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# Relationship between serum nonesterified fatty acids at calving and the incidence of periparturient diseases in Holstein dairy cows

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**Abstract**

The objective was to describe the relationship between concentration of serum nonesterified fatty acids (NEFAs) at calving and the incidence of periparturient disorders in Chilean Holstein dairy cows (*Bos taurus*). The study was conducted at two dairies (central Chile) with 700 milking cows each and similar management. Between July 2006 and March 2007, 350 cows were selected, and concentrations of serum NEFAs were determined at calving. The incidence of milk fever (MF), retained fetal membranes (RFMs), metritis, and clinical mastitis from calving to 100 d in lactation were consistently recorded. The relationship between concentration of serum NEFAs at calving and the incidence of periparturient diseases was determined using logistic regression. The main explanatory variable was concentration of serum NEFAs at calving. The incidence of MF, RFM, metritis, and mastitis was 5.4%, 15.6%, 10.8%, and 14.4%, respectively. There was no association between concentration of NEFAs at calving and the incidence of these conditions when the median value of NEFAs (0.9 mEq/L) was used as a cutoff. However, when the 75th percentile (1.2 mEq/L) was used as the cutoff, cows with values $<1.2$ mEq/L were 0.45 and 0.32 times as likely to develop clinical mastitis and MF, respectively, compared with cows with values $\geq 1.2$ mEq/L. When the 90th percentile (1.6 mEq/L) was used as a cutoff, cows with values $<1.6$ mEq/L were 0.25 times as likely to develop clinical mastitis compared with cows with values $\geq 1.6$ mEq/L. As a continuous variable, for every 0.1 mEq/L increment in NEFAs at calving, cows were 1.11 times more likely to experience clinical mastitis. In conclusion, cows with NEFA concentrations $\geq 1.2$ mEq/L had a higher incidence of clinical mastitis and MF than that of cows with values $<1.2$ mEq/L.


*Keywords:* Chile; Dairy cattle; Nonesterified fatty acids; Periparturient diseases; Postpartum period

## 1. Introduction

Fatty acids (FAs) are hydrocarbon chains of which three are attached by ester bonds to glycerol to form triglycerides in adipose tissue. Under negative energy balance or low concentrations of insulin, the secretion of hormone-sensitive lipase is stimulated, triggering lipolysis with the subsequent release of FA in its nonesterified form (nonesterified fatty acid; NEFA) to the bloodstream [1]. Just before calving, the concentration of serum NEFAs increases; however, occasionally it reaches concentrations that are detrimental to the health status of postpartum dairy cows [2]. High concentrations of serum NEFAs have been associated with an increased incidence of periparturient diseases (retained fetal membranes, ketosis, and mastitis), displacement of the abomasum, and immunosuppression in dairy cattle [3–5]. Under

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Chilean farming conditions, the association between the concentration of serum NEFAs at calving and the incidence of periparturient diseases has not been previously determined. The hypothesis of this study was that cows with higher NEFA concentrations will experience a greater incidence of periparturient diseases compared with cows with normal NEFA concentrations. The rationale for this hypothesis is that NEFAs may induce immunosuppression, which may predispose to more periparturient diseases. The objectives of this study were to characterize NEFA concentrations at calving and to determine a relationship with the incidence of periparturient disorders in Chilean Holstein dairy cows (*Bos taurus*).

## 2. Materials and methods

### 2.1. Herd and management

The study was conducted on two dairy farms located in the central area of Chile with 700 milking cows each and similar management conditions. Cows were kept under confinement, milked three times a day, and had a mature equivalent 305-d milk production of 9500 kg. Lactating cows were housed in a dry-lot system and fed a total mixed ration three times a day to meet or exceed the nutritional requirements of the National Research Council [6]. Diet was based on corn silage, alfalfa hay, and concentrate. Cows were dried-off between 50 to 70 d before expected parturition (BEP) and maintained in a dry-lot until 21 d BEP. Cows were moved to a maternity barn 3 to 5 d BEP.

