Events of elevated somatic cell counts in high-producing dairy cows are associated with daily body weight loss in early lactation

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ABSTRACT

The objective of this study was to determine associations between body weight (BW) and body condition score (BCS) variables indicating a more severe negative energy balance in early lactation and events of somatic cell counts (SCC) >250,000 cells/mL and SCC >400,000 cells/mL in dairy cows. We studied lactations from 634 primiparous and 1,086 multiparous Israeli Holstein dairy cows originating from 7 commercial dairy farms. Generalized mixed models with a random herd effect were used to quantify the effects of BW and BCS variables in early lactation on events of elevated SCC. Data were analyzed using 2 different approaches. In the first approach, only first events in a lactation were taken into account, whereas in the second approach, all events in a lactation were analyzed and repeated events from the same cow were accounted for. Although no associations were found between the different BW and BCS variables and first events of elevated SCC, associations were present between these variables and events of elevated SCC when all events were analyzed. The cumulative incidence of a lactation with multiple events of SCC >250,000 cells/mL was 8.8 and 27.7% for primiparous and multiparous cows, respectively. The odds of an event of SCC >250,000 cells/mL were 25% greater for cows belonging to the upper quartile in relative BW loss from calving to nadir BW (loss >12.3, 15.0, and 15.7% for first-, second-, and third- parity and greater cows, respectively) compared with cows losing less relative BW. Odds of an event were 44% greater for cows with ketosis when compared with cows without. The cumulative incidence of a lactation with multiple events of SCC >400,000 cells/mL was 4.1 and 14.3% for primiparous and multiparous cows, respectively. The odds of an event of SCC >400,000 cells/mL were 43% greater for cows belonging to the upper quartile in relative BW loss from calving to nadir BW

compared with cows losing less relative BW. Odds of an event were 33% greater for cows with ketosis when compared with cows without. Assuming that extreme BW loss and ketosis in early lactation indicate a more severe negative energy balance, our findings support the hypothesis that greater negative energy balance in early lactation predisposes dairy cows to udder inflammation. Considering the fact that many of the events were recurring, we stress the importance of including all events in the analysis and postulate the possibility of long-term detrimental effects of negative energy balance on udder health.

Key words: body weight, dairy cow, body condition score, somatic cell count

INTRODUCTION

Negative energy balance (NEB) is considered a physiological phenomenon in high-yielding dairy cows in early lactation (Goff and Horst, 1997). In this period, metabolic and endocrine changes drive enhanced mobilization of body fat and skeletal muscle breakdown and favor diversion of absorbed metabolites to the mammary gland to supply sufficient substrates for milk synthesis. Because practically all cows undergo a state of NEB in early lactation and eventually recover from this state, Jorritsma et al. (2003) suggested the use of the term "adaptation to NEB." They define cows as less adapted when certain risk factors, such as a longer lasting or deeper calculated NEB or certain biochemical, endocrinological, or (sub)clinical characteristics, are present. Poor adaptation or poor responsiveness to NEB has been shown to be detrimental to reproductive performance (de Vries and Veerkamp, 2000; Reist et al., 2003a; Roche et al., 2007), milk production (Reist et al., 2003b), and health status (Collard et al., 2000; Knight, 2001; Bobe et al., 2004).

Nevertheless, relatively little has been published on the relationship between poor responsiveness to NEB and udder health, and the findings in these studies have often been inconclusive. Most published studies have concentrated on the relationship between NEB and

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clinical mastitis, and very few have focused on NEB and SCC.

Kremer et al. (1993) studied the clinical course of experimentally induced *Escherichia coli* mastitis in ketonemic cows compared with nonketonemic cows. Clinical symptoms were generally more severe in the ketonemic cows. Furthermore, whereas clinical symptoms in nonketonemic cows were negatively related to the preinfection chemotactic response of polymorphonuclear leukocytes, this was not the case in ketonemic cows. In contrast to these findings, Perkins et al. (2002) did not find an effect of feed restriction on the clinical symptoms of experimentally induced endotoxin mastitis. These results were similar to those reported by Kornalijnslijper et al. (2003), who found no correlations between the metabolic status, production level, or feeding regimen and the severity of experimental *E. coli* mastitis, except for relatively more severe mastitis in cows with BHBA concentrations above 1.4 mmol/L.

