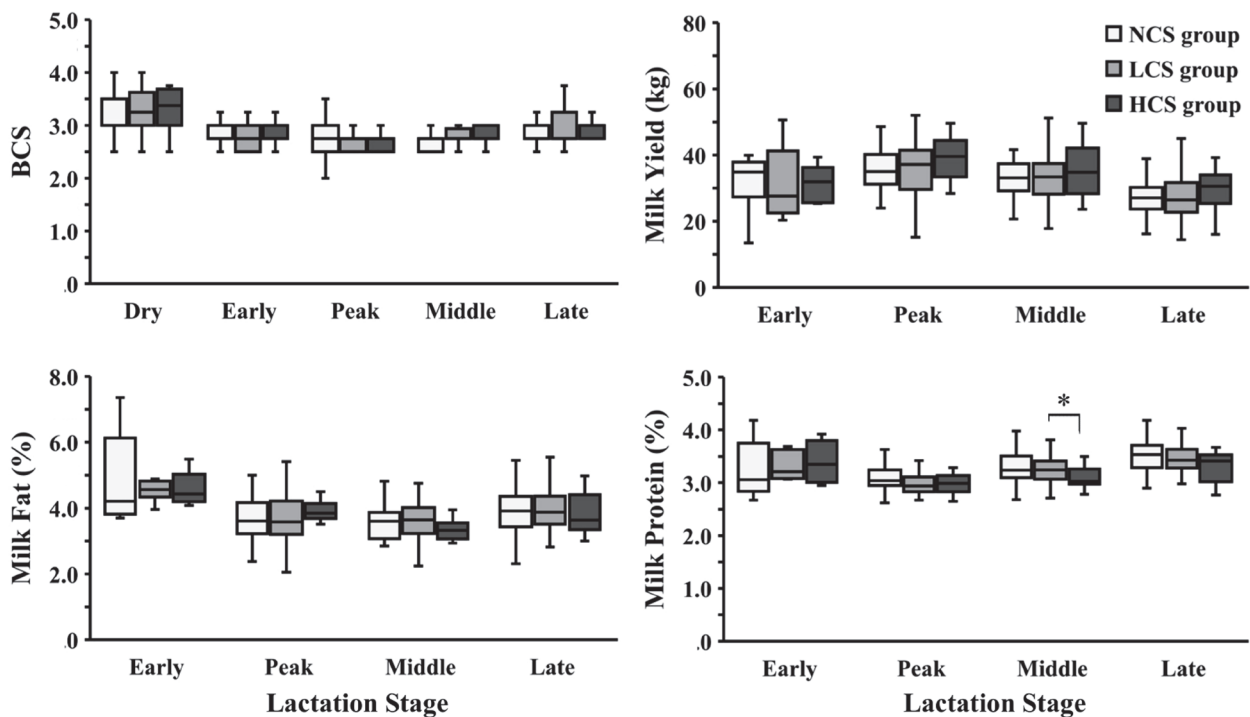

Fig. 5. Box plots representing the body condition scores (BCS), milk yield, and proportions of milk fat and milk protein of the herds fed non-corn silage (NCS group, n=12), CS with <30% of dry matter (DM) (LCS group, n=14), and CS with ≥30% of DM (HCS group, n=4) diets. For the BCS, the number of cows in the three groups are the same as in Fig. 2. For the milk yield and composition, which exclude the dry period, the number of cows in the three groups were 9, 8, and 5; 41, 47, and 15; 39, 66, and 17; and 62, 77, and 15, respectively. Median and quartiles are displayed in the box. Upper and lower bars represent maximum and minimum values, respectively. *Significant differences ($P<0.05$) between the two groups.
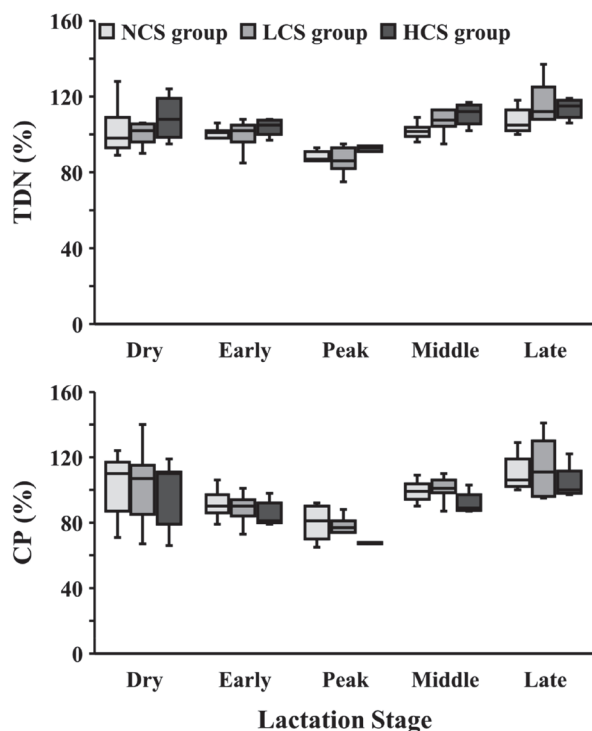
Fig. 6. Box plots representing the total digestible nutrient (TDN) and crude protein (CP) sufficiency rates to meet the Nutrient Requirements of Dairy Cattle guidelines (NRC) for dairy cows in the herds fed non-corn silage (NCS group, n=12), CS with <30% of dry matter (DM) (LCS group, n=14), and CS with ≥30% of DM (HCS group, n=4) diets. Median and quartiles are displayed in the box. Upper and lower bars represent maximum and minimum values, respectively.