Within 12 h postpartum, cows were routinely processed. This evaluation included body condition score (BCS; scale 1 to 5, in 0.25-point increments), udder score (for presence of edema), reproductive tract status (trauma or lacerations), and whether the cow developed retained fetal membranes (RFMs). If cows developed either RFMs or clinical hypocalcemia (milk fever; MF), they were treated and remained in the hospital barn until recovery. After processing, cows were moved to a postpartum lot for approximately 21 d and fed a diet higher in forage neutral detergent fiber (NDF) than that fed to cows in other stages of lactation. In addition, cows were scored for body condition consistently at dry-off and 100 and 250 d in milk. Reproductive management consisted of a voluntary waiting period of 50 d. Both dairies used 100% artificial insemination and visual heat detection. Both dairy farms used the same dairy herd management computer program (Dairy COMP 305; Valley Agricultural Software, Tulare, CA, USA); therefore, information and records were consistent and comparable.

### 2.2. Study design

To find a difference of 12% for the total incidence of the periparturient disorders RFMs, mastitis, metritis, and MF between a group of cows with high concentrations of serum NEFAs (>1.0 mEq/L; 50% of total incidence of disorders listed) and low concentrations of serum NEFAs (≤1.0 mEq/L; 38% of total incidence of disorders listed) at calving [3] with a 95% confidence and 80% power, a sample size of 310 cows was calculated [7]. A total of 350 cows that calved between July 2006 and March 2007 were selected at freshening (175 cows from each herd). Cows were identified using ear tag numbers. Data for parity were also obtained at calving and recorded.

A blood sample was obtained from the coccygeal vessels within 12 h of calving. Sample was taken immediately after the first milking for the fresh cow (at 8:00 AM, 4:00 PM, or 12:00 AM), and centrifuged at 3000 × *g* for 10 min. Serum was collected and stored in plastic vials at –20 °C until analysis. Concentrations (mEq/L) of serum NEFAs were determined by an enzymatic colorimetric method (Randox, Crumlin, UK).

The incidence of MF, RFMs, metritis, and clinical mastitis until 100 d in lactation was consistently recorded. The diseases under study were considered based on the accuracy and consistency of recording of health data. Definition of diseases was based on criteria used by the farm veterinarian and previously published information [8]. Milk fever was defined as any recumbent cow within 72 h after parturition exhibiting anorexia, nervous symptoms, staggering, varying degrees of unconsciousness, and good response to intravenously administered calcium (recovery from recumbence state and nervous symptoms). Retained fetal membranes were defined as visible fetal membranes at the vulva or detection of fetal membranes in the vagina by vaginal examination more than 24 h after calving. Metritis was defined as the presence of a foul-smelling vaginal discharge obtained by transrectal palpation within the first 10 d postpartum. Clinical mastitis was defined as visibly abnormal milk, with or without abnormal changes of the mammary gland determined during the milking process. A new case was considered after a period of 14 d of normal milk. Incidence of clinical mastitis was evaluated up to 100 d in milk. Retained fetal membranes diagnosis, evaluation of metritis by transrectal palpation, and udder health assessment were part of a monitoring health program conducted at both farms for fresh cows during the first 13 d postpartum.

Table 1
Descriptive statistics of variables used in the current study to determine the relationship between NEFA concentrations at calving and the incidence of periparturient diseases in dairy cows.

| Variable | Number | Mean | SD | Median | Minimum | Maximum |
|---|---|---|---|---|---|---|
| NEFA at calving (mEq/L) | 347 | 0.90 | 0.47 | 0.86 | 0.15 | 2.45 |
| Parity | 350 | 2.00 | 1.50 | 2.00 | 1.00 | 8.00 |
| BCS at calving | 346 | 3.60 | 0.34 | 3.75 | 2.25 | 4.25 |

Table 2
Incidence of periparturient diseases in dairy cows based on median, percentile 75, and percentile 90 of concentration of serum NEFAs at calving as a cutoff value.

