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Estimations of prepartum feed intake and its effects on transition metabolism and subsequent milk production

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ABSTRACT

The objectives of this study were to identify factors associated with prepartum DMI, evaluate the performance of linear models to estimate prepartum DMI using different classes of predictors, and investigate the consequences of different levels of prepartum DMI on transition metabolism and lactation performance. Individual feed intake of nulliparous ($n = 100$) and parous cows ($n = 173$) was measured by automatic feeding bins from d -35 to 98 relative to calving. Rumination and physical activities were monitored by wearable sensors. Blood metabolites were measured on d -21 , -10 , -3 , 0 , 3 , 7 , 10 , 14 , and 21 . Body weight (BW) and body condition score (BCS) were assessed throughout the study. The average prepartum DMI as percentage of BW (DMIpBW) was calculated for each cow and used as dependent variable of linear models. Parity, prepartum BCS and BW, milk production in the previous lactation (M305) and at dry-off (MYDO), and length of the dry period were associated with DMIpBW and explained 41% of the variation in all cows, and 49% in parous cows. Estimations of DMIpBW were improved when data on prepartum rumination and blood metabolites were added in the predictive models. In the latter, the adjusted R-Sq increased to values between 47 and 61%, and selected models performed consistently in a 5-fold cross-validation analysis. To evaluate the implications of DMIpBW to transition metabolism and performance, cows were ranked within parity and classified into terciles as low (LFI), moderate (MFI), or high feed intake (HFI). The mean DMI was 1.44, 1.70, and $1.91 \pm 0.01\%$ of BW, respectively. No differences in BW were observed in nulliparous cows, but all 3 groups of parous cows differed (LFI = 892, MFI = 849, HFI = 798 ± 8 kg). The proportion of cows with BCS > 3.5 at enrollment differed among all groups, and averaged 67.4, 55.1, and $36.5 \pm 6\%$, respectively. For parous cows, M305 and

MYDO differed among all groups and averaged 9,808, 10,457, and $11,182 \pm 233$ kg, and 18.1, 23.1, and 26.2 ± 1 kg/d, respectively. After calving, DMI (LFI = 20.9, MFI = 21.9, and HFI = 22.1 ± 0.2 kg/d) and milk yield (LFI = 36.7, MFI = 38.2, and HFI = 38.3 ± 0.4 kg/d) was lower in LFI cows compared with the other 2 groups. Postpartum EBAL differed among all groups and averaged -2.79 , -1.63 , and -0.66 ± 0.3 Mcal/d for LFI, MFI, and HFI, respectively. During the transition period, LFI cows had higher serum concentrations of NEFA, BHB, Cl (prepartum only), and AST (postpartum only), and lower serum concentrations of cholesterol, P, GLDH, GGT (prepartum only), AST (prepartum only), urea (parous only), and SOD activity (parous only). In conclusion, a low level of prepartum DMI was associated with fatter and heavier cows, lower milk production in previous lactation, important adjustments in energy metabolism, and moderate losses in DMI and milk yield in the subsequent lactation. Moreover, the inclusion of prepartum rumination activity and target blood metabolites into predictive models improved the estimations of prepartum DMI.

Key words: feeding behavior, prediction, transition period, dry period

INTRODUCTION

Most dairy cows experience a certain degree of feed intake reduction in the last weeks of pregnancy. The resulting suppression in energy intake summed to increased energy requirements to support the final stages of fetal growth and onset of lactation lead cows to negative energy and nutrient balances (Grummer et al., 2004; Santos et al., 2010). Previous research has shown negative associations between reduced prepartum dry matter intake (DMI) and immunity and postpartum health outcomes of dairy cows. Hammon (2006) and collaborators observed that cows within the lowest quartile of prepartum DMI had decreased neutrophil function beginning in the week before parturition, which remained impaired until the third week of lactation. They also showed that cows diagnosed with puerperal metritis or subclinical endometritis had a lower prepartum DMI in comparison to healthy

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The list of standard abbreviations for JDS is available at adsa.org/jds-abbreviations-24. Nonstandard abbreviations are available in the Notes.

cows. Huzzey et al. (2007) described that, for every 1 kg reduction in DMI in the week before calving, cows are approximately 3 times more likely to be subsequently diagnosed with metritis. Each 0.1% decrease in DMI (as % of BW) during the last 3 d preceding calving increased by 8% the odds of cows to develop metritis (Pérez-Báez et al., 2019a) and by 10% the odds of cows to develop mastitis (Pérez-Báez et al., 2019b). In addition, reduced prepartum feed intake was associated with an increased likelihood of metabolic and digestive disorders in the early postpartum such as fatty liver (Bertics et al., 1992), ketosis (Pérez-Báez et al., 2019b), indigestion, and displaced abomasum (Pérez-Báez et al., 2021). The associations between prepartum feed intake and subsequent milk production outcomes also have been explored, but results are inconsistent (Bertics et al., 1992; Olsson et al., 1998; Agenäs et al., 2003; Roche, 2007; Winkelman et al., 2008).

The main causes of variability in prepartum DMI are still largely unknown, which complicates the ability to estimate individual DMI values (Hayirli et al., 2002; Pascottini et al., 2020; NASEM, 2021). Moreover, many studies have focused on the decline in feed intake in the week preceding calving rather than the overall level of feed intake during the prepartum period. Thus, further research exploring potential factors associated with variation in prepartum feed intake and its consequences to transition cow biology is needed. Our objectives were to identify factors associated with prepartum DMI, evaluate the performance of linear models to estimate prepartum DMI using different classes of predictors, and investigate the consequences of different levels of prepartum DMI on transition metabolism and lactation performance. We hypothesized that cows with reduced levels of prepartum DMI would have impaired transition metabolism and subsequent milk production. In addition, we hypothesize that previous milk production, prepartum BCS, BW, wearable sensor information on rumination and physical activities, and target blood metabolites would be associated with prepartum DMI and individually improve the performance of predictive models estimating the average DMI during the prepartum period.

MATERIALS AND METHODS

This retrospective cohort study used data from a randomized controlled study evaluating the effects of 2 forms of supplementary trace minerals on health and performance of dairy cows (Mion et al., 2022; 2023), and used a convenience sample size of 100 nulliparous and 173 multiparous Holstein cows. All research methods were approved by the Animal Care Committee of the University of Guelph (Protocol #4064).

Animals, Housing, and Management

This study was conducted at the Ontario Dairy Research Centre (Elora, ON, Canada). Cows were enrolled 45 ± 3 d before the expected calving date and followed through 98 DIM. They were housed in a freestall barn equipped with mattress beds covered with chopped wheat straw, and automatic feed bins (Insentec B. V.) for evaluation of feed intake and feeding behavior. The prepartum TMR was identical for all cows, except for the source of trace minerals (Se, Co, Cu, Mn, and Zn), which was either in an organic or an inorganic form. The diet was delivered once a day, and the amount offered was adjusted daily to allow approximately 8% of refusals. Two days before the expected calving date, or when demonstrating signs of calving, cows were moved to maternity box stalls with chopped wheat straw bedding, where they were housed individually until 7 DIM. In the maternity stalls, diet was delivered using a feed cart equipped with an electronic scale (Super Data Ranger, American Calan) to measure the amount offered. Refusals were weighed daily and used to calculate daily feed intake. Postpartum freestall pens were also equipped with automatic feed bins and mattress beds covered with chopped wheat straw. All lactating cows received the same postpartum TMR, except for the source of trace minerals, and it was delivered twice a day. Feed samples of both pre- and postpartum TMRs were collected weekly to determine the dry matter content and used to create monthly composites for analyses of chemical composition. Ingredients and chemical composition of pre- and postpartum diets are described by Mion et al. (2022).