that dairy cows that are solely fed a CS diet as roughage were treated more frequently for ketosis compared to cows that are fed only alfalfa hay. Belyea *et al*. [2] also documented increases in the incidences of ketosis and DA when multiparous dairy cows were fed roughage including only a CS diet (≥60% of DM in the total diets) in comparison with cows that were fed roughage consisting of both hay and CS (approximately 30% of DM) diets. However, these experiments involved excessive proportions of CS in the diet, which may not necessarily correspond to the diets used on commercial dairy farms. Nonetheless, Coppock [10] indicated that increased amounts of CS feeding during the prepartum transition period was a risk factor for the development of DA although the effects of the CS diet on metabolic diseases during various lactation stages was not discussed. Therefore, information concerning the characteristics of disease incidence in dairy herds that are fed CS diets is limited.

In the present study, the incidence rates of ketosis and DA were higher in the CS herd compared to in the NCS herd, which was similar to the results observed in the case of cows that were experimentally overfed a CS diet [2, 45]. In the analysis of the ketosis risk factors using Fisher's exact tests, among the herd data, mean parity was selected as a risk factor, which corresponds to findings of previous reports [34, 46]. Via a backward stepwise selection performed during the logistic regression analysis, the proportion of CS in the diets with ≥30% of DM was also selected and identified as the most important and reliable risk factor for developing clinical ketosis. These results indicate that an increase in the proportion of CS in the diet, especially with ≥30% of DM, is a major risk factor for the development of ketosis in dairy herds in Iwate Prefecture. Moreover, previous studies also identified risk factors for ketosis, including an increase in the previous 305-day milk yield, decreases in mean milk protein levels, a longer dry period, and an increase in the length of the previous lactation period [31, 34, 46]. One reason why these factors were not significant in the present study is that herd data, and not the

data associated with individual cows, were used; further, only the incidences of clinical ketosis recorded in medical records was considered in the present study.

Concerning the analysis of the risk factors for DA via Fisher's exact tests, the proportion of CS in the diets with ≥20% of DM, CS feeding during dry and lactation periods, the feeding style, and the number of rearing dairy cows were selected as risk factors. However, these variables failed to fit the logistic regression model. An increase in the proportion of CS feeding during the prepartum transition period has previously been identified as a risk factor for the development of DA [10, 38] in cases in which the cows were fed a CS diet with approximately >50% of DM. The association of CS feeding during the dry period with the incidence of DA was unclear in the present study because the proportions of CS feeding during dry periods in the present study approximately included ≤20% of DM, which may not be a risk factor for DA. Furthermore, despite the larger herd sizes, lower parity, failure in lead feeding, and high maximum daily milk yield that have also previously been reported as being risk factors for DA [7, 38, 42], these relationships may not have been strong in the present study due to our use of herd- and not individual cow-level data.