| Disease | Disease incidence, % | | | | | |
|---|---|---|---|---|---|---|
| | NEFA 50% (0.9 mEq/L) | | NEFA 75% (1.2 mEq/L) | | NEFA 90% (1.6 mEq/L) | |
| | <0.9 mEq/L | ≥0.9 mEq/L | <1.2 mEq/L | ≥1.2 mEq/L | <1.6 mEq/L | ≥1.6 mEq/L |
| RFMs | 11.6 | 19.1 | 15.4 | 15.1 | 14.5 | 21.2 |
| Metritis | 8.8 | 11.6 | 10.4 | 9.6 | 9.6 | 15.2 |
| Mastitis | 10.2 | 21.8 | 12.2[a] | 27.4[b] | 13.0[a] | 39.4[b] |
| MF | 4.8 | 7.5 | 3.6[a] | 13.7[b] | 5.4 | 12.1 |

[a,b]Within a row and within a NEFA cutoff value, incidences without a common superscript differed ($P \leq 0.05$).

### 2.3. Statistical analysis

Variables of the current study were summarized through descriptive statistics. The relationship between the concentrations of NEFAs at calving and the risk of periparturient diseases until 100 d postpartum was modeled with logistic regression. The main explanatory variable was the concentration of serum NEFAs at calving. Several models were constructed using the concentration of serum NEFAs at calving as dichotomized variable (high and low) based on the median value and 75th and 90th percentiles of the sample. Sensitivity and specificity at each cutoff value and 95% confidence intervals were calculated. In addition, several models using NEFA as a continuous variable (per 0.1 mEq/L) were built. If the model was significant, further analysis was conducted to determine if the relationship was linear through the entire distribution. This was assessed by testing the association between NEFA (per each 0.1 mEq/L) and the diseases within several ranges of NEFA.

Other explanatory variables included in the models were farm (1 vs. 2), parity (primiparous: 1 calving; multiparous: >1 calving), and BCS at calving. When not significant, the main variable (concentration of NEFAs at calving) was forced to remain in the model. Models with the best fit based on the –2 log likelihood were determined. Receiver-operator characteristic curves to select the optimum cut point were built. Data analysis was conducted using SAS for Windows [9].

## 3. Results

The mean concentration of NEFAs at calving was $0.90 \pm 0.47$ mEq/L (Table 1). Because of laboratory constraints, only 347 samples were properly analyzed, therefore this number was used for the statistical analysis. Median value and 75th and 90th percentiles for the concentration of NEFAs at calving were 0.9, 1.2, and 1.6 mEq/L, respectively. The incidence of MF, RFMs, metritis, and mastitis were 5.4%, 15.6%, 10.8%, and 14.4%, respectively. The proportion of cases for each disease based on the three cutoff values defined in the current study were different from each other (Table 2). Sensitivity, specificity, and 95% confidence interval (CI) for each cutoff point for RFMs, metritis, MF, and clinical mastitis is shown, respectively, in Table 3.

There was no significant difference in disease risk between cows with NEFAs above or below the median (Table 4). However, when the 75th percentile (1.2 mEq/L) was used as the cutoff point, cows with NEFAs <1.2 mEq/L were 0.45 (95% CI, 0.22 to 0.92) and 0.32 (95% CI, 0.11 to 0.90) times as likely to develop clinical mastitis and MF, respectively, compared with cows with values ≥1.2 mEq/L, accounting for the effects of farm, parity, and BCS (Table 5). When the 90th percentile (1.6 mEq/L) was used as the cutoff point, cows with values <1.6 mEq/L were 0.25 (95% CI, 0.10 to 0.61) times as likely to develop clinical mastitis compared with cows with values ≥1.6 mEq/L (Table 6).