Body weight was measured weekly during the prepartum period and daily in the postpartum period using a walkthrough scale (DeLaval). Lactating cows were milked twice daily, initially using a portable bucket milking system (DeLaval) in the maternity pens, and then in a rotary parlor (DeLaval) when cows were moved to the postpartum freestall pens. Both milking systems had milk meters that automatically recorded milk production at each milking. Daily milk yields were retrieved from the farm management software until 98 DIM and summarized by week, 1 to 14 relative to calving. Milk composition was evaluated once a month at a DHIA testing laboratory (Lactanet, Guelph, ON, Canada). Energy-corrected milk was calculated using the following equation: $\text{ECM} = [(0.327 \times \text{kg of milk}) + (12.95 \times \text{kg of milk fat}) + (7.20 \times \text{kg of milk protein})]$. Energy balance (EBAL) was estimated using equations provided in NRC (2001). In addition to energy intake and net energy used for maintenance, prepartum EBAL considered the net energy requirements for pregnancy, and postpartum EBAL considered the net energy requirements for milk production. Body condition score was assessed visually

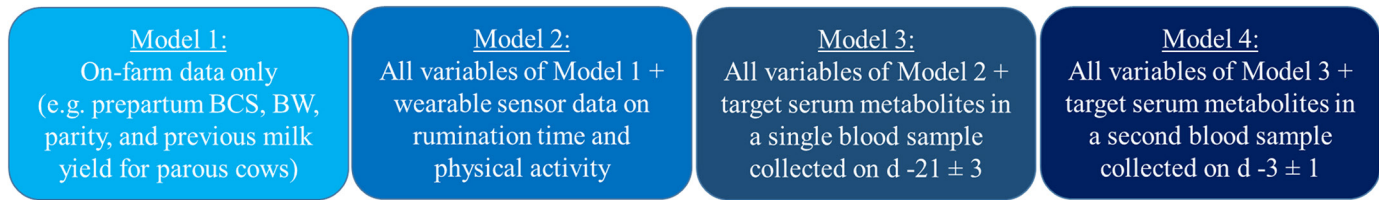


Figure 1. Design of 4 linear regression models built to explain the variation in average feed intake as percentage of body weight in the last 5 wk of pregnancy (DMI_pBW). From model 1 to 4, groups of independent variables were added to evaluate the gain in explanation of the variability of the dependent variable DMI_pBW. Model 1 used only on-farm information. Model 2 considered all variables of Model 1 and the information collected from wearable sensors on rumination and physical movement activities. Model 3 considered all variables of Model 2, in addition to concentrations of blood metabolites measured on d -21 ± 3. The metabolites considered were BHB, NEFA, cholesterol, total protein, albumin, urea, Ca, P, Mg, Cl, haptoglobin, AST, GGT, GLDH, GPx, SOD, and FRAP. Model 4 included all variables of Model 3 and the blood metabolites measured on d -3 ± 2, the change in their concentration from d -21 to d -3, and the average of the 2 measurements. For all models, only variables that were significant ($P \leq 0.05$) in a univariable model were included in the multivariable model. When 2 highly correlated variables were eligible for a multivariable model, only the one with the highest effect was included to avoid collinearity problems.

on d -45, -21, 3, 23, 35, and 65 ± 3 relative to calving using a 1–5 scale with 0.25 increments (Ferguson et al., 1994).

Experimental Design

Dry matter intake information was summarized weekly as the average daily feed intake on that week (kg of DMI/d). For the prepartum period specifically, weeks were determined retrospectively according to the date of calving (study d 0). Week -1 comprised d -1 to -7 relative to calving, week -2 comprised d -8 to -14 relative to calving, and so on. Dry matter intake as percentage of BW (DMI_pBW) was calculated for each cow dividing the weekly average of daily DMI by the cow's BW of the respective week. The average DMI_pBW in the last 5 wk of pregnancy (DMI_pBW) was then used as a dependent variable to identify factors associated with prepartum feed intake variation (Figure 1), and to classify cows and evaluate its impact on transition metabolism and subsequent performance. For the latter, cows were ranked within parity according to average prepartum DMI_pBW and grouped into terciles, as follows: low prepartum feed intake (LFI), moderate prepartum feed intake (MFI), and high prepartum feed intake (HFI). The sample size of 91 cows per tercile group would allow us to detect a 15% increase in the average concentration of NEFA in serum during the transition period (mean = 0.47; SD = 0.154 mmol/L), and a 2 kg/d difference in average energy corrected milk in the first 14 wk (mean = 42; SD = 4.7 kg/d) with a statistical power of 80% and confidence of 95%.

Rumination and Physical Activities

Rumination activity (RumA) and physical activity (PhyA) were monitored continuously from d -28 ± 3 relative to the expected calving to 30 ± 3 DIM using

electronic loggers (Hr-TAG-LD, SCR Engineers Ltd.) attached to neck collars. Sensors recorded both activities in 2-h intervals, which were summed to obtain daily values of RumA (min/d) and PhyA (AU/d). Daily values were used to calculate weekly averages, and weekly averages of wk -3 and -1 were used to calculate the changes in RumA (RumACh) and changes in PhyA (PhyACh) from wk -3 to -1.

Blood Sampling and Metabolites Analyses

Blood samples were collected from the coccygeal blood vessels on d -21 ± 3, -14 ± 3, -10 ± 1, -7 ± 1, -3 ± 1 before the expected calving date and on d 0, 3, 7, 10, 14, and 21 ± 3 relative to calving. Sterile polyethylene terephthalate tubes were used to collect plasma (BD Vacutainer™ with sodium heparin) and serum (BD Vacutainer™ for trace element testing with serum clot activator). After centrifugation, plasma and serum samples were harvested and stored at -20°C until laboratory analyses. Concentrations of glucose, cholesterol, non-esterified fatty acids (NEFA), β-hydroxybutyrate (BHB), total protein (TP), albumin, aspartate aminotransferase (AST), gamma-glutamyl transferase (GGT), glutamate dehydrogenase (GLDH), haptoglobin, Ca, Cl, and Mg were measured in serum collected on d -21, -10, -3, 0, 3, 7, 10, 14, and 21 relative to calving. Ferric reducing ability of plasma (FRAP) and ceruloplasmin were evaluated in plasma collected on d -21, -14, -10, -7, -3, 0, 3, 7, 10, 14, and 21. Additionally, the activity of superoxide dismutase (SOD) and glutathione peroxidase (GPx) was measured in plasma collected on d -21, -3, 0, 3, 7, 14, and 21. A detailed description of analytical methods was provided by Mion et al. (2022; 2023). Sample from d -21 and -3 were used to calculate the absolute changes in concentration of metabolites (Ch) from d -21 to -3 and the average value of the 2 samples.

Statistical Analyses

Four linear regression models were built to explain the variation in DMIPBW (Figure 1). From model 1 to 4, groups of independent variables were added to evaluate the gain in explanation of the variability of the dependent variable DMIPBW. Model 1 used only on-farm information: parity of the cow (nulliparous, primiparous, multiparous), BW deviation from the parity average at enrollment (**BWD**), BCS at enrollment and BCS change (**BCSCh**) during the dry period, calf birth body weight, twinning, stillbirth, dietary trace mineral source (SMT or OTM), and season (1 to 5, each representing a 3-mo interval). For parous cows, 305-d milk production (**M305**) and length of previous lactation, milk yield at dry-off (**MYDO**), and length of dry period were also considered in Model 1. Model 2 considered all variables of Model 1 and the information collected from wearable sensors: average daily RumA (min/d) on wk -3, -2, and -1, RumACh from wk -3 to wk -1, average daily PhyA (AU/d) on wk -3, -2, and -1, and PhyACh from wk -3 to -1. Model 3 considered all variables of Model 2, in addition to concentrations of blood metabolites measured on d -21 ± 3 . The metabolites considered were BHB, NEFA, cholesterol, total protein, albumin, urea, Ca, P, Mg, Cl, haptoglobin, AST, GGT, GLDH, GPx, SOD, and FRAP. Model 4 included all variables of Model 3 and the blood metabolites measured on d -3 ± 2 , the change in their concentration from d -21 to d -3, and the average of the 2 measurements. The units of each independent variable are presented in the Supplementary Table 1. For all models, only variables that were significant ($P \leq 0.05$) in a univariable model were included in the multivariable model. When 2 highly correlated variables ($r > 0.5$) were eligible for a multivariable model, only the one with the highest effect on the dependent variable was included to avoid collinearity problems. After the characterization of a full model with all eligible variables, 2 methods of variable selection were performed using the GLMSelect procedure of SAS on Demand (SAS Institute Inc., Cary, NC). First, a backward elimination technique was used, starting with the full model and deleting variables based on the Bayesian information criterion (**BIC**) statistic. Then, to evaluate the predictive value of all eligible independent variables of each model, a 5-fold cross-validation was performed using the Least Absolute Shrinkage and Selection Operator (**LASSO**) method. Fit statistics of all models were reported and compared. In addition, the list of selected variables and their estimates were reported.

To evaluate the effects of DMIPBW terciles, dependent variables were analyzed using mixed linear regression models in the GLIMMIX procedure of SAS on Demand. Our models included the fixed effects of the tercile group (LFI, MFI, HFI), parity (parous, nulliparous), time (either

day or week), their interactions, source of trace mineral supplement, and season, and the random effect of cow nested within group. For milk production responses, the estimated breeding values of the respective trait and BW of the cow were added as covariates in a second model. The covariance structures were tested and the one that resulted in the lowest BIC was used. Residuals were evaluated for normality and homogeneity of variances, and data were transformed when assumptions were not met. Post-hoc adjustments of probability values for pairwise comparisons were performed using the Tukey method.

RESULTS

Estimations of DMIPBW Using Linear Models

The DMIPBW mean (\pm SD) and median were 1.714% ($\pm 0.269\%$) and 1.696%, respectively. For the linear Model 1, the independent variables that were significant in the univariable models and included in the multivariable model were parity, diet, BWD, BCS, and BCSCh. The adjusted R^2 for the full model was 0.407 and the root MSE was 0.207 (Table 1). When the backward elimination technique was applied, all independent variables were retained in the final model except for BCSCh, and resulted in an adjusted R^2 of 0.41 and the root MSE of 0.207. In the 5-fold cross-validation model, BCSCh was again the only variable removed from the final model, resulting in similar adjusted R-Sq and root MSE. The sum of the 5 predicted residual sum of squares (CVPRESS), which represent an estimate of the prediction error, was 0.043 and similar to the MSE of the training model (Table 1). The cross-validation Model 1 and coefficient estimates were:

$$\text{DMIPBW} = 2.344436 + (\text{BWD} \times -0.00123) + (\text{BCS} \times -0.186459) + \text{Parity adjustment} + \text{Diet adjustment}$$

where the parity adjustment was -0.105864, 0.197067, and 0 for nulliparous, primiparous, and multiparous, respectively, and the diet adjustment was 0 and 0.046134 for inorganic and organic trace minerals, respectively.

