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Association of dry matter intake and energy balance prepartum and postpartum with health disorders postpartum: Part II. Ketosis and clinical mastitis

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ABSTRACT

The main objective of this study was to determine the association of dry matter intake as percentage of body weight (DMI%BW) and energy balance (EB) prepartum (−21 d relative to parturition) and postpartum (28 d) with ketosis (n = 189) and clinical mastitis (n = 79). For this, DMI%BW and EB were the independent variables and ketosis and clinical mastitis were the dependent variables. A secondary objective was to evaluate prepartum DMI%BW and EB as predictors of ketosis and clinical mastitis. For this, ketosis and clinical mastitis were the independent variables and DMI%BW and EB were the dependent variables. Data from 476 cows from 9 experiments were compiled. Clinical mastitis was diagnosed if milk from 1 or more quarters was abnormal in color, viscosity, or consistency, with or without accompanying heat, pain, redness, or swelling of the quarter or generalized illness, during the first 28 d postpartum. Ketosis was defined as the presence of acetoacetate in urine that resulted in any color change [5 mg/dL (trace) or higher] in the urine test strip (Ketostix, Bayer, Leverkusen, Germany). Cows that developed ketosis had lesser DMI%BW and lesser EB on d −5, −3, −2, and −1 than cows without ketosis. Each 0.1-percentage point decrease in the average DMI%BW and each 1-Mcal decrease in the average of EB in the last 3 d prepartum increased the odds of having ketosis by 8 and 5%, respectively. Cut-offs for DMI%BW and EB during the last 3 d prepartum to predict ketosis were established and were ≤1.5%/d and ≤1.1 Mcal/d, respectively. Cows that developed ketosis had lesser postpartum DMI%BW and EB and greater energy-corrected milk (ECM) than cows without ketosis. Cows that developed clinical mastitis had lesser DMI%BW but similar prepartum EB compared with cows without clinical mastitis. Each 0.1-percentage point decrease in the average DMI%BW and each 1-Mcal decrease in the average EB in the last 3 d prepartum increased the odds of having clinical mastitis by 10 and 8%, respectively. The average DMI%BW and EB during the last 3 d prepartum produced significant cut-offs to predict clinical mastitis postpartum, which were ≤1.2%/d and ≤1.0 Mcal/d, respectively. Cows that developed clinical mastitis had lesser postpartum DMI%BW from d 3 to 15 and on d 17; greater EB on d 18, from d 21 to 23, and on d 26; and lesser ECM. The main limitation in this study is that the time-order of disease relative to DMI%BW and ECM is inconsistent such that postpartum outcomes were measured before and after disease, which was diagnosed at variable intervals after calving. In summary, measures of prepartum DMI were associated with and were predictors of ketosis and clinical mastitis postpartum, although the effect sizes were small.

Key words: transition period, dry matter intake, ketosis, mastitis, dairy cow

INTRODUCTION

The decrease in prepartum DMI and the insufficient DMI postpartum lead to a state of negative nutrient balance characterized by increased lipid mobilization in the form of nonesterified fatty acids (**NEFA**) and an increase in ketone bodies such as BHB (Drackley, 1999; Grummer et al., 2004; French, 2006). Several studies have found a detrimental effect of ketosis on milk yield (Rajala-Schultz et al., 1999; Ospina et al., 2010a; Chapinal et al., 2012); however, the effect was found to be conditional on parity, the day of onset of subclinical ketosis, and the peak BHB concentration in blood (Ospina et al., 2010a; Chapinal et al., 2012; McArt et

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al., 2012). Postpartum hyperketonemia has also been associated with postpartum diseases such as displaced abomasum and metritis and with decreased fertility and increased culling, which incur significant economic losses to dairy producers (Ospina et al., 2010a; Chapinal et al., 2011; McArt et al., 2013b). Several risk factors for postpartum ketosis have been determined. McArt et al. (2013a) showed that cows with increased BCS, a male calf, increased prepartum NEFA (≥0.30 mEq/L), decreased calving ease, stillbirth, and increased parity had higher risk of developing ketosis during the first 16 d postpartum. Moreover, a previous study observed a negative correlation between prepartum DMI and subclinical ketosis postpartum, and 1-kg decrease in the average daily DMI prepartum increased the risk of subclinical ketosis 2.2 times (Goldhawk et al., 2009). Nonetheless, a comprehensive study of the association of prepartum DMI, including DMI as a percentage of BW (**DMI%BW**), and prepartum energy balance (**EB**) with ketosis postpartum is still lacking.

Furthermore, clinical mastitis in one of the main diseases on dairy farms in the United States, with an incidence that ranges from 16 to 27% (USDA-NAHMS, 2018). Clinical mastitis is associated with reduced milk yield and fertility and increased culling, causing substantial economic losses to dairy farms (Santos et al., 2004; Hadrich et al., 2018). The average cost per case of clinical mastitis ranges from US$95 to $211, depending on the etiology (Bar et al., 2008; Cha et al., 2011).

Cows with ketosis have higher odds of having clinical mastitis (Raboisson et al., 2014). Immune cells are exposed to high NEFA and BHB concentrations during the first weeks of lactation, which has been shown to be associated with decreased neutrophil function (Suriyasathaporn et al., 1999; Hammon et al., 2006). Suriyasathaporn et al. (2000) proposed that hyperketonemia affects the udder defense by affecting leukocyte phagocytosis, cytokine production, and migration. This is in agreement with the results from Hammon et al. (2006), who showed that the killing ability of neutrophils was negatively correlated with NEFA concentrations in the week of calving. In addition, clinical mastitis postpartum has been linked to decreased glucose and increased NEFA and BHB concentrations prepartum (Jánosi et al., 2003; Moyes et al., 2009; Schwegler et al., 2013).

Given the relationship of NEFA and BHB with clinical mastitis and the association between DMI, EB, NEFA, and BHB, we hypothesized that a reduction in DMI%BW and EB during the transition period would be associated with ketosis and clinical mastitis postpartum. Therefore, our main objective was to evaluate the association of pre- and postpartum DMI%BW and EB with ketosis and clinical mastitis postpartum. A secondary objective was to evaluate the use of prepartum DMI%BW and EB as predictors of ketosis and clinical mastitis postpartum.

MATERIALS AND METHODS

Study design, housing, measurement, and calculation of DMI, milk yield, BW, BCS, and EB are described in detailed in a companion paper (Pérez-Báez et al., 2019). In summary, data were from a total of 476 cows (139 primigravid and 337 multigravid) from 9 experiments conducted at the University of Florida dairy unit, located in the city of Hague, Florida. This was a convenience sample; therefore, no a priori sample size calculation was performed. For continuous variables, approximately 200 cows in the affected group (ketosis, n = 198; Table 1) would be needed to detect statistical differences with an effect size of 0.2 (e.g., difference in DMI of 0.8 kg/d when prepartum SD is 4 kg of DMI/d), α of 0.05, and β of 0.2. In the case of clinical mastitis (n = 79; Table 1), we would be able to detect statistical differences with an effect size of 0.3 (e.g., difference in DMI of 1 kg/d when prepartum SD is 4 kg of DMI/d), α of 0.05, and β of 0.2.