Table 3
Sensitivity, specificity, and 95% CI for median, percentile 75, and percentile 90 of serum NEFAs at calving as a cutoff value.

| Disease | Disease incidence | | | | | |
|---|---|---|---|---|---|---|
| | NEFA 50% (0.9 mEq/L) | | NEFA 75% (1.2 mEq/L) | | NEFA 90% (1.6 mEq/L) | |
| | Sensitivity, % (95% CI) | Specificity, % (95% CI) | Sensitivity, % (95% CI) | Specificity, % (95% CI) | Sensitivity, % (95% CI) | Specificity, % (95% CI) |
| RFMs | 37.8 (23.6–51.9) | 47.8 (41.6–54.0) | 75.6 (63.0–88.1) | 24.5 (19.5–29.8) | 86.7 (76.7–96.6) | 10.0 (6.3–13.8) |
| Metritis | 43.3 (25.6–61.1) | 49.2 (43.2–55.3) | 76.7 (61.5–91.8) | 24.6 (19.4–29.8) | 86.7 (74.5–98.8) | 10.2 (6.6–13.9) |
| Mastitis | 31.9 (18.6–45.2) | 46.6 (40.3–52.8) | 59.6 (45.5–73.6) | 21.5 (16.3–26.6) | 74.5 (62.0–86.9) | 7.7 (4.4–11.0) |
| MF | 38.9 (16.4–61.4) | 49.3 (43.4–55.2) | 44.4 (21.5–67.4) | 22.5 (17.5–27.4) | 83.3 (66.1–100) | 10.1 (6.6–13.7) |

Table 4
Logistic regression models for the incidence of periparturient diseases in dairy cows using the median value of serum NEFAs at calving (0.9 mEq/L) as a cutoff value.

| Dependent variable | Explanatory variables | Adjusted OR | 95% CI OR | P value |
|---|---|---|---|---|
| RFMs | NEFA (<0.9 vs. ≥0.9) | 0.61 | 0.30–1.23 | 0.17 |
| | Parity (mult. vs. prim.) | 1.24 | 0.60–2.57 | 0.56 |
| | BCS at calving (for each point) | 0.68 | 0.23–2.00 | 0.49 |
| | Farm (1 vs. 2) | 1.07 | 0.76-1.35 | 0.78 |
| Metritis | NEFA (<0.9 vs. ≥0.9) | 0.72 | 0.31–1.67 | 0.45 |
| | Parity (mult. vs. prim.) | 1.0 | 0.43–2.32 | 0.99 |
| | BCS at calving (for each point) | 0.79 | 0.21–2.88 | 0.72 |
| | Farm (1 vs. 2) | 1.24 | 0.52–2.32 | 0.53 |
| Mastitis | NEFA (<0.9 vs. ≥0.9) | 0.57 | 0.28–1.17 | 0.12 |
| | Parity (mult. vs. prim.) | 1.95 | 0.90–4.22 | 0.08 |
| | BCS at calving (for each point) | 5.53 | 1.35–22.60 | 0.01 |
| | Farm (1 vs. 2) | 1.13 | 0.72–2.21 | 0.71 |
| MF | NEFA (<0.9 vs. ≥0.9) | 1.21 | 0.40–3.65 | 0.73 |
| | Parity (mult. vs. prim.) | 4.57 | 1.00–21.00 | 0.05 |
| | BCS at calving (for each point) | 2.32 | 1.79–2.94 | 0.01 |
| | Farm (1 vs. 2) | 1.02 | 0.88–1.98 | 0.67 |

OR, odds ratio; mult., multiparous; prim., primiparous.

When NEFA concentrations at calving were modeled as a continuous variable, for every 0.1 mEq/L increment in NEFAs at calving, cows were 1.11 times (95% CI, 1.03 to 1.19) more likely to experience clinical mastitis (Table 7). However, this association was not linear, as cows with a 0.1 mEq/L increase in serum NEFAs at calving within different ranges of NEFA at calving had different odds ratios for the incidence of clinical mastitis (Table 8). For example, within the range of 0.15 to 0.5 mEq/L, the odds ratio for clinical mastitis was 1.21 (95% CI, 0.48 to 3.06). Within the range of 0.9 to 1.2 mEq/L, the odds ratio was 0.53 (95% CI, 0.20 to 1.38), and for the concentration ≥ 1.2 mEq/L, the odds ratio was 1.19 (95% CI, 1.0 to 1.41). Two-way interactions between NEFA (as binary explanatory variable) and farm (1 and 2), parity (primiparous and multiparous), and BCS at calving (<3.75, ≥3.75) were not associated with the risk of diseases.