Collard et al. (2000) did not find an association between the occurrence of clinical mastitis (CM) and calculated NEB-related variables. Zadoks et al. (2001) reported no effect of BCS change on infection with *Staphylococcus aureus* or *Streptococcus uberis*. On the other hand, Ruegg and Milton (1995) found that cows that were diagnosed with CM lost more BCS between calving and nadir than cows that were not diagnosed with CM. János et al. (2003) found that elevated serum BHBA levels 1 to 3 d postpartum were associated with an increased risk for CM caused by environmental pathogens. More recently, Berry et al. (2007) found that neither BCS nor BCS change affected the likelihood of an animal contracting CM, although a nonlinear relationship between the rate of BCS change from calving to nadir and CM in early lactation was evident. The probability of an animal contracting CM increased at a declining rate as the rate of BCS loss became greater, and greater odds of CM during lactation were associated with greater BW at calving. Rezamand et al. (2007) studied associations among BW and BCS and the occurrence of a new subclinical IMI during the periparturient period. Cows with a new IMI had greater BCS, BW, and BW loss in early lactation compared with cows that did not develop a new IMI.

Somatic cell count concentration is a measure of the number of leukocytes in milk that have been directed from the blood to the udder as a response to inflammation. For this reason, and because of the established genetic relationships, it is often used as a proxy for CM (Banos et al., 2006). Berry et al. (2007) studied the association between BCS, BW, and SCC in seasonally calving dairy cows. Increased BCS at calving was as-

sociated with reduced SCC in first- and second-parity cows but with greater SCC in older cows. Furthermore, increased BCS and BW loss in early lactation was associated with lower average SCC and a reduced probability of high test-day SCC. Nyman et al. (2008) found that serum concentrations of metabolites, indicating a more severe NEB, were associated with greater SCC at the first test milking in primiparous dairy cows.

The objective of this study was to quantify associations between BW and BCS variables indicating a more severe NEB in early lactation and events of SCC >250,000 cells/mL (EV250) and SCC >400,000 cells/mL (EV400) in dairy cows.

MATERIALS AND METHODS

Study Design and Population

The study was designed as an observational, prospective cohort study and was conducted in a convenience sample of 7 typical Israeli commercial dairy farms from different geographical areas in Israel. The study population consisted of Israeli Holstein cows, held under zero grazing in open sheds. All farms fed a TMR in ad libitum amounts and bred exclusively by AI. The farms included in the study used automated walk-through scales for the measurement of daily BW (SAE, Kibbutz Afikim, Israel) connected to the farm computer (Afi-farm computerized milking and management software, Kibbutz Afikim) and were willing to participate in the study. Herd size ranged from 251 to 824 cows.

Farms included are members of the Israel Cattle Breeders Association (ICBA), perform monthly milk recordings, and participate in the Herd Health Program of "Hachaklait," the Mutual Society for Veterinary Services (Caesarea Industrial Park, Israel). Within this program, all cows are routinely examined at 5 to 14 d postpartum for uterine disease. During this examination, sick cows and cows with an average daily milk production of <25 kg are also checked for ketosis (urine acetoacetic acid concentration ≥ 1.5 mmol/L). These data, alongside management, health, and reproduction data, are recorded on the farm computer by using either NOA (ICBA) or Afifarm management software. Geographical area, size, and milk production of the participating farms have been described previously (van Straten et al., 2008).

During the months of March and June 2006, scales on all farms were calibrated. Scales were additionally calibrated approximately every 4 mo. For each farm, the study period started after initial calibration, included 1 yr of calvings, and ended approximately 10 mo after the last cow included in the study calved.

Table 1. Definitions of variables used in the analysis

Variable	Definition
Milk fever	Periparturient recumbency, treated with calcium
Stillborn calf	Calf dead within 24 h of calving
Retained placenta	Placental membranes visible in vulva at least 24 h after calving
Metritis	Abnormal vaginal discharge with or without systemic disease at routine postpartum inspection
Displaced abomasum	Clinically diagnosed by attending veterinarian
Ketosis	Urine acetoacetic acid concentration ≥ 1.5 mmol/L ¹
Summer calving	Calving in the months June to September

¹Ketostix (Bayer, Dublin, Ireland).