Health Disorders

Detailed paper and electronic health records were kept for each cow. Each cow underwent a complete physical examination before enrollment in the initial trials, and cows showing signs of disease or disorders such as mastitis, lameness, digestive disorders, or pneumonia were not enrolled in the trials. Additionally, each cow underwent scheduled complete physical examinations by a trained herdsperson or by a veterinarian from the College of Veterinary Medicine Food Animal Reproduction and Medicine Service (**FARMS**) at the University of Florida on d 4, 7, and 12 postpartum. Furthermore, cow attitude was monitored daily prepartum by a member of the research team when cows were individually fed at 0600 and 1800 h and throughout the day when feed was pushed manually using a shovel every 2 h from 0800 to 2000 h. Any cow showing signs of depression, inappetence, lethargy, altered stride, or inflammation of the mammary gland underwent a physical examination by a trained herdsperson or by a FARMS veterinarian. Cows that became sick during the prepartum period were excluded. In addition to cow attitude, daily milk yield was monitored postpartum, and cows with a decrease in milk yield greater than 10% underwent a physical examination by a trained herdsperson or by a FARMS veterinarian. The veterinarians from FARMS performed physical examinations and provided supervi-

sion and training of herd personnel performing clinical diagnosis and treatment of postpartum cows at least once a week. Additionally, FARMS veterinarians were called to assist with or confirm clinical diagnosis or treatment of postpartum cows throughout the weekdays and weekends. Only disease events occurring during the first 28 DIM were used in this study. We first retrieved the electronic health records and then confirmed the information using the paper health records. Cows with mismatched information or with a disease diagnosis prepartum were excluded from the study. The health disorders recorded were ketosis, clinical mastitis, calving disorders (dystocia, twins, stillbirths), and metritis. Ketosis was defined as the presence of acetoacetate in urine that resulted in any color change [5 mg/dL (trace) or higher] in the urine test strip (Ketostix, Bayer, Leverkusen, Germany). The test strip has 90% sensitivity and 86% specificity using blood BHB concentration ≥1.4 mmol/L (Carrier et al., 2004). This means that after each test, 10% of the cows with ketosis would not be diagnosed with ketosis and 14% of the cows without ketosis would be diagnosed with ketosis. The chance of classifying cows as not having ketosis was decreased even further because cows were systematically tested at 4, 7, and 12 d postpartum. Of the cows never diagnosed with ketosis (n = 287), 17.4, 15.3, and 25.4% never produced a urine sample at 4, 7, and 12 d postpartum, respectively. Nine cows (3.1%) never produced a urine sample; therefore, they were removed from the analysis. Misclassification in this scenario would bias the estimates toward the null hypothesis. Cows were also tested for ketosis if they showed signs of depression, inappetence, lethargy, or a decrease in milk yield greater than 10%. On average, cows were tested 2.4 ± 0.72 (range: 0–3) times during the first 4 wk of lactation. Herein, no attempt was made to distinguish between subclinical and clinical ketosis. Clinical mastitis was diagnosed if milk from 1 or more quarters was abnormal in color, viscosity, or consistency, with or without accompanying heat, pain, redness, or swelling of the quarter, or generalized illness. Trained farm employees actively diagnosed clinical mastitis during forestripping at each milking, and it was confirmed by the herdsperson, the FARMS veterinarians, or both. Cows diagnosed with mild or moderate clinical mastitis were treated with intramammary antibiotics. Cows with severe clinical mastitis also received intramuscular antibiotics, intravenous nonsteroidal anti-inflammatories, and hypertonic saline solution in addition to intramammary antibiotics. Detailed information about calving and uterine disorders is presented in a separate companion paper (Pérez-Báez et al., 2019). Cows suffering from ketosis, clinical mastitis, or metritis were treated according to the farm standard operating procedure (http://animal.ifas.ufl.edu/facilities/du/).

Statistical Analysis

To evaluate the association of prepartum and postpartum DMI%BW and EB with ketosis and clinical mastitis, we analyzed the data using ANOVA for repeated measures using the MIXED procedure of

Table 1. Frequency of ketosis and mastitis by study diagnosed during the first 28 d postpartum

Study	Disease	Frequency	Percentage, %	MPP[1]	IQR[2]
1	Mastitis	3	0.6	6 (2–14)	6
	Ketosis	38	8.0	7 (2–28)	8
2	Mastitis	9	1.9	9.5 (1–28)	15.5
	Ketosis	17	3.6	4 (4–12)	3
3	Mastitis	4	0.8	4.5 (1–9)	2.3
	Ketosis	3	0.6	7 (4–13)	4.5
4	Mastitis	4	0.8	2 (1–7)	1.5
	Ketosis	7	1.5	7 (3–12)	3
5	Mastitis	17	3.6	6 (1–26)	5
	Ketosis	9	1.9	7 (1–9)	3
6	Mastitis	5	1.1	4 (2–8)	4
	Ketosis	16	3.4	7 (4–13)	4
7	Mastitis	20	4.2	8 (1–22)	3
	Ketosis	19	4.0	7 (1–12)	3
8	Mastitis	9	1.9	4 (1–26)	6
	Ketosis	36	7.6	6 (1–26)	7.3
9	Mastitis	8	1.7	13 (4–27)	11
	Ketosis	44	11.7	7 (1–27)	3
Total cases	Mastitis	79	16.5	7 (1–28)	6.3
	Ketosis	189	39.7	7 (1–28)	5

[1]Median days postpartum when the disease was diagnosed (minimum–maximum in parentheses).
[2]Interquartile range.

SAS version 9.4 (SAS Institute Inc., Cary, NC). The data were divided into 2 periods, prepartum and postpartum. The dependent variables were prepartum DMI%BW and EB and postpartum DMI%BW, EB, and ECM. The independent variable was 1 of the 2 disorders (ketosis or clinical mastitis), and they were modeled separately; cows that developed ketosis were compared with cows that did not develop ketosis, and cows that developed clinical mastitis were compared with cows that did not develop clinical mastitis. Cows that did not develop ketosis could have developed any other disorder, including clinical mastitis. Likewise, cows that did not develop clinical mastitis could have developed any other disorder. Other studies have used healthy cows as the comparison group (Huzzey et al., 2007). However, this would introduce selection bias; therefore, this could artificially increase the differences in the measures of DMI between the groups and inflate the estimates in a prediction model. Although the focus of this study was the comparison between cows affected by ketosis and clinical mastitis and unaffected cows, a comparison with healthy cows (i.e., cows that did not have any disorder diagnosed in the first 28 d postpartum) was also performed for comparison with the previous literature. The models also included the fixed effects of parity (primigravid vs. multigravid), BCS in the last week prepartum (<3.75 vs. ≥3.75), day relative to calving (prepartum: d −21 to −1; postpartum: d 1 to 28), heat stress abatement (cool, hot without evaporative cooling, and hot with evaporative cooling), and 2-way interactions between disorder and other covariates, and cow was nested within experiment as a random effect. First-order autoregressive, compound symmetry, and unstructured covariance structures were tested, and the first-order autoregressive was selected because it resulted in the smallest Akaike's information criterion.