The inclusion of sensor data in Model 2 improved the performance obtained in Model 1 (Table 1). In addition to the independent variables included in model 1, RumAWk-1 and RumACh were included in the full model. Compared with Model 1, the adjusted R^2 increased to 0.469 and the root MSE reduced to 0.195 in the full model. After backward elimination, BCSCh and RumACh were removed, resulting in similar adjusted R-Sq and MSE. The 5-fold cross-validation resulted in an adjusted R^2 of 0.471 and a root MSE of 0.195, with the following model and coefficient estimates:

Table 1. Fit statistics of linear models predicting the average dry matter intake as percentage of body weight during the prepartum period in all cows enrolled

Item ¹	Model 1	Model 2	Model 3	Model 4
Number of cows used	273	242	182	182
Dependent mean	1.714	1.719	1.721	1.721
<i>Full model</i>				
Root MSE	0.207	0.195	0.174	0.163
R-Square	0.421	0.487	0.590	0.657
Adj R-Sq	0.407	0.469	0.548	0.602
AIC	-577.1	-537.4	-436.4	-452.7
BIC	-849.7	-778.7	-614.5	-626.1
C(p)	7.0	9.0	18.0	26.0
PRESS	12.1	9.6	7.5	6.0
ASE	0.042	0.037	0.027	0.023
<i>Backward elimination</i>				
Root MSE	0.207	0.195	0.172	0.162
R-Square	0.420	0.482	0.581	0.632
Adj R-Sq	0.410	0.469	0.557	0.609
AIC	-579.0	-539.2	-446.3	-468.1
BIC	-851.7	-780.8	-626.4	-648.0
C(p)	5.1	7.1	7.8	9.2
PRESS	12.0	9.5	6.5	5.2
ASE	0.042	0.037	0.028	0.024
<i>Lasso 5-fold cross validation</i>				
Root MSE	0.207	0.195	0.178	0.167
R-Square	0.420	0.486	0.561	0.623
Adj R-Sq	0.409	0.471	0.527	0.581
AIC	-579.0	-539.3	-431.7	-449.3
BIC	-851.7	-780.7	-612.8	-629.0
C(p)	5.1	7.1	21.8	27.6
ASE	0.042	0.037	0.029	0.025
CVEX PRESS	0.043	0.039	0.039	0.031

¹Root MSE = square root of the mean square error; R-square = coefficient of determination; Adj R-Sq = adjusted coefficient of determination; AIC = Akaike information criterion; BIC = Bayesian information criterion; C(p) = Mallows' C(p) statistic; PRESS = predicted residual sum of squares; CVEX PRESS = predicted residual sum of squares of the external cross-validation; ASE = average square errors.

$$\text{DMI}_{\text{pBW}} = 2.131451 + (\text{BWD} \times -0.00119) + (\text{BCS} \times -0.179365) + (\text{RumAWk-1} \times 0.000453) + (\text{RumACh} \times 0.00029) + \text{Parity adjustment} + \text{Diet adjustment}$$

where the parity adjustment was -0.102532, 0.177437, and 0 for nulliparous, primiparous, and multiparous, respectively, and the diet adjustment was 0 and 0.046038 for inorganic and organic trace minerals, respectively.

In addition to the independent variables of Model 2, serum concentrations of NEFA, BHB, glucose, cholesterol, TP, urea, Cl, Mg, and GLDH on d -21 were included in the full Model 3. The inclusion of serum metabolites increased the adjusted R^2 to 0.548 and reduced the root MSE to 0.174 (Table 1). After backward elimination, parity, diet, BCS, BWD, RumAWk-1, NEFA-21, BHB-21, GLDH-21, and Cl⁻21 were retained, resulting in an adjusted R^2 and root MSE of 0.557 and 0.172, respectively. The 5-fold cross-validation resulted in an adjusted R^2 of 0.527 and a root MSE of 0.178, with the following model and coefficient estimates:

$$\text{DMI}_{\text{pBW}} = 3.085467 + (\text{BCS} \times -0.150037) + (\text{BWD} \times -0.000849) + (\text{RumAWk-1} \times 0.000157) +$$

$$(\text{RumACh} \times 0.000138) + (\text{NEFA-21} \times -0.222006) + (\text{BHB-21} \times -0.000148) + (\text{Cl}^-21 \times -0.010438) + (\text{Urea-21} \times 0.018337) + (\text{GLDH-21} \times 0.001236) + (\text{Mg-21} \times 0.010388) + \text{Parity adjustment} + \text{Diet adjustment}$$

where the parity adjustment was -0.036925, 0.146721, and 0 for nulliparous, primiparous, and multiparous, respectively, and the diet adjustment was 0 and 0.004619 for inorganic and organic trace minerals, respectively.

In Model 4, serum concentrations of glucose, Cl, and Mg on d -3, the average concentration of NEFA and TP on d -21 and -3, and the changes in serum concentration of NEFA, BHB, cholesterol, GLDH, and GGT from d -21 to -3 were added to the list of independent variables in the full model, which resulted in an adjusted R^2 of 0.602 and root MSE of 0.163 (Table 1). After backward elimination, BCS, BWD, average NEFA, BHB-21, GLDH-21, Mg-21, glucose-3, Cl⁻21, and Cl⁻3 were retained in the model, resulting in an adjusted R^2 of 0.609 and root MSE of 0.162. The 5-fold cross-validation resulted in an adjusted R^2 of 0.581 and a root MSE of 0.167, with the following model and coefficient estimates:

$$\begin{aligned} \text{DMI}_{\text{pBW}} = & 3.311334 + (\text{BWD} \times -0.000899) + (\text{BCS} \\ & \times -0.137419) + (\text{RumAWk-1} \times 0.000103) + (\text{RumACh} \\ & \times 0.00011) + (\text{average NEFA} \times -0.432281) + (\text{average} \\ & \text{urea} \times 0.024701) + (\text{CholesterolCh} \times -0.038567) \\ & + (\text{GGTCh} \times -0.001061) + (\text{BHB-21} \times 0.000119) \\ & + (\text{GLDH-21} \times 0.001217) + (\text{Mg-21} \times 0.081132) + \\ & (\text{Mg-3} \times 0.069876) + (\text{Cl}^-21 \times -0.007158) + (\text{Cl}^-3 \times \\ & -0.001061) + (\text{glucose-3} \times 0.000308) + \text{Diet adjustment} \\ & + \text{Season adjustment} \end{aligned}$$

where the parity adjustment was -0.01461, 0.13379, and 0 for nulliparous, primiparous, and multiparous, respectively, and the diet adjustment was 0 and 0.004515 for inorganic and organic trace minerals, respectively.

When nulliparous cows were excluded, the adjusted R^2 in Models 1 and 2 was slightly higher than when all cows were used (Table 2). However, Models 3 and 4, which included information of blood metabolites, performed similarly to when all cows were used (Table 2).

The best Model 1 for cross-validation purposes in parous cows and the coefficient estimates were:

$$\text{DMI}_{\text{pBW}} = 1.876112 + (\text{BWD} \times -0.001181) + (\text{BCS}$$

$$\begin{aligned} & \times -0.117004) + (\text{MY at dry-off} \times 0.015173) + (\text{length} \\ & \text{of dry-off} \times -0.001601) + \text{Parity adjustment} + \text{Diet} \\ & \text{adjustment} \end{aligned}$$

where the parity adjustment was 0.137689 and 0 for primiparous and multiparous, respectively, and the diet adjustment was 0 and 0.027447 for inorganic and organic trace minerals, respectively.

The best Model 2 for cross-validation purposes in parous cows and the coefficient estimates were:

$$\begin{aligned} \text{DMI}_{\text{pBW}} = & 1.619922 + (\text{BWD} \times -0.001055) + (\text{BCS} \\ & \times -0.088231) + (\text{MY at dry-off} \times 0.012622) + (\text{length} \\ & \text{of dry-off} \times -0.000635) + (\text{Wk-1RumA} \times -0.000414) \\ & + (\text{RumACh} \times 0.00011) + \text{Parity adjustment} + \text{Diet} \\ & \text{adjustment} \end{aligned}$$

where the parity adjustment was 0.113451 and 0 for primiparous and multiparous, respectively, and the diet adjustment was 0 and 0.006511 for inorganic and organic trace minerals, respectively.