Receiver-operator characteristic (ROC) analysis for RFMs and metritis was not significant. However, ROC analysis for MF and mastitis indicated an association of NEFA concentration with these diseases. Results of ROC analysis for MF showed an estimated area under the curve of 62.7% (Table 9, Fig. 1). Concentration of serum NEFAs at calving with the best combination of sensitivity and specificity as cutoff value for MF was 0.96 mEq/L. Cows with concentration of NEFAs at calving ≥0.96 mEq/L were 2.6 times more likely to experience MF than were cows with NEFAs <0.96 mEq/L ($P < 0.05$). Per each 0.1 mEq/L increment of NEFAs at calving, the odds ratio of experiencing MF fever was 1.10 times. Results of ROC analysis for clinical mastitis showed an estimated area under the curve of 59.6% (Table 10, Fig. 2). Concentration of serum NEFAs at calving with the best combination of sensitivity (48%) and specificity (61%) as cutoff value for clinical mastitis was 0.93 mEq/L. Cows with concentration of NEFAs at calving ≥0.93 mEq/L were 2.52 times more likely to experience clinical mastitis than were cows with NEFAs <0.93 mEq/L ($P < 0.05$).

Table 5
Logistic regression models for the incidence of periparturient diseases in dairy cows using the percentile 75 of serum NEFAs at calving (1.2 mEq/L) as a cutoff value.

| Dependent variable | Explanatory variables | Adjusted OR | 95% CI OR | P value |
|---|---|---|---|---|
| RFMs | NEFA (<1.2 vs. ≥1.2) | 1.29 | 0.56–3.00 | 0.54 |
| | Parity (mult. vs. prim.) | 1.29 | 0.62–2.66 | 0.49 |
| | BCS at calving (for each point) | 0.89 | 0.31–2.62 | 0.84 |
| | Farm (1 vs. 2) | 0.89 | 0.34–2.45 | 0.88 |
| Metritis | NEFA (<1.2 vs. ≥1.2) | 1.05 | 0.40–2.77 | 0.91 |
| | Parity (mult. vs. prim.) | 1.02 | 0.43–2.37 | 0.96 |
| | BCS at calving (for each point) | 0.92 | 0.25–3.30 | 0.89 |
| | Farm (1 vs. 2) | 1.11 | 0.89–1.90 | 0.66 |
| Mastitis | NEFA (<1.2 vs. ≥1.2) | 0.45 | 0.22–0.92 | 0.03 |
| | Parity (mult. vs. prim.) | 1.93 | 0.89–4.20 | 0.09 |
| | BCS at calving (for each point) | 6.17 | 1.55–24.53 | 0.009 |
| | Farm (1 vs. 2) | 1.08 | 0.67–1.58 | 0.45 |
| MF | NEFA (<1.2 vs. ≥1.2) | 0.32 | 0.11–0.90 | 0.03 |
| | Parity (mult. vs. prim.) | 4.08 | 0.88–18.89 | 0.07 |
| | BCS at calving (for each point) | 12.68 | 1.19–135.18 | 0.03 |
| | Farm (1 vs. 2) | 1.33 | 0.77–2.56 | 0.65 |

OR, odds ratio; mult., multiparous; prim., primiparous.

Table 6
Logistic regression models for the incidence of periparturient diseases in dairy cows using the percentile 90 of serum NEFAs at calving (1.6 mEq/L) as a cutoff value.