Data Editing and Statistical Analyses

All data editing and analyses were performed using SAS version 9.1 (SAS Institute, 2006). Results were considered to be of statistical significance if the relevant *P*-value was < 0.05 .

Calving, Management, and Production Data. Calving and management data were retrieved from the farm computer (Afifarm or NOA management software). Definitions of the variables used are summarized in Table 1. Calving data included the variables milk fever, stillbirth, retained fetal membranes, metritis, displaced abomasum, and ketosis. A BCS (5-point scale) was assigned by the attending veterinarian at 5 to 14 d after calving (**BCS1**) and 40 to 60 d postpartum (**BCS50**). Test-day data, including quantity (kg) and composite SCC (cells/mL) from a maximum of 10 and a minimum of 6 monthly milk recordings per cow, were retrieved from the ICBA central computer.

Daily BW Data Editing. Cows were automatically weighed on their way back from the milking parlor 3 times a day. The 3 measurements were arithmetically averaged to one value for further analysis. Daily BW measurements from the first 150 d of lactation were included in the analysis. Each subject (a cow within a given lactation) was assigned a unique identification number constructed by its herd book number, farm number, and parity number. For generating variables representing BW changes in early lactation, individual BW measurements were first smoothed using penalized cubic splines. In this procedure, a nonparametric model with penalized least squares estimates for each subject was used. The model contained the time unit day in lactation as the single smoothing variable. As many unique design points as BW measurements were used for each time series, and the trade-off between goodness of fit and smoothness was determined by minimizing the generalized cross-validation function (PROC TPSPLINE, SAS Institute, 2006). A detailed description of the model can be found elsewhere (van Straten et al., 2008).

BW-Related Variables. Variables reflecting absolute or relative BW and BW changes were considered for inclusion as independent variables in the different

models. These variables were chosen based on a priori hypotheses regarding their significance as variables reflecting the severity of NEB experienced by an individual. Body weight at calving was defined as the smoothed value for BW (kg) at calving. Body weight at nadir was defined as the smoothed value for BW (kg) at the nadir BW. Absolute BW changes were calculated by subtracting the smoothed BW of any given day from the smoothed BW at calving. Relative BW change was calculated as

$$\text{Relative BW change} = \frac{BW_C - BW_T}{BW_C},$$

where BW_T is the smoothed BW on any given day in lactation and BW_C is BW at calving. For analyses in the present study, we used absolute and relative BW loss from calving to nadir BW (**LCN** and **RLCN**, respectively). Absolute and relative BW losses in the first 10 d and first 20 d of lactation were also considered for inclusion as independent variables in the models. For each cow, the number of days from calving to smoothed nadir BW was determined.

The variables LCN and RLCN were also dichotomized. If, for a certain cow, any of the values of these variables belonged to the upper quartile, indicating a greater absolute or relative BW loss, the value of the corresponding dichotomous variable was set to 1. Otherwise, the value was set to 0. Upper quartiles were determined for first-parity cows, second-parity cows, and third-parity and greater cows separately because significant differences were found in these variables across the different parity groups.

Data Imputation. On 2 farms, BCS50 was not available. Because this variable showed strong and significant relationships with the some of the outcomes of interest, and because we wanted to include the data from these farms in the analyses, we decided to predict the missing values of BCS50 on these 2 farms by using a linear regression model applied to the data from the remaining farms. From these data, univariate relationships between BCS50 and all other variables possibly associated with BCS50 were established. Significant

variables were then entered sequentially in a linear regression model. The adjusted R^2 of the final model was 0.41. The final model predictors and their coefficients were then used to calculate the predicted BCS50 by using the following formula:

$$\begin{aligned} \text{BCS50}_p = & 0.205 + (0.567 \times \text{BCS1}) + (0.284 \\ & \text{first parity}) + (0.147 \times \text{second parity}) - (0.002 \\ & \times \text{LCN}) + (0.001 \times \text{NBW}) + (0.065 \\ & \times \text{summer calving}) - (0.088 \times \text{ketosis}), \end{aligned}$$

where BCS50_p is the predicted value for BCS50, LCN is BW loss (kg) from calving to nadir, and NBW is BW (kg) at nadir. The BCS50_p was used only for cows from the farms where BCS50 was not available. The effect of using BCS50_p instead of BCS50 was evaluated by comparing the final models including BCS50_p with identical models in which BCS50_p was replaced by BCS50. The effect was considered insubstantial if the coefficients for variables in the former model were within the 95% confidence intervals of the corresponding variables in the latter model or if variables significant in the former model retained the direction of effect but lost only significance in the latter model.