As an example, the initial model to evaluate the association between prepartum DMI%BW and ketosis was

$$\begin{aligned}\text{DMI\%BW prepartum} &= \text{ketosis} + \text{day}\\ &+ \text{heat stress abatement} + \text{BCS} + \text{parity} + \text{ketosis}\\ &\times \text{day} + \text{ketosis} \times \text{season} + \text{ketosis} \times \text{BCS}\\ &+ \text{ketosis} \times \text{parity} + \text{cow (experiment)}.\end{aligned}$$

The disorder of interest was forced into the model, but other variables were removed from the model by stepwise backward elimination according to Wald statistics criterion when $P > 0.05$. When an interaction was detected, mean separation was assessed using the SLICE option in the MIXED procedure, and multiple comparisons were performed using the Tukey-Kramer adjustment method in SAS.

To evaluate the use of prepartum DMI%BW and EB as predictors of ketosis and clinical mastitis, each disorder was considered the dependent variable and DMI%BW and EB were considered as independent variables. These data were analyzed by logistic regression with the GLIMMIX procedure of SAS. The objective was to assess whether measures of prepartum DMI%BW or EB were associated with the odds of ketosis or clinical mastitis. In this case, each disease or disorder was the dependent variable, and the measures of prepartum DMI%BW or EB were assessed separately in different models as independent variables. For this purpose, the variables average DMI%BW or EB in the last 14, 7, and 3 d prepartum and the reduction from d −8 to −1 and from d −4 to −1 were created. Univariable and multivariable models were performed. The univariable models included cow nested within experiment as a random variable. Measures of DMI%BW or EB with $P < 0.20$ were selected for inclusion in the multivariable logistic regression models. Multivariable models also included parity (primigravid vs. multigravid), prepartum BCS (<3.75 vs. ≥3.75; Gearhart et al., 1990), and heat stress abatement (cool, hot without evaporative cooling, and hot with evaporative cooling) and cow nested within experiment as a random effect. Two-way interaction terms of measures of DMI%BW and EB with $P \leq 0.05$ and other covariates were tested. A stepwise backward elimination was performed, and explanatory variables with $P > 0.05$ according to the Wald statistics criterion were removed from the model.

When a measure of DMI%BW or EB prepartum was found to be significant ($P \leq 0.05$) after addition to the logistic regression model containing other covariates, we assessed its contribution to the predictive ability of the logistic regression model by comparing the area under the curve (**AUC**) of a receiver operating characteristic curve of the model with and without the measures of DMI%BW or EB using the ROCCONTRAST statement of the LOGISTIC procedure of SAS as previously reported (Vergara et al., 2014). An AUC ≤0.50 was considered noninformative, AUC between 0.50 and 0.70 was considered to have low accuracy, AUC between 0.70 and 0.90 was considered accurate, and AUC between 0.9 and 1.0 was considered highly accurate (Swets, 1988). Finally, we determined cut-off values for measures of DMI%BW and EB prepartum with $P \leq 0.05$ for predicting ketosis and clinical mastitis postpartum using receiver operating characteristic curves, and the cut-off with the greatest Youden's J statistic, which combines the values for sensitivity and specificity, was chosen. The sensitivity, specificity, and

overall accuracy of applying the cut-off to predict ketosis and clinical mastitis were calculated. Statistical significance was considered when $P \leq 0.05$. The main limitation in this study is that the time-order of disease relative to DMI%BW and ECM is inconsistent such that postpartum outcomes were measured before and after disease, which was diagnosed at variable intervals after calving. A limitation of the current study is that we did not perform external validation of our predictive models; therefore, future validation studies are needed.

An additional ANOVA for repeated measures was performed to evaluate which disease or disorder had the strongest association with prepartum DMI%BW and EB. The dependent variables were prepartum DMI%BW or EB. As independent variables we included all of the diseases or disorders evaluated postpartum (i.e., calving disorders, metritis, ketosis, digestive disorders, mastitis, and lameness) and other covariates such as parity, BCS, day relative to parturition, and heat stress abatement. Cow was nested within experiment as a random effect. Independent variables were removed from the model by stepwise backward elimination according to Wald statistics criterion when $P > 0.05$.

RESULTS

The frequency of ketosis and clinical mastitis is depicted in Table 1. Results for the comparison between cows that developed ketosis or clinical mastitis and healthy cows are presented in the supplemental files (https://doi.org/10.3168/jds.2018-15879). The association of pre- and postpartum DMI with ketosis and mastitis is shown in Supplemental Table S1. The association of pre- and postpartum DMI, DMI%BW, EB, and ECM with ketosis and mastitis compared with healthy cows is shown in Supplemental Tables S2 and S3, respectively. The association of pre- and postpartum DMI with ketosis and mastitis is shown in Supplemental Figure S1. The interaction between ketosis and parity on postpartum DMI is shown in Supplemental Figure S2. The association of pre- and postpartum DMI, DMI%BW, EB, and ECM with ketosis compared with healthy cows is shown in Supplemental Figure S3. The interaction between ketosis and parity on prepartum DMI and EB compared with healthy cows is shown in Supplemental Figure S4. The interaction between ketosis and parity on ECM compared with healthy cows is shown in Supplemental Figure S5. Dry matter intake (% of BW) according to disease status related to ketosis is shown in Supplemental Figure S6. The association of pre- and postpartum DMI, DMI%BW, EB, and ECM with mastitis compared with healthy cows is shown in Supplemental Figure S7. Dry matter intake (% of BW) according to disease status related to mastitis is shown in Supplemental Figure S8.

Association of Prepartum DMI%BW and EB with Ketosis

Ketosis was associated with a lesser DMI%BW ($P < 0.01$) in the last 3 wk prepartum (Table 2). There was an interaction ($P < 0.01$) with day, which showed that DMI%BW for cows that developed ketosis was lesser compared with cows that did not develop ketosis from d −17 to −1 ($P \leq 0.01$; Figure 1A). There was an interaction ($P < 0.01$) between ketosis and day on EB prepartum (Table 2). Cows with ketosis had lesser EB on d −5, −3, −2, and −1 (Figure 1B). There was an interaction ($P = 0.04$) between ketosis and parity on EB prepartum. The EB for primigravid cows that developed ketosis was similar to that for primigravid cows that did not develop ketosis (3.4 ± 0.7 vs. 2.9 ± 0.4 Mcal/d; $P = 0.56$; Figure 2A), whereas the EB for multigravid cows that developed ketosis was lesser than that for multigravid cows that did not develop ketosis (1.6 ± 0.3 vs. 2.9 ± 0.3 Mcal/d; $P < 0.01$; Figure 2B).

Prepartum DMI%BW and EB as Predictors of Ketosis

Of the variables evaluated, parity and heat stress abatement were the only predictors of ketosis postpartum. Multigravid cows had increased odds of developing ketosis postpartum compared with primigravid cows (odds ratio, **OR**: 4.7; 95% CI: 2.3–7.9; $P < 0.01$). Cows that were in heat stress without evaporative cooling during the prepartum period had lower odds of developing ketosis postpartum compared with cows that were in heat stress with evaporative cooling (OR: 0.39; 95% CI: 0.2–0.7; $P < 0.01$), but the odds of having ketosis were not different between cows that were not in heat stress and cows that were in heat stress with evaporating cooling (OR: 1.5; 95% CI: 0.9–2.4; $P = 0.10$). The average DMI%BW and EB during the last 3 d prepartum were explanatory variables for ketosis. For each 0.1-percentage point decrease in the average DMI%BW in the last 3 d prepartum, the odds of having ketosis increased by 8%. For each 1-Mcal decrease in the average EB in the last 3 d prepartum, the odds of having ketosis increased by 5% (Table 3).