| Dependent variable | Explanatory variables | Adjusted OR | 95% CI OR | P value |
|---|---|---|---|---|
| RFMs | NEFA (<1.6 vs. ≥1.6) | 0.98 | 0.31–3.10 | 0.97 |
| | Parity (mult. vs. prim.) | 1.27 | 0.61–2.64 | 0.52 |
| | BCS at calving (for each point) | 0.84 | 0.30–2.47 | 0.76 |
| | Farm (1 vs. 2) | 1.03 | 0.30–3.10 | 0.90 |
| Metritis | NEFA (<1.6 vs. ≥1.6) | 0.52 | 0.16–1.75 | 0.29 |
| | Parity (mult. vs. prim.) | 0.94 | 0.40–2.24 | 0.90 |
| | BCS at calving (for each point) | 0.80 | 0.23–2.86 | 0.73 |
| | Farm (1 vs. 2) | 0.77 | 0.45–2.45 | 0.79 |
| Mastitis | NEFA (<1.6 vs. ≥1.6) | 0.25 | 0.10–0.61 | 0.002 |
| | Parity (mult. vs. prim.) | 1.72 | 0.78–3.79 | 0.17 |
| | BCS at calving (for each point) | 5.40 | 1.35–21.71 | 0.01 |
| | Farm (1 vs. 2) | 1.09 | 0.55–2.39 | 0.57 |
| MF | NEFA (<1.6 vs. ≥1.6) | 0.53 | 0.15–1.85 | 0.32 |
| | Parity (mult. vs. prim.) | 4.15 | 0.90–19.16 | 0.06 |
| | BCS at calving (for each point) | 16.23 | 1.51–174.08 | 0.02 |
| | Farm (1 vs. 2) | 0.89 | 0.45–2.99 | 0.68 |

OR, odds ratio; mult., multiparous; prim., primiparous.

Per each 0.1 mEq/L increment of NEFAs at calving, the odds ratio for clinical mastitis was 1.10 times.

## 4. Discussion

In the current study, NEFAs were measured at calving because it was not feasible to obtain samples before parturition. From a practical point of view, NEFA at calving is much easier to evaluate than samples obtained before parturition. The hypothesis of this study was that cows with higher concentrations of NEFAs at calving will experience a greater incidence of periparturient diseases compared with that in cows with lower concentrations of NEFAs. The results of this study partially substantiated this hypothesis, as cows with NEFAs <1.2 mEq/L were one-half to one-third as likely to develop clinical mastitis and MF, respectively, compared with cows with ≥1.2 mEq/L. Similar results were found by Dyk et al. [3]. However, in that study, using 1650 dairy cows from 95 farms in Michigan, cows with ≥1.0 mEq/L NEFAs had a higher incidence of RFMs, ketosis, and clinical mastitis than did cows with <1.0 mEq/L serum NEFAs. This value is very similar to what the ROC analysis provided in the

Table 7
Logistic regression models for the incidence of periparturient diseases in dairy cows using serum NEFAs at calving as a continuous variable (for each 0.1 mEq/L).

| Dependent variable | Explanatory variables | Adjusted OR | 95% CI OR | P value |
|---|---|---|---|---|
| RFMs | NEFA at calving (for each 0.1 mEq/L) | 1.016 | 0.95–1.88 | 0.64 |
| | Parity (mult. vs. prim.) | 1.49 | 0.75–2.97 | 0.25 |
| | BCS at calving (for each point) | 0.65 | 0.26–1.64 | 0.36 |
| | Farm (1 vs. 2) | 1.32 | 0.33–3.01 | 0.98 |
| Metritis | NEFA at calving (for each 0.1 mEq/L) | 1.003 | 0.93–1.08 | 0.94 |
| | Parity (mult. vs. prim.) | 0.92 | 0.42–1.93 | 0.80 |
| | BCS at calving (for each point) | 0.74 | 0.25–2.17 | 0.59 |
| | Farm (1 vs. 2) | 1.04 | 0.55–2.78 | 0.74 |
| Mastitis | NEFA at calving (for each 0.1 mEq/L) | 1.11 | 1.03–1.19 | 0.003 |
| | Parity (mult. vs. prim.) | 2.05 | 0.92–4.60 | 0.07 |
| | BCS at calving (for each point) | 3.55 | 0.56–22.40 | 0.17 |
| | Farm (1 vs. 2) | 0.96 | 0.56–2.33 | 0.78 |
| MF | NEFA at calving (for each 0.1 mEq/L) | 1.05 | 0.95–1.16 | 0.30 |
| | Parity (mult. vs. prim.) | 4.37 | 1.00–20.10 | 0.05 |
| | BCS at calving (for each point) | 19.3 | 1.80–207.00 | 0.01 |
| | Farm (1 vs. 2) | 1.21 | 0.58–2.45 | 0.57 |

OR, odds ratio; mult., multiparous; prim., primiparous.