Table 2. Fit statistics of linear models predicting the average dry matter intake as percentage of body weight during the dry period in parous cows only

Item ¹	Model 1	Model 2	Model 3	Model 4
Number of cows used	173	155	114	114
Dependent Mean	1.795	1.797	1.810	1.810
<i>Full model</i>				
Root MSE	0.208	0.197	0.182	0.175
R-Square	0.508	0.561	0.613	0.670
Adj R-Sq	0.487	0.531	0.550	0.586
AIC	-361.2	-335.4	-256.8	-261.0
BIC	-533.4	-488.7	-364.9	-362.3
C(p)	8.0	11.0	17.0	24.0
PRESS	7.8	6.7	6.5	6.6
ASE	0.041	0.036	0.028	0.024
<i>Backward elimination</i>				
Root MSE	0.207	0.197	0.181	0.173
R-Square	0.501	0.549	0.592	0.636
Adj R-Sq	0.489	0.534	0.557	0.593
AIC	-365.0	-341.1	-264.6	-271.6
BIC	-537.6	-495.5	-376.4	-381.8
C(p)	4.1	5.0	8.4	11.4
PRESS	7.7	6.3	6.0	3.9
ASE	0.042	0.037	0.030	0.027
<i>Lasso 5-fold cross validation</i>				
Root MSE	0.207	0.201	0.202	0.180
R-Square	0.506	0.540	0.477	0.616
Adj R-Sq	0.489	0.515	0.448	0.561
AIC	-362.8	-331.9	-242.4	-261.5
BIC	-535.1	-486.4	-358.3	-372.9
C(p)	6.4	14.1	31.1	20.9
ASE	0.041	0.038	0.038	0.028
CVEX PRESS	0.046	0.046	0.055	0.038

¹Root MSE = square root of the mean square error; R-square = coefficient of determination; Adj R-Sq = adjusted coefficient of determination; AIC = Akaike information criterion; BIC = Bayesian information criterion; C(p) = Mallows' C(p) statistic; PRESS = predicted residual sum of squares; CVEX PRESS = predicted residual sum of squares of the external cross-validation; ASE = average square errors.

Prepartum Characteristics According to Terciles of DMIPBW

As per experimental design, DMIPBW was different between tercile groups and averaged 1.44, 1.70, and 1.91 \pm 0.013% for cows in the LFI, MFI, and HFI groups, respectively. When prepartum DMIPBW was evaluated as weekly repeated measures in the last 5 wk of gestation, an interaction ($P = 0.03$) between tercile group, parity, and time was observed (Figure 2A). Within parity, tercile groups had very distinct feed intake; however, the difference between groups was larger in parous than in nulliparous cows and varied over time. Similar results were obtained when DMI was evaluated as kg of DMI/d (Figure 2B). The average intake was 11.6, 13.2, and 14.2 \pm 0.14 kg/d for LFI, MFI, and HFI groups, respectively, but differences among groups were larger in parous than in nulliparous cows and varied slightly over time (Figure 2B).

The tercile group interacted with both parity ($P < 0.01$) and time ($P < 0.01$) for BW in the last 5 wk of gestation (Figure 2C). Within nulliparous, BW did not differ between groups and averaged 708, 699, and 680 \pm 10 for LFI, MFI, and HFI, respectively. However, within parous cows, all 3 groups differed in BW and averaged 892, 849, 798 \pm 8 for LFI, MFI, and HFI, respectively. The prepartum BCS was different among all 3 groups, and averaged 3.73, 3.62, 3.54 \pm 0.024 for LFI, MFI, and HFI, respectively. No interactions were observed between groups and parity or between group and time or between group, parity, and time. The proportion of cows with BCS >3.5 on d -45 also differed among all groups, and averaged 67.4, 55.1, and 36.5 \pm 6% for LFI, MFI, and HFI, respectively. No interaction between group and parity was observed.

Prepartum EBAL followed a similar pattern of prepartum DMI, and a triple interaction ($P = 0.03$) between group, parity, and time was observed (Figure 2D). Within parity, differences between all tercile groups were evident, but those differences were larger in parous (LFI = 2.2, MFI = 6.3, and HFI = 8.6 \pm 0.2 Mcal/d) than in nulliparous cows (LFI = 0.2, MFI = 1.8, and HFI = 3.1 \pm 0.3 Mcal/d) and varied over time.

Physical activity in the last 3 wk of gestation did not differ between tercile groups. However, an interaction ($P < 0.01$) between group and time was observed for rumination activity (Figure 2E). In wk -3 and -2, LFI cows had a shorter ($P \leq 0.05$) rumination time (min/d) than HFI cows, but both groups did not differ from MFI cows. However, the difference between groups increased and rumination time differed ($P < 0.05$) among all groups in wk -1, being longer for HFI (481 \pm 11 min/d), followed by the MFI (453 \pm 10 min/d) and LFI (412 \pm 11 min/d) groups. When rumination activity was evaluated as min/

kg of DMI, LFI cows had longer ($P < 0.01$) prepartum rumination time than the other 2 groups (LFI = 41.7, MFI = 37.9, and HFI = 37.0 \pm 0.9 min/kg of DMI) (Figure 2F).

For parous cows, there were important differences regarding previous lactation performance and length. Previous milk production by 305-d differed ($P < 0.01$) among all 3 groups and averaged 9,808 \pm 233, 10,457 \pm 191, and 11,182 \pm 197 kg for LFI, MFI, and HFI parous cows, respectively. Similarly, milk yield at dry-off differed among all groups and averaged 18.1 \pm 1.0, 23.1 \pm 0.8, and 26.2 \pm 0.8 kg/d for LFI, MFI, and HFI parous cows, respectively. Lactation length was shorter for HFI parous cows than MFI parous cows, and tended to be shorter compared with LFI parous cows (LFI = 350 \pm 9, MFI = 359 \pm 7, and HFI = 329 \pm 7). Length of the dry period, however, was longer for LFI parous cows than for the other 2 groups (LFI = 51.8 \pm 1.3, MFI = 47.3 \pm 1.0, and HFI = 47.8 \pm 1.1).

Postpartum Performance According to Terciles of DMIPBW

Differences in DMI between tercile groups continued in the postpartum period (Figure 3AB). As % of BW, an interaction ($P < 0.01$) between group and parity was observed. In parous cows (all multiparous cows after calving), all 3 groups differed (LFI = 3.14, MFI = 3.40, and HFI = 3.61 \pm 0.03%), whereas in nulliparous cows (all primiparous cows after calving) only LFI and HFI differed (LFI = 3.01, MFI = 3.12, and HFI = 3.18 \pm 0.04%). As kg/d, DMI was lower ($P < 0.01$) for LFI compared with the other 2 groups (LFI = 20.9, MFI = 21.9, and HFI = 22.1 \pm 0.2 kg/d) and did not interact with parity or time. Postpartum rumination time did not differ ($P = 0.29$) between tercile groups; however, when adjusted to DMI, LFI cows had longer ($P = 0.03$) rumination time per kg of DMI than HFI cows and both groups did not differ from MFI cows (LFI = 31.1, MFI = 29.2, and HFI = 28.4 \pm 0.8 min/kg of DMI).

An interaction between tercile group and parity ($P < 0.01$), and between tercile group and time ($P = 0.05$) were observed for postpartum BW (Figure 3C). Although no differences in postpartum BW were observed in nulliparous cows (LFI = 608, MFI = 614, and HFI = 607 \pm 7 kg/d), HFI parous cows were lighter than the other 2 groups of parous cows (LFI = 754, MFI = 732, and HFI = 694 \pm 7 kg/d). As for postpartum BCS, no differences between groups or interaction with parity or time were observed, and averaged 3.3 \pm 0.03. However, cows in the LFI group had a greater change in BCS during the first 35 DIM than cows in the MFI group, and both groups did not differ from the HFI group (LFI = -0.25; MFI = -0.13; HFI = -0.20 \pm 0.03; $P = 0.03$).