The AUC from the model containing parity and heat stress abatement was 0.63 (95% CI: 0.58–0.68) and increased to 0.72 (95% CI: 0.68–0.76) when the average DMI%BW in the last 3 d prepartum was included in the ketosis-predicting model. The AUC increased

Table 2. Association of prepartum (−21 to −1 d) and postpartum (1 to 28 d) DMI, DMI as percentage of BW (DMI%BW), energy balance (EB), and ECM with ketosis (Ket) postpartum according to multivariable analysis[1]

Item	Prepartum		*P*-value			Postpartum		*P*-value		
	Ket	No Ket	Ket	Day	Ket × day	Ket	No Ket	Ket	Day	Ket × day
DMI%BW	1.54 ± 0.03	1.67 ± 0.02	<0.01	<0.01	<0.01	2.45 ± 0.04	2.84 ± 0.03	<0.01	<0.01	<0.01
EB, Mcal/d	2.6 ± 0.4	2.9 ± 0.2	0.29	<0.01	<0.01	−8.1 ± 0.5	−3.2 ± 0.3	<0.01	<0.01	<0.01
ECM, kg/d	—	—	—	—	—	37.2 ± 1.0	32.6 ± 0.6	<0.01	<0.01	<0.01

[1]Day = day relative to parturition; ket × day = interaction between ketosis and day. Values are LSM ± SEM.

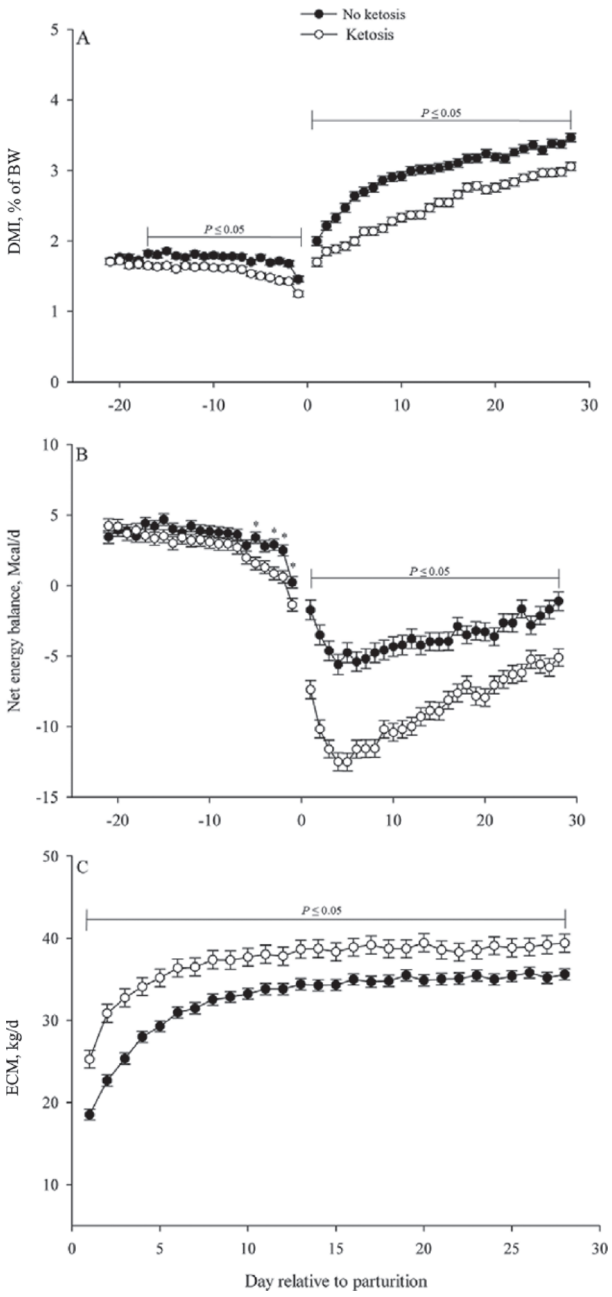

Figure 1. Association of ketosis postpartum (n = 189) with (A) DMI (% of BW), (B) energy balance (EB, Mcal/d) during the transition period (from −21 to 28 d), and (C) ECM (kg/d) during the first 28 d postpartum. Values are LSM ± SEM. Prepartum DMI (% of BW): ketosis, $P < 0.01$; day relative to parturition, $P < 0.01$; interaction between ketosis and day, $P < 0.01$. Prepartum EB: ketosis, $P = 0.29$; day relative to parturition, $P < 0.01$; interaction between ketosis and day, $P < 0.01$. Postpartum DMI (% of BW): ketosis, $P < 0.01$; day relative to parturition, $P < 0.01$; interaction between ketosis and day, $P < 0.01$. Postpartum EB: ketosis, $P < 0.01$; day relative to parturition, $P < 0.01$; interaction between ketosis and day, $P < 0.01$. ECM: ketosis, $P < 0.01$; day relative to parturition, $P < 0.01$; interaction between ketosis and day, $P < 0.01$. The main limitation in this study is that the time-order of disease relative to DMI and milk yield is inconsistent such that postpartum outcomes were measured before and after disease, which was diagnosed at variable intervals after calving. *$P \leq 0.05$.

Table 3. Effect of each unit decrease in average prepartum DMI, each 0.1-percentage point decrease in average DMI as percentage of BW (DMI%BW), and each unit decrease in average energy balance (EB) in the last 3 d prepartum on postpartum ketosis and clinical mastitis in the first 28 d postpartum

	DMI%BW			EB, Mcal/d		
Disorder	OR[1]	95% CI	*P*-value	OR	95% CI	*P*-value
Ketosis	1.08	1.03–1.13	<0.01	1.05	1.01–1.10	0.03
Clinical mastitis	1.10	1.03–1.16	<0.01	1.08	1.02–1.14	<0.01

[1]Odds ratio.

from 0.63 to 0.72 (95% CI: 0.67–0.76) when EB was included in the ketosis-predicting model. The AUC was different ($P \leq 0.05$) in both model comparisons. The average DMI%BW and EB during the last 3 d prepartum produced ($P < 0.01$) cut-offs to predict ketosis postpartum, which were ≤1.5%/d and ≤1.1 Mcal/d, respectively (Table 4).

Association of Postpartum DMI%BW, EB, and ECM with Ketosis

During the postpartum period, cows that developed ketosis had lesser DMI%BW than cows that did not develop ketosis ($P < 0.01$; Table 2). Although there was an interaction ($P < 0.01$) between ketosis and day on postpartum DMI%BW, the DMI%BW for cows that developed ketosis was less than that for cows that did not develop ketosis throughout the entire postpartum period, with the difference being more pronounced from d 5 to 17 (Figure 1A).

Cows that developed ketosis had lesser EB ($P < 0.01$) compared with cows that did not develop ketosis (Table 2). Although there was an interaction ($P < 0.01$) between ketosis and day on postpartum EB, the EB for cows that developed ketosis was less ($P < 0.01$) than that for cows that did not develop ketosis throughout the entire postpartum period, with the difference being more pronounced from d 1 to 8 (Figure 1B).