Table 8
Logistic regression model for the incidence of clinical mastitis in dairy cows based on the concentration of serum NEFAs at calving as a continuous variable (for each 0.1 mEq/L) within several NEFA ranges to determine linearity of the association.

| NEFA range (mEq/L) | Explanatory variables | Adjusted OR | 95% CI OR | P value |
|---|---|---|---|---|
| 0.15–0.5 | NEFA at calving (for each 0.1 mEq/L) | 1.21 | 0.48–3.06 | 0.68 |
| | Parity (mult. vs. prim.) | 1.94 | 0.19–19.9 | 0.57 |
| | BCS at calving (for each point) | 0.81 | 0.05–13.9 | 0.88 |
| | Farm (1 vs. 2) | 1.04 | 0.55–2.56 | 0.79 |
| 0.51–0.9 | NEFA at calving (for each 0.1 mEq/L) | 1.31 | 0.75–2.30 | 0.33 |
| | Parity (mult. vs. prim.) | 1.04 | 0.29–3.70 | 0.94 |
| | BCS at calving (for each point) | 6.33 | 0.46–87.3 | 0.16 |
| | Farm (1 vs. 2) | 0.86 | 0.45–2.77 | 0.45 |
| 0.91–1.2 | NEFA at calving (for each 0.1 mEq/L) | 0.53 | 0.20–1.38 | 0.19 |
| | Parity (mult. vs. prim.) | 1.57 | 0.35–7.6 | 0.55 |
| | BCS at calving (for each point) | 20.7 | 0.80–530 | 0.06 |
| | Farm (1 vs. 2) | 1.21 | 0.79–2.83 | 0.58 |
| >1.2 | NEFA at calving (for each 0.1 mEq/L) | 1.19 | 1.0–1.41 | 0.04 |
| | Parity (mult. vs. prim.) | 8.32 | 0.89–78.2 | 0.06 |
| | BCS at calving (for each point) | 6.80 | 0.46–101 | 0.16 |
| | Farm (1 vs. 2) | 1.11 | 0.79–3.1 | 0.78 |

OR, odds ratio; mult., multiparous; prim., primiparous.

Table 9
Summary of ROC analysis for milk fever in dairy cows.

| Parameter | Number | Estimate | 95% CI estimate | SEM | P value | OR | 95% CI OR | OR per 0.1 mEq/L NEFA | 95% CI OR |
|---|---|---|---|---|---|---|---|---|---|
| Intercept | 279 | −3.68 | −4.81, −2.55 | 0.57 | 0.0001 | NA | NA | NA | NA |
| NEFA (mEq/L) | 279 | 0.96 | 0.03, 1.89 | 0.47 | 0.04 | 2.60 | 1.03, 6.6 | 1.10 | 1.0, 1.20 |

OR, odds ratio; NA, not applicable.

current study (∼0.95 mEq/L); however, the value of 1.2 mEq/L offered a more remarkable cutoff value, because odd ratios for mastitis and MF were highly significant. In addition, although the value of 0.95 mEq/L provided the best combination of sensitivity and specificity, for the type of disease studied in the current research a slightly higher sensitivity than specificity is desirable. In other words, we preferred

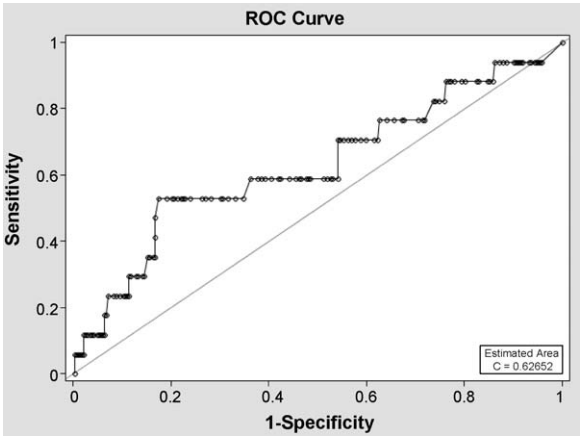


Fig. 1. Receiver-operator characteristic curve for MF in dairy cows.