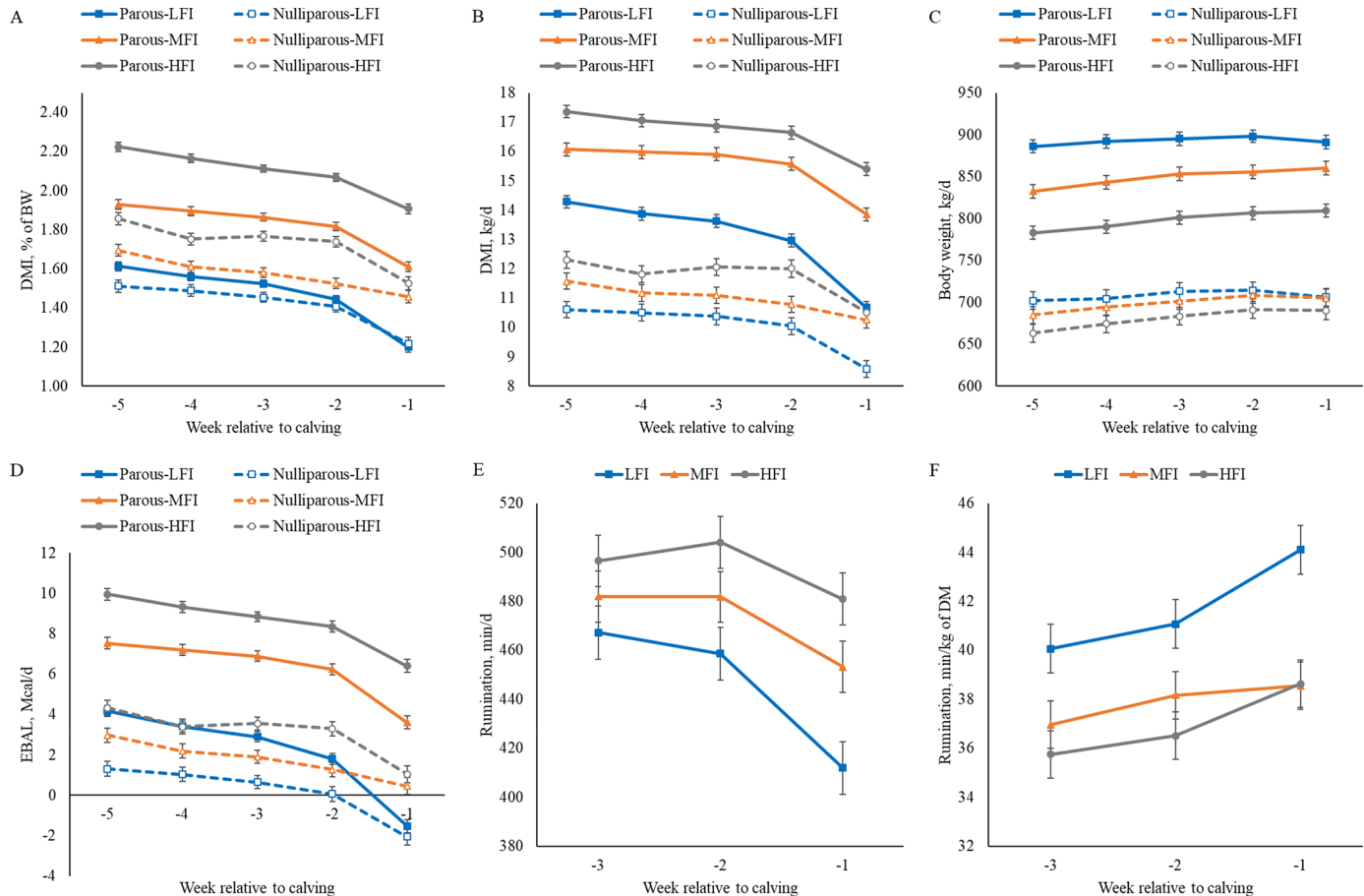


Figure 2. Dry matter intake (DMI) as % of body weight (panel A) and kg/d (panel B), body weight (panel C), energy balance (panel D), and rumination activity as min/d (panel E) and min/kg of DM (panel F) in prepartum cows classified according to averaged DMI as % of BW in the last 5 wk of pregnancy, as follows: low feed intake (LFI), moderate feed intake (MFI), and high feed intake (HFI).

Milk production was lower ($P = 0.02$) for LFI cows than for the other 2 groups (LFI = 36.7, MFI = 38.2, and HFI = 38.3 ± 0.4 kg/d) despite adding or not EBV and prepartum BW as covariates in the model (Figure 3D). For ECM, an interaction ($P = 0.058$) between tercile group and time was observed, and LFI cows had lower yields than MFI cows on wk 11 to 14, and lower yields than HFI cows on wk 13 and 14 (Figure 3E). For postpartum EBAL, there was an effect of group ($P < 0.01$) and it was different among all groups (LFI = -2.79 , MFI = -1.63 , and HFI = -0.66 ± 0.3 Mcal/d) (Figure 3F).

Blood Metabolites during Transition According to Terciles of DMipBW

Concentrations of NEFA in serum differed ($P < 0.01$) according to tercile groups and were higher for LFI cows than for MFI and HFI cows (LFI = 0.51, MFI = 0.44, and HFI = 0.41 ± 0.014 mmol/L) (Figure 4A). An interaction ($P < 0.01$) between tercile group and day was

observed for concentrations of BHB in serum (Figure 4B). On d-21, LFI cows tended ($P = 0.06$) to have lower concentrations of BHB in serum than the other 2 groups; however, from d -3 to 10 relative to calving concentrations of BHB were higher in LFI cows than in the other 2 groups (Figure 4B).

An effect ($P < 0.01$) of tercile group was observed for concentrations of cholesterol in serum, which was lower for LFI cows than for HFI cows (LFI = 2.23, MFI = 2.38, and HFI = 2.44 ± 0.048 μ mol/L) (Figure 4C). Cows in the MFI group tended ($P = 0.07$) to have higher concentrations of cholesterol than cows in the LFI group but did not differ from cows in the HFI. No differences in concentration of glucose in serum between tercile groups were observed.

There was an interaction ($P < 0.01$) between tercile group and parity for concentrations of urea in serum (Figure 5A). In parous cows, concentration of urea was lower in LFI than in the other 2 groups of cows (Figure 5B). In nulliparous, however, no differences were observed

(Figure 5C). There were no differences in concentration of TP, albumin, and globulin in serum between tercile groups.

Concentrations of GLDH in serum differed ($P < 0.01$) according to tercile groups and were lower for LFI cows than for the other 2 groups (LFI = 1.13, MFI = 1.23, and HFI = 1.22 ± 0.022 U/L) (Figure 6A). Although no interaction with time was observed ($P = 0.14$), the differences in GLDH were more distinct in the prepartum period than in the postpartum period. For concentrations of GGT in serum, an interaction ($P = 0.05$) between group and time was observed (Figure 6B). Concentrations of GGT in serum of LFI cows were lower than in the other 2 groups between d -21 and -3, but no differences were observed from calving to d 14. On d 21, however, it was lower for HFI cows than in the other 2 groups. A triple interaction ($P = 0.03$) between tercile group, parity, and time was observed for concentrations of AST in serum (Figure 6C). In nulliparous, concentrations of AST were similar over time in all tercile groups. However, in parous cows,

concentration of AST in the LFI group was lower on d -21 and -10 but higher on d 7 and 10 compared with the other 2 groups. No differences in concentrations of haptoglobin and ceruloplasmin were observed between tercile groups.

A triple interaction ($P = 0.03$) between tercile group, parity, and time was observed for the activity of SOD in plasma (Figure 7A). In parous cows, SOD activity was distinctly higher in HFI cows than in the other 2 groups (Figure 7B). However, no major differences were observed between tercile groups of nulliparous cows (Figure 7C). No differences in the activity of GPx in plasma and FRAP were observed between tercile groups.

Concentration of P in serum differed ($P = 0.02$) between tercile groups, and it was lower for LFI cows than for HFI cows (LFI = 1.76, MFI = 1.80, and HFI = 1.84 ± 0.017 mmol/L) (Figure 8A). On average, MFI cows had intermediate values and did not differ from the other 2 groups. An interaction between tercile and group ($P = 0.04$) was observed for concentrations of Cl in serum