The ECM for cows that developed ketosis was greater ($P < 0.01$) than that for cows that did not develop ketosis (Table 2). There was an interaction ($P < 0.01$) between ketosis and day on ECM, which showed that cows that developed ketosis had greater ECM compared with cows that did not develop ketosis throughout the entire postpartum period, with the difference being more pronounced from d 1 to 7 ($P \leq 0.05$; Figure 1C). There was an interaction ($P < 0.01$) between ketosis and parity on ECM. The ECM for primigravid cows that developed ketosis was greater than that for primigravid cows that did not develop ketosis (38.1 ± 1.8 vs. 28.6 ± 0.9 kg/d; $P < 0.01$; Figure 3A), whereas the ECM for multigravid cows that developed ketosis was similar to that for multigravid cows that did not develop ketosis (36.2 ± 0.8 vs. 36.6 ± 0.8 kg/d; $P = 0.72$; Figure 3B).

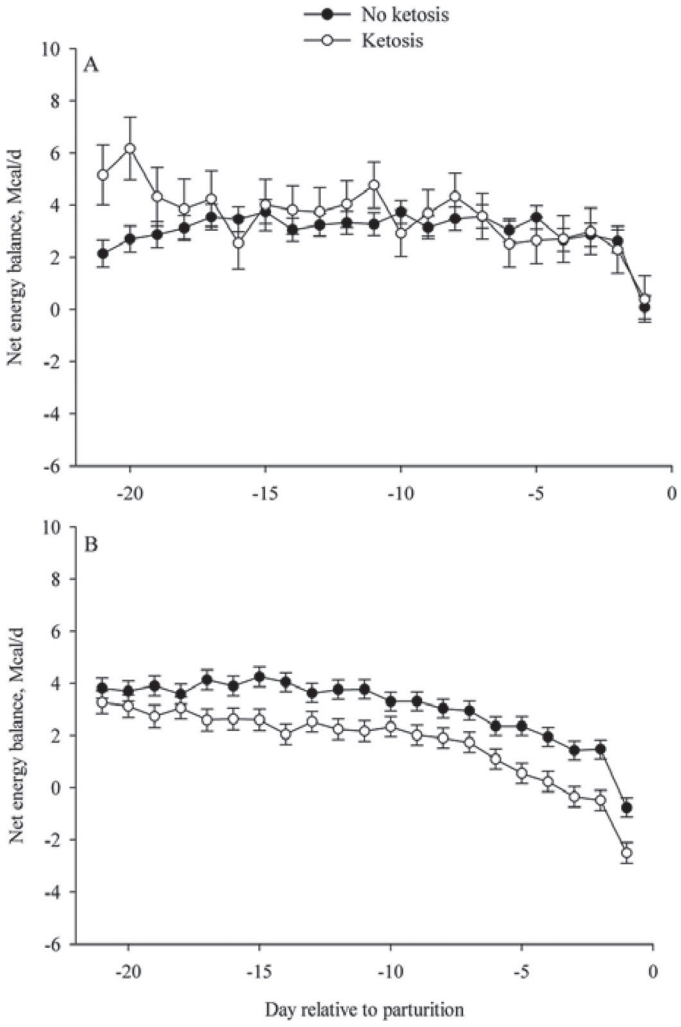

Figure 2. Interaction ($P \leq 0.01$) between ketosis and parity on energy balance (Mcal/d) in (A) primigravid and (B) multigravid cows during the prepartum period (from −21 to −1 d). Values are LSM ± SEM.

Table 4. Cut-offs of DMI as percentage of BW (DMI%BW) and energy balance (EB) to predict ketosis postpartum[1]

Item	Cut-off	Se, %	Sp, %	PPV, %	NPV, %	Acc, %	AUC	*P*-value
DMI%BW	≤1.5	71	51	48	74	64	0.63	<0.01
EB, Mcal/d	≤1.1	65	53	47	71	61	0.60	<0.01

[1]Se = sensitivity; Sp = specificity; PPV = positive predicted value; NPV = negative predictive value; Acc = accuracy; AUC = area under the curve.

Association of Prepartum DMI%BW and EB with Clinical Mastitis

During the prepartum period, cows that developed clinical mastitis had lesser DMI%BW compared with cows that did not develop clinical mastitis (1.56 ± 0.04 vs. 1.65 ± 0.02%/d; $P = 0.05$; Table 5). Clinical mastitis was not associated with prepartum EB, although cows that had clinical mastitis had numerically lower EB than cows that did not have clinical mastitis (1.8 ± 0.4 vs. 2.6 ± 0.2; $P = 0.08$ Mcal/d; Table 5).

Prepartum DMI%BW and EB as Predictors of Clinical Mastitis

Of the covariates evaluated, parity and heat stress abatement were the only significant predictors of clinical mastitis postpartum. Multigravid cows had increased odds of developing clinical mastitis postpartum compared with primigravid cows (OR: 2.5; 95% CI: 1.1–4.9; $P = 0.03$). Cows that were not in heat stress prepartum had decreased odds of developing clinical mastitis postpartum compared with cows in heat stress with (OR: 0.3; 95% CI: 0.1–0.6; $P < 0.01$) or without (OR: 0.2; 95% CI: 0.1–0.5; $P < 0.01$) evaporating cooling. There was no difference between cows that were in heat stress with and without evaporating cooling (OR: 0.8; 95% CI: 0.5–1.5; $P = 0.56$). Of the measures of DMI evaluated, the average DMI%BW and EB in the last 3 d prepartum were the only significant explanatory variables for clinical mastitis. For each 0.1-percentage point decrease in average DMI%BW in the last 3 d prepartum, the odds of developing clinical mastitis increased by 10%. For each 1-Mcal decrease in the average EB in the last 3 d prepartum, the odds of having clinical mastitis increased by 8% (Table 3).

When the average DMI%BW and EB in the last 3 d prepartum were included in the clinical mastitis-predicting model containing only parity and heat stress abatement as explanatory variables, the AUC increased from 0.69 (95% CI: 0.64–0.73) to 0.71 (95% CI: 0.68–0.76) and from 0.69 to 0.72 (95% CI: 0.63–0.76), respectively. The differences between the AUC were not statistically significant ($P \geq 0.10$). The average DMI%BW and EB during the last 3 d prepartum produced significant ($P < 0.01$) cut-offs to predict clinical mastitis postpartum, which were ≤1.2%/d and ≤1.0 Mcal/d, respectively (Table 6).