Relationship Between EV2500 or EV400 and Other Covariates. Cutoff points for events were chosen so that it would be possible to compare our results with similar work (Berry et al., 2007) but were also based on previous work routinely done on data from high-producing dairy farms in Israel ("Hachaklait" Herd Health Department, unpublished data). Two approaches were used. In the first approach, only first events of elevated SCC occurring during lactation were considered. In other words, cows were categorized as either having experienced an event of elevated SCC during their lactation (multiple events were ignored) or not having experienced an event of elevated SCC during their lactation. In the second approach, all events of elevated SCC occurring during lactation were considered. In both cases, a generalized mixed model was used to study the effects of BW and BCS variables of interest alongside several other independent variables (herd, parity, stage of lactation, summer calving, and calving diseases) on the odds of an event of elevated SCC. Parity was divided into 2 index variables, first and second and greater. Each lactation was divided into 3 stages: early lactation (≤ 90 DIM), midlactation (91 to 180 DIM), and late lactation (>180 DIM). Because we expected a correlation between cows in a given herd as well as a correlation between repeated measurements of SCC from the same cow in the case of repeated events, we modeled farm as a random in-

tercept effect and incorporated a correlation matrix in the error term (marginal effect) for the case of repeated measurements. Generalized mixed models take on the following form:

$$g(\mathbf{Y}) = \mathbf{X}\beta + \mathbf{Z}\gamma + \varepsilon,$$

where g is a link function, $\mathbf{Y}$ is the vector of observations, β is an unknown vector of fixed-effect parameters with known design matrix $\mathbf{X}$, γ is an unknown vector of random-effect parameters with known design matrix $\mathbf{Z}$, and ε is an unknown random error vector whose elements are no longer required to be independent. In the case of repeated events of elevated SCC, ε is a complex error term that includes a correlation matrix $\mathbf{R}$ representing the within-cow correlation of SCC measurements. The covariance structure chosen for $\mathbf{R}$ was autoregressive because we expected a decaying temporal dependency between measurements. The link function we used was the natural log of the odds of a cow experiencing an event of elevated SCC. Model building was performed in a stepwise manner. Initially, possible independent variables other than BW and BCS variables were entered consecutively into the model and remained if the corresponding P was <0.05 . Body condition score and BW variables of interest were then entered consecutively. Only variables with a significance level of $P < 0.05$ remained in the model. Finally, biologically plausible 2-way interactions between variables in the model were tested. Significance of the fixed effects was determined using the F -test (PROC GLIMMIX, SAS Institute, 2006).

Milk Production. Average daily milk production was estimated from monthly test-day data by using a mixed model with a marginal effect to account for repeated measurements from the same cow. Farm was modeled as a random intercept effect and the correlation matrix used for $\mathbf{R}$ was autoregressive. Model building was performed in a stepwise manner. Initially, possible independent variables other than BW and BCS variables were entered consecutively into the model and remained if the corresponding P was <0.05 . Body condition score and BW variables of interest were then entered consecutively. Only variables with a significance level of $P < 0.05$ remained in the model. Finally, biologically plausible 2-way interactions between variables in the model were tested. The model we used was as follows:

$$\begin{aligned} Y = & \text{parity (2 index variables)} + \text{MIM (10 index} \\ & \text{variables)} + (\text{parity} \times \text{MIM}) + \text{MOT (12 index} \\ & \text{variables)} + \text{SSCL (4 index variables)} + \text{SC (2 index} \\ & \text{variables)} + \text{MET} + \text{farm (random)} + e, \end{aligned}$$

Table 2. Number of lactations (%) with 0, 1, or >1 events of SCC >250,000 cells/mL or SCC >400,000 cells/mL in first- and second-parity and greater cows with or without extreme relative BW loss from calving to nadir BW

