# Monitoring Negative Energy Balance in Transition Dairy Cows for Herd Results

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## Abstract

Non-esterified fatty acids **(NEFA)** and -hydroxybutyrate **(BHB)** are energy metabolites that can be used as markers of excessive negative energy balance in dairy cows during the transition period. When sampled in the appropriate time frame pre- and postpartum, NEFA and BHB concentrations above certain thresholds are associated with negative downstream outcomes, such as increased risk of disease, and decreased milking and reproductive performance at the individual cow level. The BHB concentrations can be measured qualitatively or quantitatively with several tests of varying sensitivities and specificities, both cow-side or in laboratories. Currently, NEFA concentrations can only be measured quantitatively in laboratories. At the cow level, the following metabolite concentrations are associated with negative downstream outcomes: pre-partum BHB $\geq$ 0.6 to 0.8 mmol/L; pre-partum NEFA $\geq$ 0.3 to 0.5 mEq/L; postpartum NEFA $\geq$ 0.7 to 1 mEq/L; and postpartum BHB $\geq$ 1.0 to 1.4 mmol/L. At the herd level, negative downstream outcomes can be seen when more than 15 to 25% of the individual cows sampled (given the appropriate sample size) are above the metabolite concentrations shown above. Herd level sensitivity is adversely affected by low cow-level test sensitivity, especially at lower prevalences and smaller sample sizes. Rational metabolite testing protocols can lead to appropriate identification of herd level risk for transition cow problems. Based on this, preventive and corrective management strategies, including treating individual cows with propylene glycol, can be employed.

## Introduction

Most transition dairy cows visit a state of negative energy balance **(NEB)** due to increased energy demands after parturition, coupled with lagging dry matter **(DM)** intake (Hayirli et al., 2002). The ability to partition available energy for milk production early in lactation (Bauman and Currie, 1980) has made the role of energy balance a key factor in the study of milk production, reproductive performance, and disease occurrence. The metabolites non-esterified fatty acids **(NEFA)** and/or -hydroxybutyrate **(BHB)** are common measures of NEB and/or ketosis in transition animals (Duffield et al., 2009). Although some elevation of these metabolites is normal as these animals balance energy intake and energy demands, excessive elevation can indicate poor adaptation to NEB (Herdt, 2000). Identification of an objective level where NEFA and/or BHB are excessive and cause detrimental effects on health, reproduction and milk production, has been difficult due to individual animal variations, normal metabolite elevations during the transition period, and the multiple herd-level factors that can affect the outcomes of interest.

The objectives here were to: 1) identify critical thresholds above which NEFA and BHB concentrations increase the risk of disease and affect production and reproductive performance at the

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individual animal level; 2) investigate the magnitude of these associations in free-stall, total mixed ration **(TMR)**-fed herds; 3) evaluate herd-level outcomes associated with the proportion of animals sampled which were above NEFA and BHB critical thresholds; 4) evaluate sampling schemes to estimate herd level outcomes; 5) intensively measure the incidence of early lactation subclinical ketosis in high performing herds; 6) identify dry period risk factors for cows that develop ketosis; and 7) evaluate the cost:benefit of various testing schemes for subclinical ketosis and subsequent treatment of positive cows with propylene glycol.

**Materials and Methods for Multi-Herd Study**

A convenience sample of 104 farms in the Northeast USA were selected to participate in a prospective cohort study. All farms consented to participate, and this study was approved by the Cornell University Institutional Animal Care and Use Committee. To be included in the study, a herd must have: 1) had greater than 250 milking cows, 2) free-stall housing, 3) fed a TMR, and 4) participated in DHIA and/or use Dairy Comp 305 (Valley Ag. Software, 2009).

All farms received a standardized consent form, a survey, and case definitions for diseases of interest. The survey collected information on farm demographics, feeding times in relation to blood collection, voluntary waiting period, and ovulation synchronization protocols. Farm personnel were instructed to document any incident cases of the diseases of interest: displaced abomasum **(DA)**, clinical ketosis **(CK)**, and metritis **(MET)** and/or retained placenta **(RP)**.