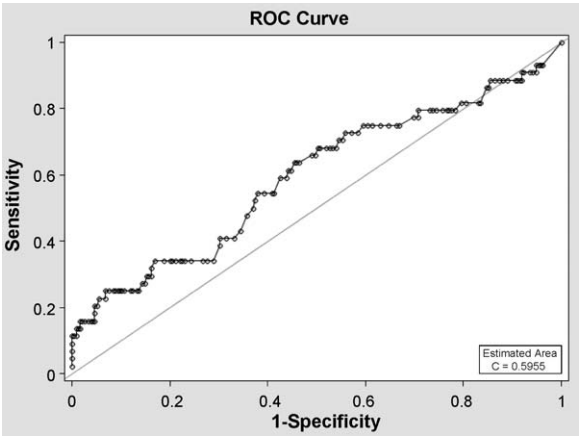


Fig. 2. Receiver-operator characteristic curve for clinical mastitis in dairy cows.

less false negatives (better sensitivity) than false positives (better specificity).

In the current study, the mean concentration of NEFAs at calving was 0.90 ± 0.47 mEq/L. This was in agreement with Vazquez-Añon et al. [10] and Bremmer et al. [11] who found similar concentrations of NEFAs at calving in U.S. Holstein cattle and with Leblanc et al. [5] in Canadian Holstein cattle. Conversely, other U.S. studies have found higher concentrations of NEFAs at calving (1.1 to 1.2 mEq/L) [12,13] than those of the current investigation, which may be indicative of excessive fat mobilization; however, the incidence of periparturient diseases in those studies were not beyond typical values reported in the scientific literature [8].

The mechanism by which high concentrations of NEFAs at calving are related to a higher incidence of mastitis may be explained by the negative effect of NEFAs on the immune system. Indeed, overconditioned cows at calving had higher concentrations of NEFAs and lower concentrations of IgM postpartum. Furthermore, they produced less IFN-γ 7 d prepartum [4]. This immunosuppression modulated by NEFAs was also demonstrated by in vitro studies in heifers [14], cows [15], and heat-stressed cows [16]. In addition, this may explain the tendency for a higher incidence of RFMs experienced by cows with higher concentrations of NEFAs than in those with lower concentrations. Cows that experienced RFMs had significantly lower neutrophil function than that in cows without RFMs before calving and during the first 2 wk postpartum [17]. In addition, interleukin-8, a potent neutrophil chemoattractant, was lower at calving in cows with RFMs than that in cows without RFMs [17]. Perhaps periparturient neutrophil function is a determining factor in the development of RFMs in dairy cattle. The association between high NEFAs and MF is more difficult to explain. Because this is not a cause-effect relationship, possibly cows with hypocalcemia were eating less; therefore, they mobilized more fat.

In conclusion, there was a relationship between the concentrations of NEFAs at calving and the incidence of certain periparturient diseases. Cows with ≥1.2 mEq/L were more likely to experience clinical mastitis and MF than cows with <1.2 mEq/L. However, this proposed cutoff value for NEFA concentrations, which demonstrated a better relationship with incidence of periparturient diseases, was moderately higher than that found in previous studies (1.0 mEq/L). In the light of current results, NEFAs at calving may be used for monitoring nutritional management and to predict the risk of periparturient diseases to take preventive measures.

Table 10
Summary of ROC analysis for clinical mastitis in dairy cows.

| Parameter | Number | Estimate | 95% CI estimate | SEM | P value | OR | 95% CI OR | OR per 0.1 mEq/L NEFA | 95% CI OR |
|---|---|---|---|---|---|---|---|---|---|
| Intercept | 279 | –2.56 | –3.31, –1.82 | 0.38 | 0.0001 | NA | NA | NA | NA |
| NEFA (mEq/L) | 279 | 0.93 | 0.27, 1.57 | 0.33 | 0.04 | 2.52 | 1.31, 4.83 | 1.10 | 1.03, 1.17 |

OR, odds ratio; NA, not applicable.

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