Association of Postpartum DMI%BW, EB, and ECM with Clinical Mastitis

During the postpartum period, cows that developed clinical mastitis had lesser postpartum DMI%BW ($P < 0.01$) compared with cows that did not develop clinical mastitis (2.55 ± 0.07 vs. 2.76 ± 0.03%/d; $P < 0.01$; Table 5). The interaction ($P < 0.01$) between clinical mastitis and day relative to calving was significant, which showed that postpartum DMI%BW for cows that developed clinical mastitis was less than that for cows that did not develop clinical mastitis from d 3 to 15 ($P \leq 0.05$) and on d 17 ($P < 0.01$) postpartum (Figure 4A). Cows that developed clinical mastitis had similar postpartum EB compared with cows that did not develop clinical mastitis (−3.8 ± 0.7 vs. −4.9 ± 0.4 Mcal/d; $P = 0.17$; Table 5). However, the interaction ($P < 0.01$) between clinical mastitis and day relative to calving was significant, which showed that cows that developed clinical mastitis had greater postpartum EB on d 18 ($P = 0.05$), from d 21 to 23 ($P \leq 0.05$), and on d 26 ($P = 0.05$) compared with cows that did not develop clinical mastitis (Figure 4B). The ECM for cows that developed clinical mastitis was less ($P < 0.01$) than that for cows that did not develop clinical mastitis (28.7 ± 1.3 vs. 33.6 ± 0.6 kg/d; $P < 0.01$; Table 5). The interaction ($P < 0.01$) between clinical mastitis and day on ECM was significant, which showed that cows that developed clinical mastitis had lesser ECM throughout the entire postpartum period, being more pronounced from d 5 to 10 ($P \leq 0.05$), compared with cows that did not develop clinical mastitis (Figure 4C).

Association of Prepartum DMI%BW and EB with Postpartum Disorders

When all diseases or disorders were included in the model, ketosis remained associated with prepartum DMI%BW and EB ($P \leq 0.01$) and mastitis remained associated with prepartum DMI%BW ($P = 0.05$; Table

Table 5. Association of prepartum (−21 to −1 d) and postpartum (1 to 28 d) DMI, DMI as percentage of BW (DMI%BW), energy balance (EB), and ECM with clinical mastitis postpartum according to multivariable analysis[1]

Item	Prepartum		*P*-value			Postpartum		*P*-value		
	Mastitis	No mastitis	Mastitis	Day	Mastitis × day	Mastitis	No mastitis	Mastitis	Day	Mastitis × day
DMI%BW	1.56 ± 0.04	1.65 ± 0.02	0.05	<0.01	0.56	2.55 ± 0.07	2.76 ± 0.03	<0.01	<0.01	<0.01
EB, Mcal/d	1.8 ± 0.4	2.6 ± 0.2	0.08	<0.01	0.39	−3.8 ± 0.7	−4.9 ± 0.4	0.17	<0.01	<0.01
ECM, kg/d	—	—	—	—	—	28.7 ± 1.3	33.6 ± 0.6	<0.01	<0.01	<0.01

[1]Day = day relative to parturition; mastitis × day = interaction between mastitis and day.

6; Perez et al., unpublished), and the direction of the association was the same as reported herein. Metritis was associated with prepartum EB ($P = 0.04$; Table 6 of Perez et al., 2019).

DISCUSSION

In this study we showed that cows that developed ketosis or mastitis had decreased prepartum DMI%BW and that the average DMI%BW and EB in the last 3 d prepartum were predictive of ketosis and clinical mastitis, although the effect sizes were small. Furthermore, cut-offs for prediction of ketosis and clinical mastitis were established, although the accuracy was low. Postpartum DMI%BW and EB were decreased in cows that developed ketosis, whereas ECM was increased in primiparous cows that developed ketosis. Postpartum

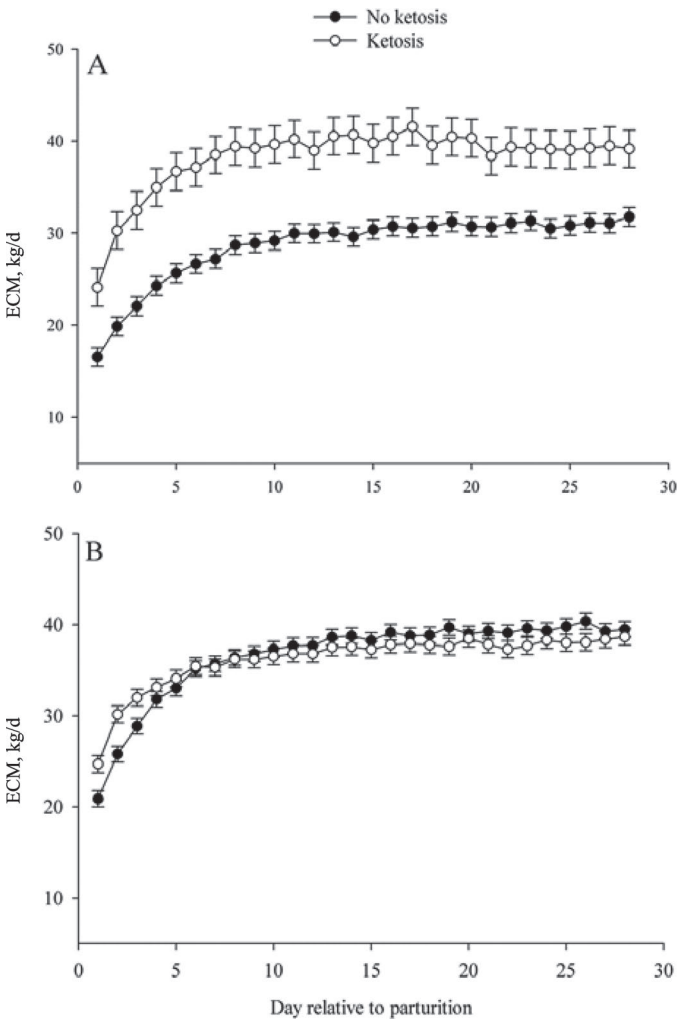

Figure 3. Interaction ($P < 0.01$) between ketosis and parity on ECM (kg/d) in (A) primigravid and (B) multigravid cows during the postpartum period (from 1 to 28 d). Values are LSM ± SEM.

Table 6. Cut-offs of DMI as percentage of BW (DMI%BW) and energy balance (EB) to predict mastitis postpartum[1,2]

Item	Cut-off	Se, %	Sp, %	PPV, %	NPV, %	Acc, %	AUC	*P*-value
DMI%BW	≤1.2	50	66	23	87	64	0.61	<0.01
EB, Mcal/d	≤1.0	67	50	21	88	53	0.59	<0.01

[1]Se = sensitivity; Sp = specificity; PPV = positive predicted value; NPV = negative predictive value; Acc = accuracy; AUC = area under the curve.

[2]*P*-values ≤0.05 were considered significant.

DMI%BW and ECM were decreased in cows that developed clinical mastitis. Previous studies showed that cows with subclinical ketosis had reduced DMI during the last week prepartum (Goldhawk et al., 2009). Dry matter intake as percentage of BW prepartum was lesser for cows that developed ketosis postpartum starting from d −17 prepartum, but when we analyzed the association between ketosis and absolute DMI, DMI was similar in cows with and without ketosis (Supplemental Figure S1, https://doi.org/10.3168/jds.2018-15879). Cows that developed ketosis had similar prepartum DMI but lower DMI%BW compared with cows that did not develop ketosis because cows with ketosis were 72 kg heavier than cows that did not develop ketosis (729 ± 87 vs. 657 ± 104); this was true for both primigravid and multigravid cows (data not shown). Therefore, after controlling for BW, DMI was reduced in cows that developed ketosis compared with the ones that did not develop ketosis. Furthermore, other researchers have shown that overconditioned cows are known to have a greater decrease in DMI prepartum and postpartum and to be predisposed to ketosis postpartum (Rukkwamsuk et al., 1999; Gillund et al., 2001).