Farms were visited once, and during the farm visit, 2 cohorts of animals were identified: those 14 to 2 days pre-partum and 3 to 14 days post-partum. Within each cohort, convenience samples of 15 apparently healthy animals were evaluated.

The evaluation included simultaneous blood collection and body condition scoring **(BCS)** (Ferguson et al., 1994). Guidelines for blood collection and sample handling were based on previous studies (Stokol and Nydam, 2006). Briefly, a plain evacuated red-top tube was used to collect 10 ml of blood from the coccygeal vein or artery. The sera from the pre-partum cohort were analyzed for NEFA and hemolysis. The sera from animals sampled post-partum were analyzed for NEFA, BHB, and hemolysis. For animals sampled, the incidence of the diseases of interest within 30 days in milk **(DIM)**, time to pregnancy within 70 days post voluntary waiting period, and Mature Equivalent 305 (ME 305) milk at 120 DIM were recorded.

*Data analysis*

Statistical analyses of data were performed using SAS version 9.1 (SAS Institute, Inc., Cary, NC 2004) and receiver operator characteristic **(ROC)** curves were obtained using MedCalc (Schoonjans, 2008). Data from the pre- and post-partum cohorts were analyzed separately. Initially, data was stratified by parity group (parity =1 or >1), but if the effect of the predictors on the outcome was similar between the 2 groups, they were pooled in the final analyses.

In summary, the analytical approach was done in 3 stages. The first stage was to identify significant risk factors with a multivariable model where the outcome was the development of any combination of the diseases of interest (DA, CK, MET, and/or RP). During the second stage, the continuous significant predictors from the multivariable model with ROC curves were analyzed to identify critical thresholds for prediction of individual diseases (e.g., DA) and any combination of the diseases. Once the range of critical thresholds predictive of disease was identified, the covariates were treated as categorical variables within this range. In the final stage, the magnitudes of the

associations between these categorical predictors with disease, reproduction, and production were evaluated. For each of these outcomes, 3 full models were evaluated: pre-partum NEFA and covariates; post-partum NEFA, BHB and covariates; and BHB with covariates.

At the herd level, the proportion of animals sampled that were above the critical thresholds was evaluated as the predictor variable and the herd level outcomes were: incidence of DA or CK in sampled animals; herd PR; and average ME 305 from sampled animals.

*Evaluation of significant risk factors*

The metabolites NEFA and/or BHB were the main risk factors at this level of analysis and were treated as continuous predictors. Parity, season, BCS, time of blood collection, and all biologically plausible 2-way interactions were evaluated as covariates in the model. They were modeled with PROC GENMOD using a Poisson distribution, a log link function, p-scale option for over-dispersion, and an exchangeable correlation matrix (Spiegelman and Hertzmark, 2005, Ospina et al., 2012). This statistical method allows for clustering of cows within herds (i.e., including herd as a random effect) while adjusting for continuous or categorical covariates. There was no adjustment for varying time spans (offset term) because the length of the time interval at risk was the same for every individual in the sample (Allison, 2007).

*ROC curves*

The continuous, significant risk factors identified in the multivariable model were evaluated using ROC curves to determine the critical threshold for predicting disease. The point on the ROC curve that has the highest combined sensitivity and specificity was considered the critical threshold. Interpretation of this critical threshold depends on the area under the curve **(AUC)**, such that if the AUC >0.7, the test is considered accurate (Swets, 1988).

*Effect on disease risk, reproduction, and production at the individual animal level*

Disease risk

Once the critical thresholds for prediction of disease (DA, CK, MET/RP, or any combination) were identified with ROC analysis, the covariates were dichotomized at the critical threshold. The risk of disease, given these categorical covariates, was evaluated with PROC Genmod, using a poisson distribution, log link function, p-scale option for over-dispersion, and an exchangeable correlation matrix (Ospina et al., 2010a).