Parity	UQRLCN ¹	Events of SCC >250,000 cells/mL			Events of SCC >400,000 cells/mL		
		0	1	>1	0	1	>1
1 (n = 634)	No (n = 486)	353 (72.6)	97 (20.0)	36 (7.4)	411 (84.6)	58 (11.9)	17 (3.5)
	Yes (n = 148)	98 (66.2)	30 (20.3)	20 (13.5)	118 (79.7)	21 (14.2)	9 (6.1)
	Total	451 (71.1)	127 (20.0)	56 (8.8)	529 (83.4)	79 (12.5)	26 (4.1)
>1 (n = 1,086)	No (n = 833)	417 (50.1)	191 (22.9)	225 (27.0)	559 (67.1)	162 (19.5)	112 (13.4)
	Yes (n = 253)	119 (47.0)	58 (22.9)	76 (30.1)	159 (62.8)	51 (20.2)	43 (17.0)
	Total	536 (49.4)	249 (22.9)	301 (27.7)	718 (66.1)	213 (19.6)	155 (14.3)
Total	1,720	987 (57.4)	376 (21.9)	357 (20.7)	1,247 (72.5)	292 (17.0)	181 (10.5)

¹UQRLCN = upper quartile in relative BW loss from calving to nadir BW. Upper quartile values were 12.3, 15.0, and 15.7% for first-, second-, and third-parity and older cows, respectively.

where Y is test-day milk production, MIM is month in milk, MOT is month of test, SSCL is SCC level, SC is summer calving, MET is metritis (limited to the first 3 monthly test days), and e is a complex error term representing the within-cow correlation of test-day results and the residual error. In this model, we used the following 4 categories for SSCL: <100,000 cells/mL, 100,000 to 200,000 cells/mL, 200,000 to 400,000 cells/mL and >400,000 cells/mL. These categories are routinely used in the analysis of data from Israeli farms ("Hachaklait" Herd Health Department, unpublished data), where significant milk loss associated with all SCC levels >100,000 cells/mL is often demonstrated. Significance of the fixed effects was determined using the *F*-test (PROC MIXED, SAS Institute, 2006).

RESULTS

Descriptive Findings

The full database included data from 2,075 cows. However, cows with an SCC $\geq$ 200,000 cells/mL on their first test day (n = 355) were excluded from the analysis. This was done to ensure that cows included in the analysis were most likely those that started out their lactation with healthy udders and to minimize the probability that BW loss in early lactation was the result of udder disease. The final database (n = 1,720) included data from 634 primiparous and 1,086 multiparous cows. The total number of events of elevated SCC found in the study is presented in Table 2. In 71.1% of the lactations of primiparous cows and 49.4% of the lactations of multiparous cows, no EV250 were recorded. The corresponding values for EVSCC400 were 83.4 and 66.1% for primiparous and multiparous cows, respectively. The cumulative incidence of a lactation with multiple EV250 in primiparous cows (2 to 8 events per lactation) was 8.8%, whereas this value in multiparous cows (2 to 9 events per lactation) was 27.7%. The cumulative incidence of a lactation with

multiple EV400 in primiparous cows (2 to 6 events per lactation) was 4.1%, whereas this value in multiparous cows (2 to 9 events per lactation) was 14.3%.

First Events of Elevated SCC

The cumulative incidence of lactations with first EV250 was 28.9% in primiparous cows and 50.6% in multiparous cows. The cumulative incidence of lactations with first EV400 was 16.6% in primiparous cows and 33.8% in multiparous cows. No associations were found between the different BW and BCS variables and the odds of a first EV250 or first EV400.