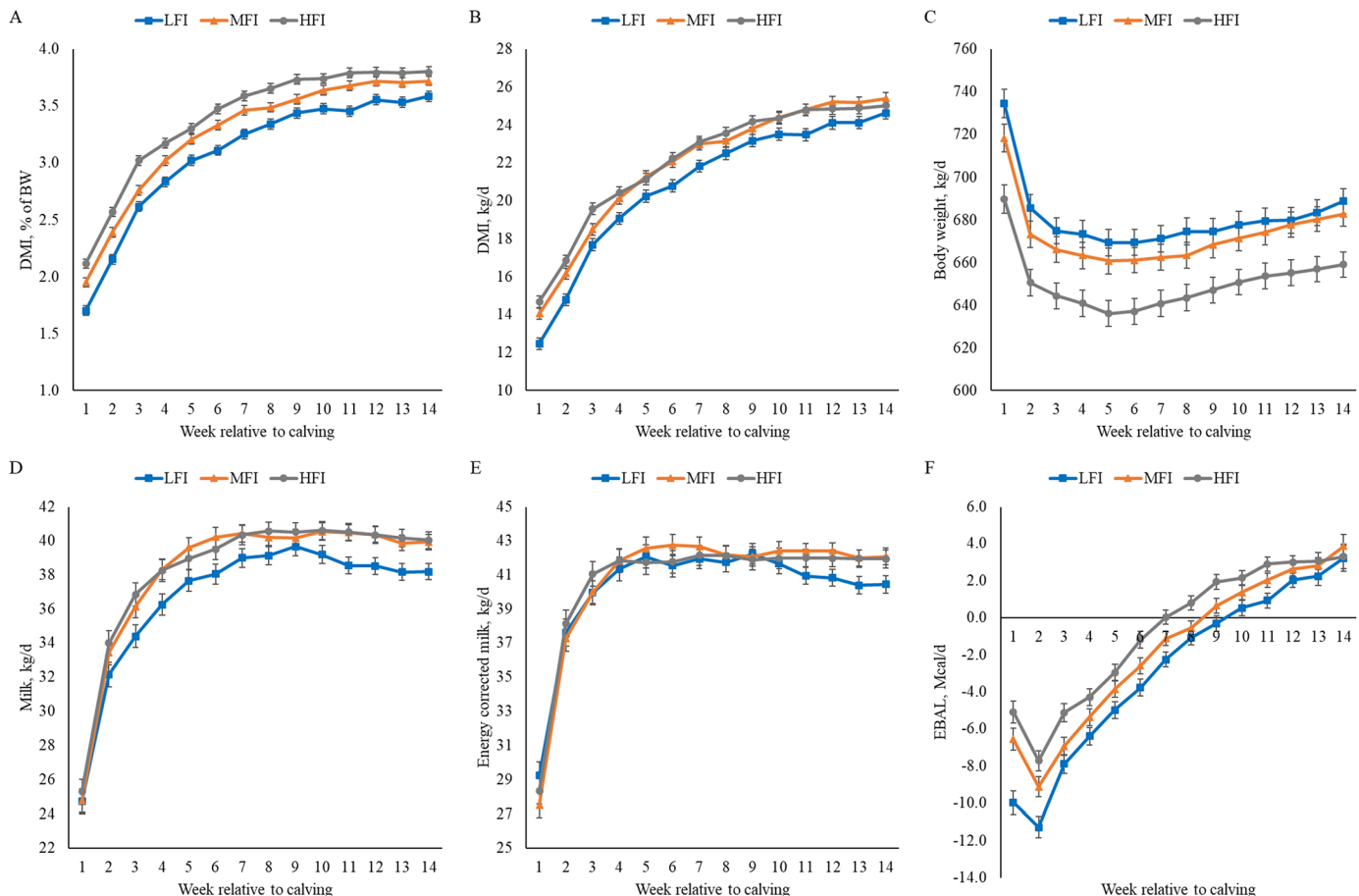


Figure 3. Dry matter intake (DMI) as % of body weight (panel A) and kg/d (panel B), body weight (panel C), yields of milk (panel D) and energy corrected milk (panel E), and energy balance (panel F) in lactating cows classified according to averaged DMI as % of BW in the last 5 wk of previous pregnancy, as follows: low feed intake (LFI), moderate feed intake (MFI), and high feed intake (HFI).

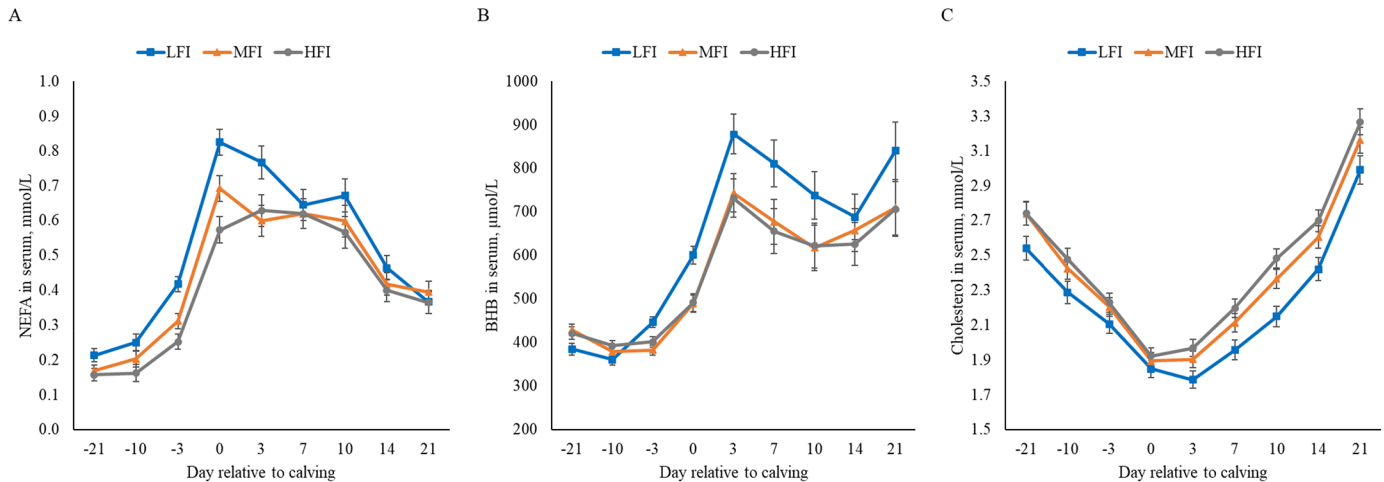


Figure 4. Concentrations of nonesterified fatty acids (panel A), β -hydroxybutyrate (panel B), and cholesterol (panel C) in serum of transition cows classified according to averaged DMI as % of BW in the last 5 wk of pregnancy, as follows: low feed intake (LFI), moderate feed intake (MFI), and high feed intake (HFI).

(Figure 8B). On d-21, LFI cows had higher Cl concentrations than HFI cows and, on d -10, LFI cows had higher Cl concentrations than the other 2 groups. No differences between groups were observed on and after d -3 (Figure 8B). In addition, there were no differences in concentrations of Ca, Mg, and K between groups during the entire transition period.

DISCUSSION

This study evaluated the utilization of novel variables to explain the variation in prepartum feed intake of Holstein cows. Additionally, it assessed the associations

between the level of DMI in the last 5 wk of pregnancy with the transition metabolism and subsequent milk production. As expected, primiparous cows ate less than multiparous cows in the prepartum and postpartum periods, which is well-established (Marquardt et al., 1977; Neave et al., 2017) and related to the increased filling capacity of the digestive tract as cows age (Smith and Baldwin, 1974). Nonetheless, our study shows there is still substantial variability in the level of prepartum feed intake among cows of the same parity, which was more evident in parous than in nulliparous cows.

Previous research has focused on understanding factors related to feed consumption in the last 3 wk pre-

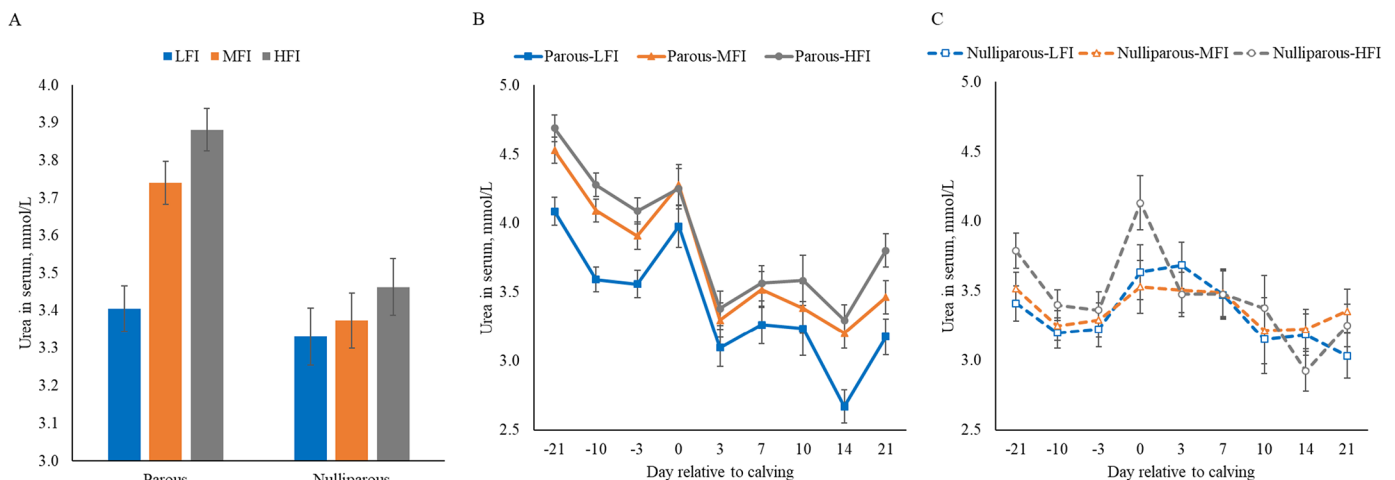


Figure 5. Concentrations of urea in serum of transition cows classified according to averaged DMI as % of BW in the last 5 wk of pregnancy, as follows: low feed intake (LFI), moderate feed intake (MFI), and high feed intake (HFI). Panel A shows average concentrations of urea per group through the entire transition period. Panel B shows concentrations of urea over time in parous cows. Panel C shows concentrations of urea over time in nulliparous cows.