Effect on reproduction

The effects of elevated NEFA and/or BHB concentrations on reproductive performance were evaluated with time-to-event analysis (PROC Phreg). Cox proportional hazard models (Cox, 1972) were analyzed accounting for clustering of cows within herds. The covariates were: BCS, parity, and ME 305 milk at 120 DIM. The ME 305 data were dichotomized based on the median production of the pre- or post-partum group. Animals culled before the end of voluntary waiting period were excluded from the analysis and those not pregnant by the end of the follow-up period were right censored. The proportional hazards assumption was checked statistically by evaluating time dependent covariates and non-informative censoring was evaluated with sensitivity analysis (Allison, 2007; SAS Institute, 1995). The categorical metabolite value selected from within the range of critical threshold predictive of disease that resulted in the smallest chance of committing a type I error was kept in the final model (Ospina et al., 2010b).

Effect on milk production

The effects of elevated NEFA and/or BHB concentrations on ME 305 milk were evaluated with

mixed effects models with herd as a random effect. The covariates were: BCS, season, and when applicable both parity, and the interaction between parity and the metabolite level. In all models, the metabolites NEFA and BHB were dichotomized and evaluated within the range of values identified as critical thresholds for prediction of disease. The categorical metabolite value that resulted in the smallest chance of committing a type I error was kept in the final model (Ospina et al., 2010b).

*Effect on disease, reproduction and production at the herd level*

The herd alarm level

Once estimates of the metabolite thresholds were established at the individual animal level, the herd alarm level, i.e., the proportion of sampled transition cows per herd with elevated pre-partum NEFA, and postpartum BHBA and NEFA concentrations that were associated with herd-level incidence of diseases, decreased pregnancy rate, and milk production was evaluated. The interaction between parity and the level of the metabolite was evaluated and the analysis was stratified by parity (parity =1 or >1) if there was a difference in the effect.

The herd alarm level consists of 2 numbers: 1) the metabolite (NEFA or BHBA) concentration threshold above which detrimental downstream outcomes are most likely to occur and 2) the proportion of animals with metabolite concentrations above this threshold that is associated with herd-level downstream outcomes. To establish the herd-alarm level, both of these parameters were evaluated concurrently. The lowest metabolite concentration and smallest proportion that yielded the smallest chance of committing a type I error and had the largest change in the outcome of interest was kept in the final model (Ospina et al., 2010c). The metabolite concentrations were evaluated within the range identified as critical thresholds associated with individual-cow health effects (Ospina et al., 2010a).

*Sampling scheme to estimate herd level outcomes*

Number of animals to sample

Based on empirical data, further data sets were simulated to estimate herd level sensitivity **(HSe)**, specificity **(HSp)**, and positive/negative predictive value of the herd alarm level for subclinical ketosis. The true prevalence of the condition was evaluated at several levels, starting with a very conservative 10% and up to 40%. These analyses were performed using formulas presented by Martin et al. (1992). The critical threshold used to decide whether a herd was positive was 15%, and the BHB concentration was >12 mg/dL (which is approximately equal to 1.2 mmol/L).

**Results of Multi-Herd Study**

*Study population*

Of the 104 herds, 4 were excluded from the study due to missing data. The 2758 cows from the remaining 100 herds were included in the study, and of these cows, 1440 were sampled pre-partum (35% heifers and 65% cows) and 1318 were sampled post-partum (37% heifers and 63% cows). The number of milking cows per herd averaged 840.

*Multivariable analysis*

In the 3 multivariable models, the metabolites were the only significant predictors of any of the diseases of interest: pre-partum NEFA (P = 0.028), post-partum NEFA (P = 0.0005), and when BHB was the only main predictor in the model (P = 0.005), it was also the only significant predictor. No other covariate or interaction term in any of the 3 multivariable models had a P-value < 0.10.

## ROC- critical thresholds for prediction of disease

The critical thresholds identified with ROC analysis are summarized in Table 1 with their AUC values. Briefly, the NEFA critical thresholds for predicting any of the diseases of interest in the pre- and post-partum cohort were 0.29 and 0.6 to 0.7 mEq/L, respectively and the BHB critical threshold was 10 to 12 mg/dL. Figure 1 is a graphical representation of an ROC curve with DA as the outcome and concentrations of post-partum NEFA as the test.