Although DMI%BW prepartum was lesser for cows that developed ketosis and the average DMI%BW in the last 3 d prepartum was a significant explanatory variable for ketosis, the effect size was quite modest. For each 0.1-percentage point decrease in the average DMI%BW in the last 3 d prepartum, there was an increase of 8% in the odds of having ketosis postpartum. The effect size for EB was similarly modest: for each 1-Mcal decrease in the average EB in the last 3 d prepartum, there was an increase of 5% in the odds of having ketosis. This contrasts with the results by Goldhawk et al. (2009), who observed that for each 1-kg decrease in DMI in the last week prepartum, there was an increase of 120% in the odds of having subclinical ketosis. It is not clear why the discrepancy exists, but a few differences between studies are worth discussing. First, Goldhawk et al. (2009) included cows with ketosis (BHB ≥1.0 mmol/L) in the first week of lactation but excluded cows from the study if BHB remained above 1.4 mmol/L in the second week postpartum. In our study, we included any cow with positive ketones in the urine during the first 4 wk of lactation, although 81% (153/189) developed ketosis in the first week of lactation. The stated purpose for removing cows with BHB ≥1.4 mmol/L in the second week of lactation in the study by Goldhawk et al. (2009) was to exclude cows with more severe or chronic ketosis, which we did not do in our study. Therefore, if anything, differences in DMI in our study should have been larger and not smaller. In the study by Goldhawk et al. (2009), cows that developed ketosis were compared with healthy cows, whereas in our study, cows that developed ketosis were compared with cows that did not develop ketosis but could have had any other disease. Nonetheless, when we compared cows that developed ketosis with healthy cows, we observed that DMI prepartum decreased only on d −3 and −2 in cows that developed ketosis (Supplemental Figure S3, https://doi.org/10.3168/jds.2018-15879). In addition, in the study by Goldhawk et al. (2009), 90% (9/10) of the cows were multigravid, whereas in our study 71% (337/476) were multigravid. We saw that multigravid cows had a greater decrease in absolute DMI prepartum (Supplemental Figure S2, https://doi.org/10.3168/jds.2018-15879), meaning that a higher proportion of multigravid cows in the study could increase the differences between cows that did and did not develop ketosis. Another difference between studies is the sample size; therefore, the small sample size in Goldhawk et al. (2009) could have led to common issues with small sample size such as inflated effect size and low reproducibility (Button et al., 2013).

An interesting finding was a decrease in the odds of ketosis in cows that were under heat stress without evaporating cooling during the prepartum period compared with cows that were under heat stress but with evaporative cooling and cows that were not under heat stress. This may be because cows that were under heat stress without evaporating cooling during the prepartum period produced approximately 5 kg/d less milk than cows that were under heat stress with evaporative cooling (Fabris et al., 2017).

In our study, we also evaluated the predictive ability of DMI%BW and EB. The predictive ability of the models for ketosis increased modestly, although significantly when the average DMI%BW and EB in the last

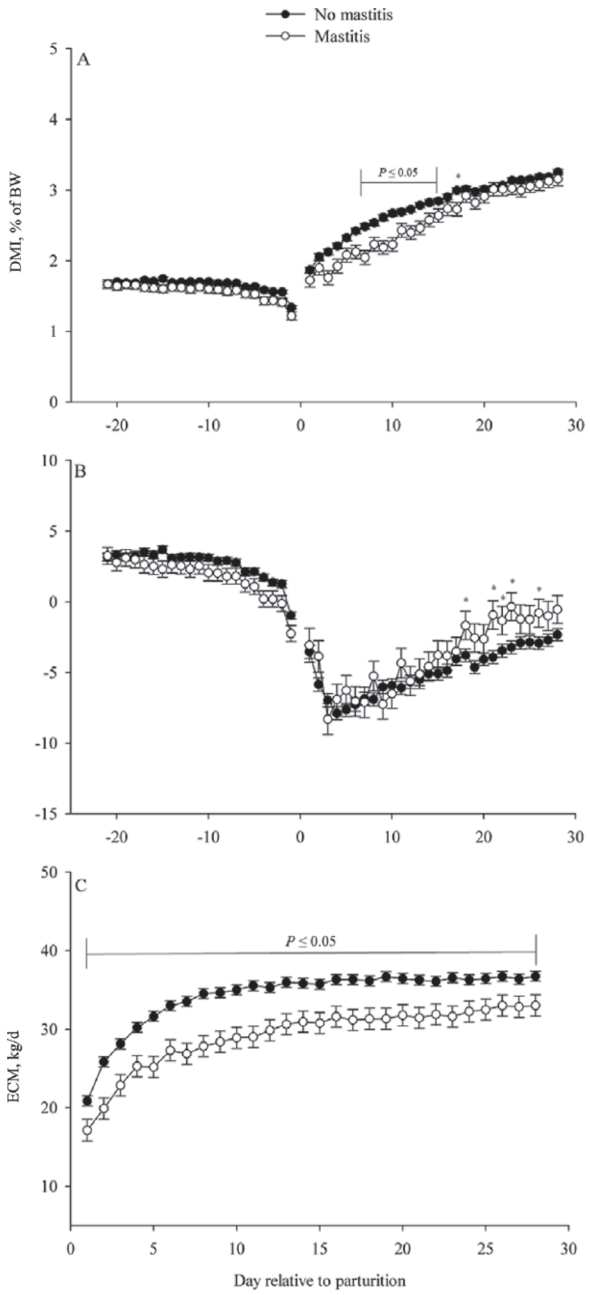

Figure 4. Association between mastitis (n = 79) and (A) DMI (% of BW), (B) energy balance (EB, Mcal/d) during the transition period (from −21 to 28 d), and (C) ECM (kg/d) during the first 28 d postpartum. Values are LSM ± SEM. Prepartum DMI (% of BW): mastitis, $P = 0.05$; day relative to parturition, $P < 0.01$; interaction between mastitis and day, $P = 0.56$. Prepartum EB: mastitis, $P = 0.08$; day relative to parturition, $P < 0.01$; interaction between mastitis and day, $P = 0.39$. Postpartum DMI (% of BW): mastitis, $P < 0.01$; day relative to parturition, $P < 0.01$; interaction between mastitis and day, $P < 0.01$. Postpartum EB: mastitis, $P = 0.17$; day relative to parturition, $P < 0.01$; interaction between mastitis and day, $P < 0.01$. ECM: mastitis, $P < 0.01$; day relative to parturition, $P < 0.01$; interaction between mastitis and day, $P < 0.01$. The main limitation in this study is that the time-order of disease relative to DMI and milk yield is inconsistent such that postpartum outcomes were measured before and after disease, which was diagnosed at variable intervals after calving. *$P \leq 0.05$.