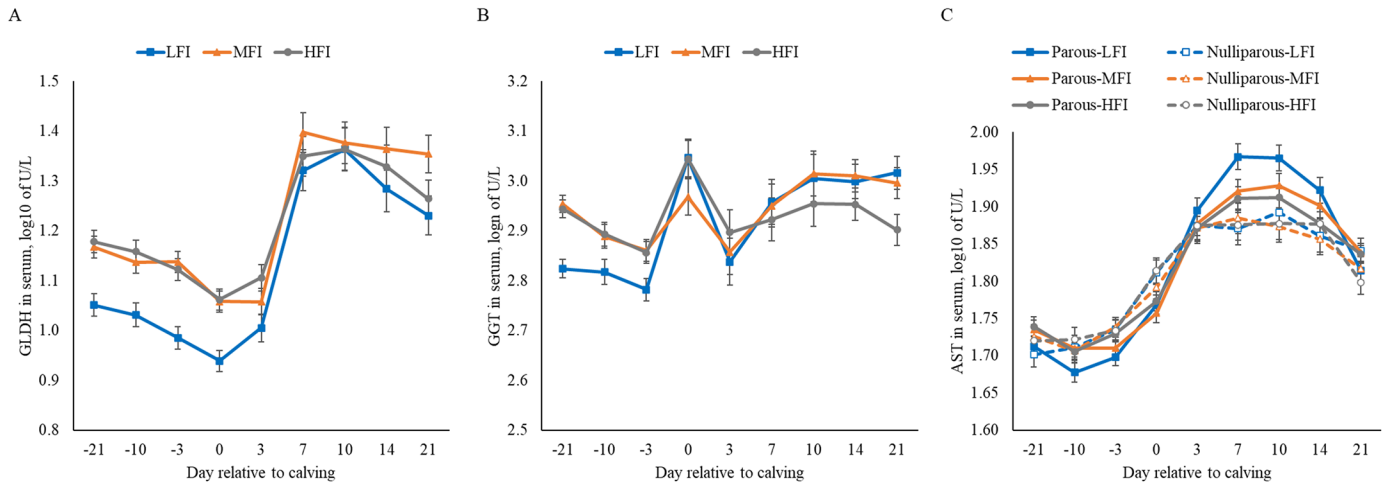


Figure 6. Concentrations of GLDH (panel A), GGT (panel B), and AST (panel C) in serum of transition cows classified according to averaged DMI as % of BW in the last 5 wk of pregnancy, as follows: low feed intake (LFI), moderate feed intake (MFI), and high feed intake (HFI).

ceding calving or with the decline in DMI as parturition approaches. Day of pregnancy and a variety of dietary (e.g., neutral detergent fiber and ether extract) and animal (e.g., parity and BCS) factors are known to affect feed intake before calving, but they were reported to account for only 18% of the variation in late prepartum DMI (Hayirli et al., 2002). In the current study, adding data on rumination time and blood metabolites improved the performance of models explaining the variation in average prepartum DMI, which was also observed for linear models predicting the decline in prepartum DMI (Santos et al., 2024). In both cases, the linear models explained near to 50% of the observed variation in all cows, and close to 60% in parous cows. The good performance of

these models is very encouraging and should be tested in different herds using different feeding strategies in prepartum cows for complete evaluation of their usefulness.

The observed negative association between BCS and prepartum feed intake has been previously reported (Hayirli et al., 2002; Daros et al., 2021), and is consistent with the temporal control of feed intake by body fatness and weight reported previously (Allen, 2014). Long-term mechanisms regulating feed intake include those related to the maintenance of body energy storage and body weight (e.g., leptin), in addition to the physiological and metabolic adaptations to late pregnancy and the onset of lactation (Allen, 2014). Leptin, for instance, is primarily produced by adipocytes and regulates energy intake and

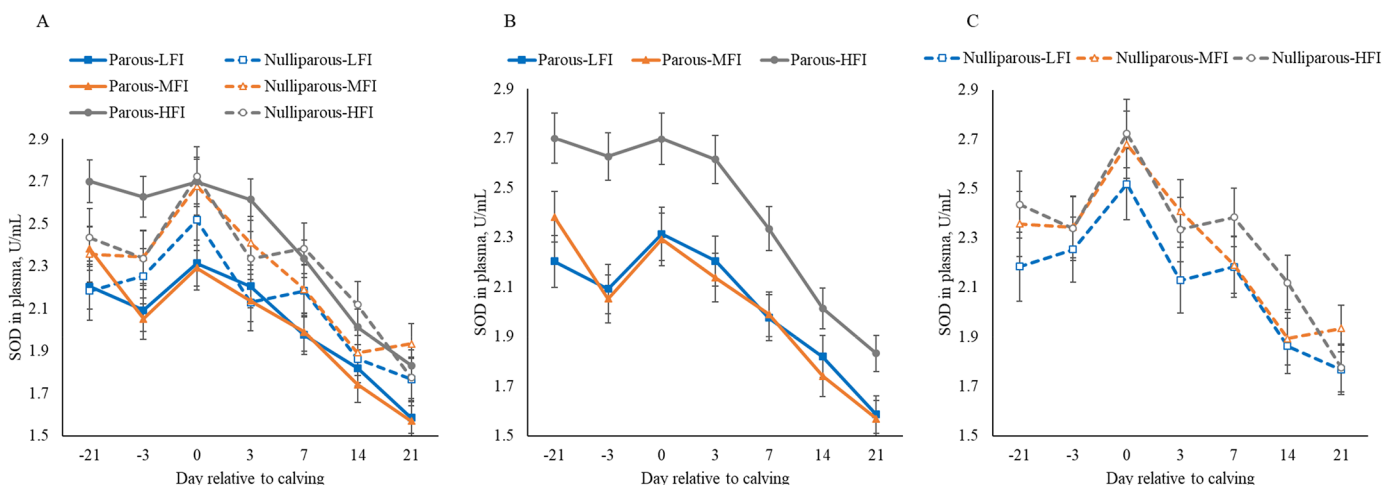


Figure 7. Activity of superoxide dismutase in plasma of transition cows classified according to averaged DMI as % of BW in the last 5 wk of pregnancy as: low feed intake (LFI), moderate feed intake (MFI), and high feed intake (HFI). Panel A shows all 6 parity-group combinations together, and panels B and C separate parous and nulliparous cows, respectively, to facilitate the visualization of data.

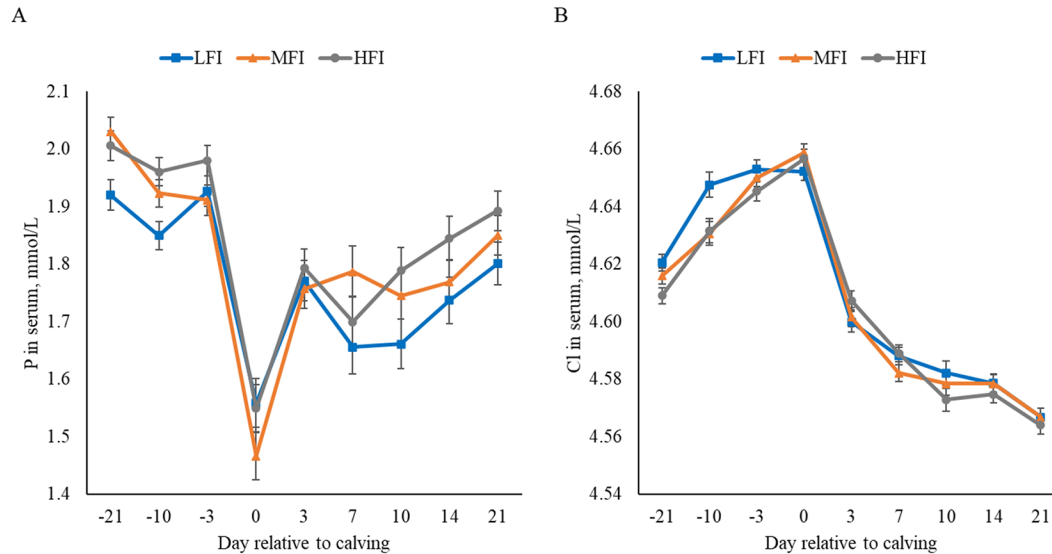


Figure 8. Concentrations of P (panel A) and Cl (panel B) in serum of transition cows classified according to averaged DMI as % of BW in the last 5 wk of pregnancy as: low feed intake (LFI), moderate feed intake (MFI), and high feed intake (HFI).

expenditure by enhancing the activity of anorexigenic neurons and reducing the activity of orexigenic neurons in the hypothalamic nuclei (Roche et al., 2008). The suppressive activity of leptin in voluntary feed consumption has been demonstrated in ruminants (Morrison et al., 2001), and previous research in prepartum dairy cows has shown that adipocyte size is positively associated with BCS and leptin expression in adipose tissue (Depreester et al., 2018). Moreover, prepartum plasma concentrations of leptin are higher in heavier (Liefers et al., 2003) and overconditioned dairy cows (Kokkonen et al., 2005; Pires et al., 2013; Mann et al., 2018). In addition to lower feed consumption during late gestation, overconditioned cows also experienced a greater reduction in feed intake during the last few days before calving (Samii et al., 2019; Santos et al., 2024). The reduced feed intake observed in overconditioned cow often results in loss of BCS before calving, which has been negatively associated with postpartum health, lactation, and reproductive performances (Chebel et al., 2018).