### Risk of disease

The risk of disease based on NEFA and BHB concentrations greater than or equal to critical thresholds are also summarized in Table 1. For example, experiencing elevated metabolite levels post-partum increased the risk of developing a DA by up to 10 times, and elevated levels of post-partum NEFA contributed the greatest risk of disease development.

### Effect on reproduction

Table 2 summarizes the results of elevated metabolite levels on reproduction with estimates for metabolites and significant covariates reported. Animals with elevated metabolite levels (within the range identified as predictors of disease) took longer to get pregnant; the hazard ratio for pregnancy within 70 days post-voluntary waiting period decreased. Figure 2 is a graphical representation of a Kaplan-Meier curve, where animals with elevated pre-partum NEFA levels took longer to get pregnant.

### Effect on production

The results of elevated metabolite concentrations are reported separately for heifers and cows sampled post-partum because the effect of the elevated metabolite thresholds on ME 305 milk was different between these 2 groups. Generally, elevated metabolite levels predicted a decrease of several hundred kilograms of ME 305 milk; however, in heifers sampled post-partum, elevated metabolite concentrations predicted an increase in ME 305 milk. The results of this analysis are summarized in Table 3 with metabolite results and significant covariates reported.

### Herd alarm levels

Table 4 summarizes the herd alarm levels, i.e., the proportion of animals sampled with NEFA or BHB concentrations above which negative downstream outcomes are more likely. The outcomes evaluated were: DA and CK incidence in sampled animals; pregnancy rate at the herd level; and ME 305 milk based on 4 test days. If more than 15% of the animals sampled had NEFA or BHB concentrations above the threshold, herds had an increase in disease, decrease in pregnancy rate, and decrease in ME 305 milk compared to herds that were below the herd alarm level.

### Herd level sampling

The HSe and HSp are very sensitive to the true prevalence of the condition of interest, the number of animals tested, and the critical threshold used to decide whether a herd is positive. Given a 15% cut-point for calling a herd positive, the HSe increases as the herd true prevalence increases, but it is largest when the sample size is 15. The Hsp does not depend on the underlying true prevalence; however, it is affected by the cut-point used to call a herd positive. Both the HSe and HSp are above 0.90 when the true prevalence is $\geq 0.3$, 15 animals are sampled, and the cut-point for calling the herd positive is $\geq 2$ animals with NEFA or BHB concentrations above the cut-point.

The herd predictive value positive **(HPV+)** and herd predictive value negative **(HPV-)** depend on the HSe, Hsp, and both the within and between

herd prevalence. The HPV+ increases as the sample size and cut-point used to determine whether a herd is positive increases, and it is $\geq 0.90$ when the true within herd true prevalence is $\geq 0.30$, regardless of between herd prevalence. The HPV- increases as within herd prevalence increases, but it generally decreases as the prevalence of positive herds increases. The HPV- is most sensitive to this change when the within herd true prevalence is <0.30. Both the HPV+ and HPV- are above 0.90 when both the within herd true prevalence are > 0.30 and 15 animals are sampled.

**Conclusions From Multi-Herd Study**

The work to this point demonstrates that excessive NEB (as measured by NEFA and BHB concentrations) in the transition period are strong predictors of clinical disease, and negative reproductive and productive performance in cattle from free-stall, TMR-fed dairy herds averaging 840 milking cows. The magnitude of the association between elevated NEFA and/or BHB and diseases of interest, measured by risk ratios, was large (range: 2.0 to 10 times more likely to get a metabolic disease when preceded by elevated NEFA or BHBA). The effects of elevated metabolite levels on reproduction decreased the hazard of pregnancy within 70 days post-voluntary waiting on average by 20%, with parity as the only other significant covariate (cows took longer to get pregnant than heifers). Milk production showed mixed results, and although further investigation about homeorhesis in heifers is warranted, there was strong evidence of significant ME 305 milk loss in cows sampled post-partum and all animals in the pre-partum cohort. When evaluated at the herd level, high levels of NEFA and BHBA were detrimental to milk production in heifers. The herd alarm levels for the concentration above which detrimental outcomes were likely were similar to individual animal levels, and the proportion of animals was on average only 15%. When evaluating herd level factors associated with sampling, it appears that at least 15 animals in