All EV250

The analysis included data from 16,406 monthly milk tests. Of these, 1,570 (9.6%) were >250,000 cells/mL and 14,836 (90.4%) were equal to or below this value. The odds of an event on any given test day were 25% greater for cows belonging to the upper quartile in relative BW loss from calving to nadir BW (loss >12.3, 15.0, and 15.7% for the first, second, and third parity and greater, respectively) compared with cows that lost less relative BW, after accounting for other effects in the model (Table 3). The odds of cows with ketosis for an event were 44% greater than those without, after adjusting for other effects in the model. Adjusted odds for cows with a BCS >3.5 between 40 to 60 d postpartum were 2.88 times higher than those for cows with a BCS between 3.5 and 2.5. The adjusted odds of an event increased as stage of lactation increased: the odds of an event occurring during the first 90 d of lactation were 73% smaller than the odds of an event occurring later than 180 d in lactation; the odds of an event occurring between 91 and 180 d of lactation were 39% smaller than the odds of an event occurring later than 180 d in lactation. The odds of an event for cows calving during the summer months were 15% smaller than those for cows calving outside these months.

Table 3. Results of generalized mixed models with random herd effects for quantifying relationships between various covariates and all events of SCC >250,000 cells/mL or SCC >400,000 cells/mL

Variable	Event of SCC >250,000 cells/mL			Event of SCC >400,000 cells/mL		
	OR ¹	95% CI ²	P-value	OR	95% CI	P-value
Parity						
1	0.38	0.32 to 0.46	<0.0001	0.33	0.26 to 0.41	<0.0001
>1	1			1		
Lactation stage (DIM)						
≤90	0.27	0.22 to 0.32	<0.0001	0.29	0.23 to 0.37	<0.0001
91 to 180	0.61	0.53 to 0.70	<0.0001	0.69	0.59 to 0.82	<0.0001
>180	1			1		
Ketosis						
Yes	1.44	1.20 to 1.72	<0.0001	1.33	1.06 to 1.67	0.0139
No	1			1		
UQRLCN ³						
Yes	1.25	1.06 to 1.47	0.0064	1.43	1.18 to 1.75	0.0003
No	1			1		
Summer calving						
Yes	0.85	0.73 to 0.99	0.0396	0.71	0.59 to 0.87	0.0007
No	1			1		
BCS at 40 to 60 d postpartum						
<2.5	1.12	0.97 to 1.29	0.1132			
>3.5	2.88	1.28 to 6.49	0.0108			
2.5 ≤ BCS ≤ 3.5	1					
BCS at calving						
<2.5				1.21	0.93 to 1.58	0.1502
>3.5				0.62	0.43 to 0.91	0.0156
2.5 ≤ BCS ≤ 3.5				1		

¹OR = odds ratio adjusted for all other covariates included in the model.²95% CI = 95% confidence interval.³UQRLCN = upper quartile in relative BW loss from calving to nadir BW. Upper quartile values were 12.3, 15.0, and 15.7% for first-, second-, and third-parity and older cows, respectively.

All EVSCC400

The analysis included data from 16,574 monthly milk tests. Of these, 912 (5.5%) were >400,000 cells/mL and 14,566 (94.6%) were equal to or below this value. The odds of an event on any given test day were 43% greater for cows belonging to the upper quartile in relative BW loss from calving to nadir BW compared with cows that lost less relative BW, after accounting for other effects in the model (Table 3). Odds of an event for cows with ketosis were 33% greater than for cows without, after adjusting for other effects in the model. Adjusted odds of an event for cows with a BCS >3.5 at calving were 38% lower than those of cows with a BCS between 3.5 and 2.5. The adjusted odds of an event increased as stage of lactation increased: the odds of an event occurring during the first 90 d of lactation were 71% smaller than the odds of an event occurring later than 180 d in lactation; the odds of an event occurring between 91 and 180 d of lactation were 31% lower than the odds of an event occurring later than 180 d in lactation. The odds of an event for cows calving during the summer months were 29% smaller than those for cows calving outside these months.

Test-Day Milk Production

Various BW and BCS variables were significantly associated with test-day milk production, although some associations were of little biological importance (Table 4). After adjusting for SCC and other covariates, test-day milk production (kg) of cows losing extreme relative BW from calving to nadir BW was not found to differ from that of cows losing less relative BW. However, test-day milk of cows with low BCS (<2.50 BCS) between 40 to 60 d postpartum was 1.59 kg greater than that of cows with a BCS between 2.50 and 3.50, after adjusting for SCC and other covariates. Test-day milk of cows with high BCS between 40 to 60 d was 4.16 kg less than that of cows with a BCS between 2.50 and 3.50. Weak associations with milk production were found for days from calving to nadir BW (0.01 kg for each additional day) and for BW (kg) at nadir BW (0.02 kg for each additional kg of BW). Daily milk production in the first 100 d of lactation in cows diagnosed with metritis was 0.76 kg smaller than in cows not diagnosed with metritis.