We found a positive association between level of prepartum DMI and rumination time. Schirmann et al. (2012) reported no correlation between daily DMI and daily rumination time in parous cows in the middle of the dry period. In our study, however, we assessed both DMI and rumination as weekly values, which might have decreased the variability observed in daily measures and increased the association between these variables. When rumination was evaluated as min per kg of DMI, then the association with the level of prepartum DMI as percentage of BW was negative. Altogether, our results suggest that the association between DMI and rumination time

is likely quadratic, with increasing rumination time as feed intake increases until the former reaches a plateau that cannot be surpassed even if feed intake continues to increase.

Because of reduced energy intake and greater BW, nulliparous and multiparous cows in LFI had the most severe NEB, which started in the last week before calving. It is known that reductions in DMI lead to a decrease in EBAL (Doepel et al., 2002), especially during the transition from pregnancy to lactation, when insufficient feed intake coincide with the increased energy requirements to support the final stage of fetal growth (Bell et al., 1995) and the subsequent onset of milk production supported by homeorhesis (Bauman and Griinari, 2003). Consequently, the degree of fat mobilization is inversely related to prepartum DMI (Holtenius et al., 2003). Not surprisingly, cows with the lowest level of prepartum DMI had greater lipid mobilization, herein characterized by higher concentrations of NEFA and BHB in serum. These cows also had higher body fatness, which could have further contributed to lipid mobilization during transition. In situations of excessive lipolysis, blood NEFA concentrations may exceed the liver's capacity to secrete triglycerides as very low-density proteins and to fully oxidize fatty acids, resulting in greater ketogenesis and accumulation of triacylglycerol in the liver (Adewuyi et al., 2005). Weber et al. (2013) showed that cows with increased total liver fat content after calving also had the lowest feed intake and the greatest NEFA and BHB concentrations during the transition period.

A severe lipomobilization state through transition has been linked to increased production of reactive oxygen

species (ROS), reactive nitrogen species, and reduced antioxidant defenses, resulting in oxidative stress (Sordillo and Raphael, 2013; Li et al., 2016). Interestingly, a high level of prepartum DMI seemed to have enhanced antioxidant capacity based on the observed greater activity of SOD, but only in parous cows. It is likely that a higher feed intake resulted in greater consumption of essential nutrients, including minerals like copper, zinc, and manganese, which are cofactors for SOD (Andrieu, 2008). Additional supplementation of trace minerals by subcutaneous injections during late pregnancy and early postpartum increased SOD activity in multiparous cows (Machado et al., 2014). The fact that this response was not observed in nulliparous cows in our study is intriguing, and might be related to the magnitude of differences prepartum feed intake among groups, comparatively larger in parous than in nulliparous, or a smaller demand for antioxidants in pregnancy heifers compared with older cows.

Concentrations of GGT, GLDH, and AST are typically used as biomarkers of liver damage, but the overall serum levels observed in our study appeared to be within a normal range for transition cows without health complications (Bertoni et al., 2008; Du et al., 2017). Nevertheless, the associations between prepartum feed intake and enzymes of hepatic damage are likely complex and changed based on the period relative to calving and parity. It is noteworthy that serum concentrations of these enzymes were all higher during the prepartum period and fluctuated less throughout the transition period in cows with HFI than in cows with LFI in the prepartum period, which might reflect the overall level of activity and functional balance of hepatocytes. The liver is also important for synthesis of cholesterol and lipoproteins (Viturro et al., 2009; Kessler et al., 2014), and we observed lower serum concentrations of cholesterol in cows with low level of prepartum feed intake. Nonetheless, adipose tissue and intestine have been suggested to be major contributors to cholesterol synthesis in ruminants (Liepa et al., 1978), and could have contributed to the observed differences in serum concentrations. Previous studies have shown that serum concentrations of cholesterol in both prepartum and postpartum periods follow a similar pattern to that observed for feed intake (Janovick et al., 2006; Walter et al., 2022).

The factors underlying the associations of urea concentrations with prepartum DMI in a parity-dependent manner are uncertain. Peripheral urea concentrations may reflect a variety of parameters, including protein intake relative to requirements, protein metabolism, and liver function (Van Saun, 2006). The larger difference in prepartum feed intake among groups of parous cows could lead to differences in ammonia absorption through the portal-drained viscera, and reduced synthesis of urea-

N (Lapierre and Lobley, 2001). In addition, previous research suggested that hepatic triglyceride accumulation is associated with impaired ureagenesis (Strang et al., 1998; Zhu et al., 2000). We did not observe any major differences in blood metabolites related to protein metabolism (i.e., albumin and total protein) in cows with distinct prepartum DMI.

Regarding concentrations of minerals in serum, we observed differences only in P and Cl. The former was lower in cows with LFI, which could be a result of reduced P intake. Deficiency in P during the transition period has been associated with reduced feed intake in the first week after calving and associated consequences to EBAL and liver fat content (Grunberg et al., 2019). It is unclear why concentrations of Cl in serum were higher during the prepartum period in cows with LFI. Although the differences in intake remained in the postpartum period, the difference in serum Cl disappeared with onset of lactation and gradual reduction in Cl blood concentrations observed in all cows.

Although in this study we did not assess disease incidence in tercile groups because of the limitations in the statistical power, we performed receiver operating characteristic (ROC) analyses to identify the optimal threshold in the level of DMI before calving with predictive ability of early postpartum clinical and subclinical health problems (data not shown). However, no significant results were found between the ROC reference curve and the curves generated by the continuous values of prepartum DMI. This indicates that long-term feed intake before calving might not have a predictive potential of health problems in the early lactation. However, other studies observed an association between lower feed intake in the last few days preceding calving and higher odds of early postpartum diseases (Pérez-Báez et al., 2019a; b, 2021).

We observed that cows with LFI in the prepartum period had reduced milk production in the following lactation. Nonetheless, the observed differences in BW and milk production in the previous lactation could represent confounding factors. The inclusion of GEBV for milk (to account for the genetic merit) and BW (to account for frame size) as covariates in the statistical models, however, did not remove the statistical difference in milk production between groups. Moreover, the lack of association between prepartum feed intake and incidence of postpartum clinical disease indicate that observed differences in milk yield were not associated with the clinical health of cows, but likely related to differences in metabolism and possibly with subclinical conditions among groups. It is noteworthy that differences in feed intake and blood metabolites remained after calving and most likely have contributed to the observed differences in milk production. Previous studies have shown that feeding level during early lactation affects milk produc-

tion and its components in later lactation (Adin et al., 2009; Jørgensen et al., 2016).

Overfeeding cows during the dry period has been linked to greater likelihood of postpartum metabolic disorders, which prompted the use of high-fiber controlled-energy diets (<1.4 Mcal/kg of DM) during the dry period (Dann et al., 2006; Janovick et al., 2011; Mann et al., 2015). These studies were based on changes in the energy density of the diets during the far-off and/or close-up periods. In the current observational study, a single total mixed ration of moderate energy density (1.45 Mcal/kg of DM) was fed for the entire duration of the prepartum period and cows ate *ad libitum*. We did not observe any negative effect in cows with HFI and consequent greater energy intake, which suggest that even cows in the HFI group were not overfed. In fact, these cows had greater postpartum feed intake, lower serum concentrations of NEFA and BHB throughout transition, and greater milk production in the subsequent lactation than cows with LFI and consequent reduced energy intake during the prepartum period. It is important to note, however, that we did not assign cows randomly to different feed intake groups and, therefore, the differences in feed intake among experimental groups are linked to other biological differences that are likely important to transition metabolism, health and subsequent performance. Nonetheless, it is important to consider the high variability in individual feed intake among cows when formulating diets that aim to meet requirements of the average cow. Energy intake below requirements during the dry period has resulted in lower milk production outcomes in the subsequent lactation in some studies (Olsson et al., 1998; Dann et al., 2006; Roche, 2007; Janovick and Drackley, 2010). Thus, our study reinforces that herds using moderate or controlled-energy diets should promote feed intake for better transition metabolism and subsequent performance. In addition to best practices in feed bunk management (DeVries, 2019), multiple dietary management strategies have been proposed recently to enhance prepartum feed intake in these conditions (Havekes et al. 2020abc).

CONCLUSIONS

Average feed intake in the last 5 wk of pregnancy was highly variable among cows and associated with their parity, body condition score, body weight, 305-d milk production in the previous lactation, milk yield at dry-off, and length of the dry period. Combined, these variables explained 41% of the variation in the individual prepartum DMI when all cows were considered, and 49% when only parous cows were considered. Estimations of average prepartum DMI using linear models were improved when data on rumination activity and certain blood metabolites were added as independent variables,

increasing the adjusted R-Sq to values between 47 and 61%. Selected models across different classes of imputed information performed consistently in 5-fold cross-validation analyses. A lower level of prepartum feed intake was also associated with reduced rumination activity before calving, important adjustments in energy metabolism during the transition period, reduced postpartum feed intake, milk production, and energy balance. Our retrospective study reinforces the importance of promoting feed intake during the prepartum period when a single moderate energy diet is fed for *ad libitum* intake.

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