that at-risk group should be sampled. It is important to note that these herds were not chosen to participate in the study due to any issues with metabolic disease. Within herds, only cows that appeared healthy were sampled, i.e., in order to be included in the study, a cow could not have already developed a DA, CK or MET. Despite these selection criteria, the prevalence of herds above the herd alarm level was 40%. Being above the herd alarm level means that more than 15% of sampled animals had BHB concentrations > 1.2 mmol/L. The distribution of herds having a given percent of sampled animals above the cow-cut point of 1.2 mmol/L is in Figure 3.

Management programs focused on minimizing the risk of these diseases and minimizing negative effects of decreased reproductive and productive performance may consider the following as general guidelines for monitoring NEFA and BHB concentrations in cattle: at least 15 animals should be sampled to determine if more than 15% of the animals sampled have NEFA concentrations $\geq 0.3$ mEq/L for cattle 14 to 2 days pre-partum; and NEFA concentrations $\geq 0.6$ mEq/L and BHB $\geq 12$ mg/dL for those 3 to 14 days post-partum.

**Introduction to Intensive Study of High Performing Herds**

Some amount of both NEFA and BHB are normal in early lactation ruminants; however, excessive amounts of either can lead to increased risk of disease, decreased reproductive performance, and decreased milk production. When compared to NEFA, ketone monitoring and evaluation can be less expensive and more practical, even though NEFA concentrations are a bit more predictive. Additionally, some cow-side ketone tests also offer a high degree accuracy. The most common fluids used for ketone tests, presented in order of increasing accuracy, are: urine (e.g. Ketostix®), milk (e.g. KetoTest™), and blood (e.g. Precision Xtra™ Meter). The blood meter, a hand-

held device originally designed for use in humans with diabetes, costs about $30, and the test strips cost around $1.50 per sample when purchased through your veterinarian. This test is very accurate (>96% sensitivity and 99% specificity) and gives a cow-side answer in about 10 seconds.

Prevalence estimates existing cases of disease - it is like a snapshot in time. In the above mentioned study, prevalence was used to evaluate the effect of subclinical ketosis **(SCK)** on herd performance. Estimating herd prevalence can be done very quickly, and it is recommended to sample at least 15 to 20 healthy animals between 3 to 14 DIM and record how many of the animals sampled have BHB concentrations $\geq 1.2$ mmol/L. For example, if 20 animals are sampled and 4 have a BHB concentration $\geq 1.2$ mmol/L, then the prevalence is 20% (4/20), and the herd is considered to be at increased risk for the negative downstream outcomes mentioned previously.

Although prevalence is a very useful measurement, the incidence of a condition sometimes gives different information. Incidence is defined as the number of cows that develop a new case of SCK divided by all the animals at risk. If, for example, 15 cows within a group of 50 sampled cows develops a new case of SCK sometime from 3 to 16 DIM, the incidence is 30% (15/50). The incidence of SCK is approximately twice the prevalence.

**Results and Discussion of Intensive Study in High Performing Herds**

A more recent study by researchers at Cornell and the University of Wisconsin followed 1,717 cows from 3 to 16 DIM in 4 free-stall, TMR fed herds (McArt et al., 2011, 2012b). Using the Precision Xtra meter, all cows that calved within the study period were monitored for SCK, defined as a BHB concentration of 1.2 to 2.9 mmol/L. Cows were tested on Mondays,

Wednesdays, and Fridays, and given this testing scheme, each cow was sampled 6 times, beginning at 3, 4, or 5 DIM and ending on 14, 15, or 16 DIM. The highest incidence of SCK occurred at 5 DIM, with 75% of cows that developed SCK testing positive for the first time from 3 to 7 DIM (Figure 4). Cows that tested SCK positive from 3 to 7 DIM were over 6 times more likely to develop a DA, 4.5 times more likely to be removed from the herd, 0.7 times as likely to conceive to first service, and made almost 5 lb/day milk per cow for the first 30 DIM than cows first testing SCK positive between 8 and 16 DIM. Thus, it is important to identify these SCK cows early in lactation in order to reduce the risk of negative downstream events.