Milk loss associated with SCC was substantial. Compared with milk production (kg) on test days associated

Table 4. Results of a linear mixed regression model for some variables significantly associated with test-day milk production¹ (milk) and corresponding 95% confidence intervals (95% CI)

Variable	Milk (kg)	P-value	95% CI
SCC × 10 ³			
100 to 200	−0.90	<0.0001	(−1.10, −0.70)
200 to 400	−1.72	<0.0001	(−2.02, −1.41)
>400	−4.59	<0.0001	(−4.95, −4.24)
<100	—	—	—
Metritis ²			
No	0.76	0.0013	(0.30, 1.22)
Yes	—	—	—
UQRLCN ³			
No	−0.45	0.1021	(−0.98, 0.09)
Yes	—	—	—
BCS 40 to 60 d postpartum			
<2.50	1.59	<0.0001	(1.13, 2.04)
>3.50	−4.16	0.0114	(−7.39, −0.94)
2.50 ≤ BCS ≤ 3.50	—	—	—
Days from calving to nadir BW	0.01	<0.0001	(0.01, 0.02)
BW (kg) at nadir BW	0.02	<0.0001	(0.02, 0.03)

¹Adjusted for farm, parity, month of lactation, parity × month of lactation, and month of test-day.

²Limited to the first 3 monthly milk recordings.

³UQRLCN = upper quartile in relative BW loss from calving to nadir BW. Upper quartile values were 12.3, 15.0, and 15.7% for first-, second-, and third-parity and older cows, respectively.

with an SCC ≤100,000 cells/mL, milk loss that could be attributed to SCC of 100,000 to 200,000, 200,000 to 400,000, and >400,000 cells/mL was 0.90, 1.72, and 4.59 kg of milk, respectively.

DISCUSSION

Because changes in energy balance in early lactation are accompanied by body fat mobilization and changes in BW, BCS and BW have been proposed as estimators for NEB (Coffey et al., 2001). Although no relationship between BW and BCS variables reflecting more severe or longer lasting NEB and first events of elevated SCC could be found, extreme (12.3 to 15.7% of BW) relative BW loss, BCS₅₀ >3.50, BCS₁ <2.50, and ketosis were associated with increased odds for events of elevated SCC when all events occurring in lactation were analyzed. To our knowledge, analysis of repeated events of elevated SCC and their associations with BW and BCS changes in early lactation have not been reported previously. In our data, taking into account only first events of elevated SCC would have led to underestimation of the associations between variables expressing a more severe NEB in early lactation and elevated SCC. Our findings stand in contrast with those reported from similar work done by Berry et al. (2007), who investigated associations between BCS and BW variables in early lactation in seasonally calving dairy cattle. Although the effects of BCS and BW on the indicators they chose as representative of udder health were found to be parity dependent, in most cases fatter and heavier cows or cows that lost the least condition during early

lactation had higher lactation average SCC and higher probability of a high test-day SCC. They found that increased BCS at calving was associated with reduced SCC in first- and second-parity cows and with greater SCC in older cows. However, the authors concluded that the effect of BCS or BW on udder health was small and of limited biological significance.

Nonetheless, the 2 studies differ on some important points. First, the cited study was done in pasture-based dairy cows with lower production levels (average 60-d milk production of 1,213 kg compared with 2,389 kg in our study). Second, different SCC variables and analysis approaches were used in both studies. The SCC variables used in the cited study were average SCC, which was the mean of all test-day records within a lactation and a dichotomous variable, high SCC, which was defined as 1 if an individual SCC test-day record >250,000 cells/mL occurred within a lactation. The first variable might not be sensitive enough to capture the effects of repeated events of elevated SCC, whereas the second variable does not necessarily differentiate between cows with one or more events of elevated SCC.