The incidence of mastitis in the present study was not significantly related to CS feeding and the other variables we examined via the generalized linear model approach. Although a previous study has identified that CS feeding during the lactation period is a risk factor for the development of clinical mastitis due to *Escherichia coli* infections [1], the influence of pathogenic bacteria was not verified in the present study. Therefore, further research is needed to clarify the relationships that exist among the incidence of mastitis, influence of a classified pathogen, and CS feeding. Previous studies have also identified an increased previous 305-day milk yield, an increased parity, and a decreased length of lactation as risk factors for mastitis [4, 18]. Furthermore, management practices that are associated with high incidence rates of clinical mastitis differed due to the effects of each species of pathogenic bacteria [1] and by the individual quarter and cow [4]. The increased exposure to environmental microorganisms due to factors, such as poor cubicle cleanliness, the use of rubber mats in cubicles, and drinking water from contaminated sources, were also identified as risk factors for mastitis [37]. In addition, factors that were not considered in the present study, such as host, environment, and pathogen characteristics and the milking hygiene, may also be related to the occurrence of mastitis; the fact that we did not include these potential moderating factors could explain why no significant factors related to mastitis were identified in the present study.

An MPT is used to assess the nutritional intake and metabolic function in dairy herds by examining the blood characteristics and metabolism of cows during each lactation stage [23, 24]. Further, an MPT serves as a useful approach for assessing feeding management, detecting periparturient diseases in dairy cows [20, 23], and evaluating their auxiliary milk production and feeding [24]; hence, MPTs were performed in the present study. Via the analysis of the MPT results, dairy cows in the HCS and LCS groups were found to exhibit higher concentrations of BHB during the early lactation stage: the median BHB concentrations were 1.2 mM (25th percentile: 0.8 mM, 75th percentile: 2.2 mM) and 1.1 mM (25th percentile: 0.8 mM, 75th percentile: 1.4 mM), respectively. In addition, the AcAc concentration in the HCS group, the median value of which was 129 μM (25th percentile: 48 μM, 75th percentile: 220 μM), tended to be higher than that of the NCS group during the early lactation stage. Subclinical ketosis is defined as a blood BHB level of 1.2–1.4 mM [13, 31] or a blood AcAc level of 125 μM [15] in the absence of clinical signs. Moreover, Suthar *et al*. [44] reported that cows with blood BHB levels above 1.1 mM in the early lactation stage had a 10.5-fold higher risk of developing clinical ketosis. Similarly, Duffield *et al*. [13] indicated that BHB levels of above 1.4 mM increased the risk of developing diseases and resulted in a substantial loss of milk yield. Our results indicated that dairy cows being fed a CS diet, especially with ≥30% of DM, were at an increased risk of developing negative energy balance and tended to exhibit hyperketonemia during the early lactation stage compared to cows that were fed an NCS diet. Although a previous study reported elevated blood NEFA levels under conditions of negative energy balance [16], no significant differences in NEFA levels were observed among the groups in the present study, and a time lag may have occurred between the elevations in the BHB and NEFA levels, as reported previously [47].

In the present study, it remains unclear why the cows in the herds that were fed a CS diet were at an increased risk of developing negative energy balance during the early lactation stages, given that the dietary nutrient composition did not differ among the groups. The reason behind this might be that the energy requirements of the cows were not satisfied in a practical way with CS feeding during the early lactation stage. For example, the quality and digestibility of CS depends on the maturity of the corn, hybrid type [33], microbial inoculation during ensiling [27], particle size [25, 26], kernel processing [9], and the presence of mycotoxins [12, 27]. In addition, a combination of CS and other forage can either improve or worsen milk production, apparent digestibility, and ruminal metabolism [5, 25]. However, these were not assessed in this study. On the other hand, Janovick *et al*. [21] reported that cows that were overfed with energy-rich diets during late gestation experienced hyperglycemia, hyperinsulinemia, and greater levels of circulating leptin prepartum, followed by a period of more severe hyperlipidemia and higher levels of ketone production due to the aggravation of insulin resistance in the adipose tissue postpartum. In the present study, higher Glu concentrations were observed in the HCS group during the dry, middle, and late lactation stages. This suggested that the cows in the HCS group were overfed during these stages and that negative energy balance might have occurred in the cows of the same group during the early lactation stage. Furthermore, the higher concentrations of BHB and AcAc and lower percentage of milk protein in the HCS group during the middle lactation stage might be a result of hyperglycemia and aggravation of insulin resistance caused by an imbalance between the energy supply and intake, as was previously reported to occur in the case of primiparous Holstein cows during the dry period [21]. Differences could easily occur between the expected and actual digestibility of a CS diet, thereby causing negative energy balance or overconsumption in herds that are fed such a diet. Further studies should better explore these characteristics of CS in order to tailor CS diets to specific circumstances.