3 d prepartum were independently included in the models containing other covariates. Therefore, although not as robust as previously reported, DMI%BW and EB in the last 3 d prepartum were shown to be predictors of ketosis. In addition, we determined cut-off values for prepartum DMI%BW and EB to see whether they could be used solely as a predictor of ketosis postpartum. The cut-off had accurate to low sensitivity (71–51%), low specificity (51–53%), low accuracy (64–61%), and low AUC (0.63–0.60). Values in parentheses are for DMI%BW and EB, respectively. Similar to our results, the cut-offs for prepartum NEFA (i.e., 0.26 mEq/L; sensitivity/specificity = 53/61%; AUC = 0.6) that were determined by Ospina et al. (2010b) to predict clinical ketosis postpartum had low sensitivity, specificity, and AUC. Therefore, although significant, these cut-offs are of limited applicability. In summary, DMI%BW and EB prepartum are significant but minor contributors to ketosis development postpartum and cannot be used reliably to identify cows that will develop ketosis postpartum.

During the postpartum period, cows that developed ketosis had lesser DMI%BW compared with cows that did not develop ketosis. Goldhawk et al. (2009) reported that cows that developed subclinical ketosis had on average 23% lower DMI in the first 2 wk postpartum compared with healthy cows. In our study, cows that developed ketosis had 12% (15.4 ± 4.6 vs. 13.8 ± 4.7 kg/d) lower DMI during the first 2 wk postpartum compared with cows that did not develop ketosis (Supplemental Figure S1, https://doi.org/10.3168/jds.2018-15879). We also compared cows that developed ketosis with healthy cows and showed that the decrease in DMI during the first 2 wk postpartum in cows that developed ketosis was 19% (16.8 ± 4.0 vs. 13.8 ± 4.7 kg/d), which is more similar to what was shown by Goldhawk et al. (2009). Another important point is that there was an interaction between ketosis and parity on ECM, showing that primigravid cows that developed ketosis produced 9.5 kg/d more ECM than primigravid cows that did not develop ketosis, whereas multigravid cows that developed ketosis had similar ECM compared with cows that did not develop ketosis. Therefore, primigravid cows that developed ketosis should be eating approximately 4.75 kg of DM/d assuming a 1:2 feed conversion of marginal milk per kilogram of DMI. Hence, when we evaluated EB, there was no interaction between parity and ketosis, and cows that developed ketosis had lower EB than cows that did not develop ketosis.

We showed that cows that developed clinical mastitis had decreased prepartum DMI%BW and that the average DMI%BW and EB in the last 3 d prepartum were predictors of clinical mastitis. Nonetheless, the ef-

fect sizes were small—10 and 8% increases in the odds of having clinical mastitis postpartum with each unit decrease in the measures of DMI%BW and EB, respectively. Moreover, the addition of DMI%BW and EB in the last 3 d prepartum to a clinical mastitis-predicting model did not significantly increase the predictive ability of the models as evaluated by the AUC, indicating that although DMI%BW and EB prepartum are significant predictors of clinical mastitis postpartum, their contribution is minor when accounting for other variables such as parity and heat stress prepartum. In addition, we determined cut-offs for DMI%BW and EB to see whether they could be used solely as a predictor of clinical mastitis postpartum, and the cut-offs resulted in low to moderate sensitivity (50–67%), specificity (66–50%), overall accuracy (64–53%), and AUC (0.61–0.59). This indicates that, although significant, these cut-offs have limited applicability. In summary, DMI%BW and EB prepartum are significant but minor contributors to clinical mastitis development postpartum and cannot be used reliably to identify cows that will develop clinical mastitis postpartum.

Furthermore, cows that develop clinical mastitis postpartum have been shown to have decreased glucose and increased NEFA and BHB concentrations prepartum (Moyes et al., 2009; Schwegler et al., 2013), which indicates a decreased DMI%BW and EB prepartum; however, to our knowledge, this is the first time that prepartum DMI%BW and EB were compared between cows that did and did not develop clinical mastitis. Previous research has shown that hyperketonemia impairs phagocytic, chemotactic, and killing ability of neutrophils and that immunosuppression peripartum is a predisposing factor for mastitis postpartum (Suriyasathaporn et al., 1999). Without a good chemotactic response, neutrophils may be less able to reach the mammary gland to fight infections, therefore predisposing cows to clinical mastitis.

During the postpartum period, the decrease in DMI%BW seen in cows that developed clinical mastitis may be explained by the inflammatory process and its association with proinflammatory cytokines such as TNF-α, IL-1, and IL-6, which lead to swelling, pain, fever, loss of appetite, and decreased feed intake (Dantzer et al., 1993; Swiergiel and Dunn, 1999; Alluwaimi, 2004). Interestingly, clinical mastitis was associated with greater EB on d 18 and 21 to 23 postpartum compared with cows that did not have clinical mastitis, although both groups were still in negative EB. This finding may be mainly because cows that developed clinical mastitis completely recovered DMI%BW after the second week of lactation but remained producing less ECM than cows that did not develop clinical mastitis. The persistent reduction in ECM among cows that developed clinical mastitis despite the recovery in DMI%BW is likely due to loss of function in the infected quarter or quarters caused by the infectious agent and by the inflammatory response against the infectious agent (Bradford et al., 2015).

A limitation of this study is that data were collected from different experiments over the years. Because different observers collected subjective data such as BCS in each trial, agreement among observers could not be compared, and treatments had been applied in each trial (i.e., heat stress abatement), which had to be controlled for in the statistical analysis. Another limitation is that the association of ketosis or mastitis with DMI%BW, EB, and ECM during postpartum period could not be evaluated before and after ketosis or mastitis diagnosis because dividing the data would have resulted in reduced sample size per day, therefore increasing the standard errors or producing unreliable standard errors. As an example, cows that developed ketosis or mastitis on d 1 would have 0 d of DMI before the disease diagnosis and 27 d of DMI after the disease diagnosis. For cows diagnosed with ketosis or mastitis from d 2 to 28, the number of days of DMI before the disease diagnosis would increase but the number of days of DMI after the disease diagnosis would decrease. Last, even if we divided our data before and after disease diagnosis, we could not infer causation because cows were not randomly assigned to develop ketosis or mastitis. Hence, herein we present the association between ketosis or mastitis development and DMI%BW and EB pre- and postpartum.

CONCLUSIONS

This study showed that ketosis and mastitis were associated with prepartum DMI%BW. The average DMI%BW and EB in the last 3 d prepartum were significant explanatory variables for ketosis and clinical mastitis postpartum, and the average DMI%BW and EB in the last 3 d prepartum significantly increased the predictive ability of ketosis-predicting models, although the effect sizes were small. Prepartum cut-offs for DMI%BW and EB to predict ketosis and clinical mastitis were established, although with low sensitivity, specificity, and overall accuracy. In addition, postpartum DMI%BW and EB were decreased in cows that developed ketosis, whereas ECM was increased in primiparous cows that developed ketosis. Postpartum DMI%BW and ECM were decreased in cows that developed clinical mastitis. The results of this study give a better understating of the role DMI%BW plays during the transition period; namely, when DMI%BW decreas-

es, the risk of ketosis and clinical mastitis increases, although the increase was small. The main limitation of this study was that the time-order of disease relative to DMI%BW and ECM was inconsistent such that postpartum outcomes were measured before and after disease, which was diagnosed at variable intervals after calving. In summary, DMI%BW and EB prepartum are significant but minor contributors to ketosis and clinical mastitis development postpartum and cannot be used reliably to identify cows that will develop ketosis and clinical mastitis postpartum.

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