Our findings indicate that cows suffering from ketosis as well as cows with extreme relative BW loss in early lactation, both indicators of cows being in a state of greater NEB, are more likely to suffer from events of elevated SCC throughout the lactation. Clinical mastitis is often a recurring event (Döpfer et al., 1999; Zadoks et al., 2001; Bar et al., 2008). The events of elevated SCC found in our study do not necessarily represent cases of CM, although it would seem more than reasonable to assume that these events are indicative of immunological

mobilization of polymorphonuclear leukocytes against udder infection (Green et al., 2004). Unfortunately, cow-level data on CM were unavailable for this study, but it is established that all farms in the study are closed farms free of *Streptococcus agalactiae*. Furthermore, on the basis of regular bacteriological milk samples from cows with CM and elevated SCC, the estimated lactational incidence of *Staph. aureus* on these farms is between 0 and 1% (Adin Shwimmer, National Service for Udder Health and Milk Quality, Israeli Dairy Board, personal communication). Nevertheless, the most likely explanation for events of elevated SCC found in this study is clinical and subclinical mastitis most probably associated with *E. coli*, coagulase-negative staphylococci, and environmental streptococci.

Although various epidemiological studies have shown associations between variables indicating a more severe NEB and udder infection (Oltenuacu and Ekesbo, 1994; Rezamand et al., 2007; Nyman et al., 2008), mechanisms that could explain these associations remain obscure. Greater concentrations of both NEFA and BHBA have been associated with impaired immune functions and mastitis in dairy cows (for a review, see Burvenich et al., 2007). Impaired production of proteins involved in inflammatory and immune responses attributable to increased lipid infiltration in the liver has also been suggested (Nyman et al., 2008).

Some studies have reported impaired neutrophil function related to NEB. Polymorphonuclear killing ability was significantly diminished in blood polymorphonuclear leukocytes isolated from cows with periparturient NEB (Hammon et al., 2006). β -Hydroxybutyrate was found to interfere with the formation of bovine neutrophil extracellular traps and bactericidal activity against mammary pathogenic *E. coli* (Grinberg et al., 2008). Although periparturient ketosis was associated with increased risk for events of elevated SCC in our study, impaired neutrophil function attributable to the direct effect of elevated ketone bodies could not easily explain events of elevated SCC occurring in later stages of lactation. Because the recruitment of blood neutrophils is apparently unhindered, it might be possible that poor adaptation to NEB causes long-term interference with the formation or function of neutrophils, or both. Impaired neutrophil function could result in chronic IMI and relapses of bacterial flare-ups, thus leading to recurrent events of elevated SCC. Additionally, other "first-line" udder defense mechanisms could be affected. These predominantly innate immunity mechanisms, involved in the early stages of bacteria elimination, may fail to contain or eradicate infection so that systemic defenses are recruited (for a review, see Rainard and Riollet, 2007).

It might be argued that within our study design, it was not possible to distinguish between cause and consequence regarding BW loss and events of elevated SCC. In other words, did extreme BW loss result in events of elevated SCC, or did events of elevated SCC result in extreme BW loss? The latter seems unlikely for the following reasons. First, cows with SCC >200,000 cells/mL on their first test day were excluded from the study. Second, upper quartile values for days to nadir BW were 53 and 109 for first- and second-parity and older cows, respectively, whereas events of elevated SCC occurred during all 10 mo of lactation. Furthermore, the number of days from calving to nadir BW was not significantly associated with events of elevated SCC in our models.

CONCLUSIONS

We demonstrated that a more severe NEB in early lactation, as indicated by extreme relative BW loss and ketosis, is associated with poor udder health. When investigating this association based on events of elevated SCC from test-day data, it is necessary to include repeated events of elevated SCC. In our case, this was made possible by the use of generalized mixed models with a complex error term. Using these models, we were able to quantify the effects of different variables on our dichotomous outcome while including a random farm effect and accounting for the repeated measurements of SCC within a lactation of the same cow. Epidemiological studies are limited in their possible contribution to understanding complex mechanisms such as those most probably playing a role in the association between NEB and udder health. With that said, a possible explanation for our findings is that first-line udder defenses involved in the early elimination of invading bacteria might be impaired because of long-term effects of NEB, resulting in enduring failure to contain or eradicate infection such that inflammation is induced and blood leukocytes are recruited.

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