In the present study, the BUN concentration in the HCS group was lower during the early and middle lactation stages, and in the LCS group, it was lower during the late stages compared to in the NCS group. Previous studies have reported that dairy cows that were solely fed a CS diet exhibited decreases in BUN concentrations [32]. Because BUN concentrations reflect the NH_3 levels and the status of microbial utilization of NH_3 in the rumen [24], overfeeding the animals with CS, which consists of high starch and low protein levels, is expected to lower BUN levels. In the present study, the dietary CP levels did not differ among the groups, primarily owing to soybean feeding that was used to control the CP levels in the CS groups. However, Hassanat *et al*. [19] indicated that because ruminal solubility and degradability properties of soybean protein are less effective compared to those of the protein from alfalfa silage, replacing alfalfa silage with a CS diet that includes soybeans reduces the concentration of NH_3 in the rumen. Therefore, the low BUN levels in the HCS group observed during the early and middle lactation stages and in the LCS group during the late stage suggest that the animals' protein requirements were not fully met and that the cows were in a negative protein balance even though the dietary CP levels were adjusted using soybeans. Although the BUN concentrations in the CS groups were lower, the NH_3 concentrations recorded during the peak and late lactation stages were higher compared to those of the NCS group. Because the main sources of blood NH_3 in dairy cows are bacterial activity in the rumen and the catabolism of amino acids in the tissue, CS diets are expected to contribute to lowering the blood NH_3 and BUN levels. A previous study suggested that triglyceride accumulation in the liver decreased the conversion of NH_3 to urea and resulted in an increased blood NH_3 concentration without an increase in BUN levels in dairy cows [49]. However, we could not generate a coherent result associated with the peripheral AST levels that corresponded with the ratio of liver lipid accumulation [8] in the CS group, and thus, further studies are required to reveal the influence of CS diet feeding on the blood fermentation parameters and on the liver functions simultaneously.

Blood AST serves as a sensitive marker of liver damage and is often used as a proxy to investigate liver function [8, 41]. Meanwhile, the relationship between blood BHB and AST levels remains unclear. Deeb *et al*. [11] showed that the blood AST levels of ketotic cows were higher than those of healthy cows; contrarily, some reports have indicated that the AST levels are not associated with the blood BHB levels or with ketosis [17, 41]. In the present study, cows that were fed CS may have been under negative energy balance without severe hepatic lipidosis during the early lactation stage. However, the reason for lower AST values in the LCS group in any of the lactation stages was not determined.

Previously, several studies reported that ketotic cows under negative energy balance have an increased risk of developing DA and mastitis. For example, serum BHB concentrations of over 1.2 mM in the first week after calving were associated with an increased risk of DA (odds ratio=2.60) [13], and BHB concentrations of over 1.7 mM, compared to lower BHB blood levels, were associated with a 6.9-fold higher risk of developing DA [44]. Moreover, the immunodepression of postpartum cows under negative energy balance can also affect the occurrence rates of metritis and mastitis. Sartorelli *et al.* [35] reported that the administration of BHB to ovines lead to a decrease in their neutrophil bactericidal activity *in vitro*, and Sato *et al.* [36] indicated that increased serum BHB concentrations in ketotic cows suppressed lymphocyte blastogenesis. Ster *et al.* [43] demonstrated that adding NEFA to peripheral blood mononuclear cells and polymorphonuclear leukocytes impaired proliferation, the release of interferon-γ, phagocytosis, and oxidative burst *in vitro*. In addition, Goff [16] determined that not only a negative energy balance but also a negative protein balance induces ketosis, fatty liver, DA, and immune suppression. Collectively, these results suggested that the cows fed diets of CS with ≥30% of DM were at an increased risk of developing negative energy and protein balances during the perinatal period, which simultaneously increased the incidence of ketosis with that of DA and might be related to clinical mastitis as reported previously [1].

In conclusion, it was suggested that feeding cows with diets comprising of CS with ≥30% of DM was a major risk factor for ketosis in Iwate Prefecture, Japan. One of the reasons behind this might be the differences between the expected and actual digestibility of a CS diet, which made it difficult for the cows to receive proper nutrition and lead to the development of negative energy and protein balances. However, because the data involved in this study was limited, further studies are required in order to verify the relationships between CS feeding and disease incidence in dairy herds. Nevertheless, it might be necessary to understand the characteristics associated with the CS diet so that feeding strategies can be tailored to specific circumstances in order to prevent negative nutrient balance.

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