The above study also looked at risk factors in dry cows to help predict which cows went on to develop ketosis between 3 and 5 DIM, as most cows first develop SCK during this time, and as mentioned above, these cows are at a higher risk for negative events than cows first developing SCK later in lactation (McArt et al., 2012c). Cows with pre-calving NEFA $\geq 0.30$ mEq/L were almost 2 times more likely to develop ketosis than cows with NEFA < 0.30 mEq/L; similarly, cows birthing male calves were 1.8 times more likely to develop ketosis than cows birthing female calves. In addition, cows with a calving ease $\geq 3$ on a scale of 1 to 5 were 2.6 times more likely to develop ketosis than cows with a calving ease < 3, cows that gave birth to a dead calf were 2.2 times more likely to develop ketosis than cows that gave birth to a live calf, and parity $\geq 3$ cows were 3 times more likely to develop ketosis than their younger herdsmates. Thus for herds that choose to focus their ketosis testing rather than test all fresh cows, special attention should be paid to cows with high pre-calving NEFA, older cows, and cows that have had difficulty birthing.

The same study evaluated the benefits of daily oral drenching of propylene glycol in cows diagnosed with SCK (McArt et al., 2011, 2012a). The first time a cow tested positive for SCK she

was randomized to either the treatment group where she received 300 mL (10 oz) of propylene glycol by oral drench once daily until she tested < 1.2 mmol/L or the control group where she was not given propylene glycol. Most cows were treated for 5 days. The SCK positive cows treated with propylene glycol were almost half as likely to develop a DA, half as likely to be removed from the herd, and on some farms, made more milk (3 lb/day/cow) in early lactation than SCK cows not given propylene glycol. In addition, SCK cows treated with propylene glycol were more likely to conceive at first service. Based on this study and the expected duration of SCK, treatment of SCK positive cows with a 5 day course of daily propylene glycol drenching is suggested.

A partial budget was developed to assess the benefit:cost ratio of different SCK testing scenarios and treatment with propylene glycol. On a herd level, the most cost-effective method depends on the herd SCK incidence. This analysis evaluated 4 different testing and treatment strategies at varying herd SCK incidences. Results indicate that at herd SCK incidences above 50%, blanket treatment of all fresh cows with 5 days of oral propylene glycol starting at 5 DIM is the most cost-effective strategy. At incidences between 15 and 50%, testing cows that are 3 through 9 DIM 2 days per week (e.g. Mondays and Thursdays) and treating SCK positive cows with 5 days of oral propylene glycol is the most cost-effective strategy; although testing all cows that are 3 through 16 DIM one day per week (e.g. Mondays) will also provide a positive return on investment. For a herd with a 40% incidence of SCK that freshens 1,000 cows per year, choosing to test cows 2 days per week and treating the positives will benefit $10,000 to $25,000 per year.

It may be easier to first conduct a SCK prevalence test (sample 15 to 20 cows) on a herd in order to approximate the herd incidence and determine the best testing and treatment plan. For those herds with an estimated incidence greater than 50%, where blanket treatment with PG is initiated, repeated prevalence testing may be necessary after management changes are implemented to determine if treating all fresh cows remains the best option. For herds with an incidence from 15 to 50%, either the one day per week or 2 day per week testing strategies will allow for repeated monitoring of herd incidence; however, it is important to remember that herds that choose to test cows from 3 to 9 DIM should assume that they are only identifying 80% of the cows that will develop SCK between 3 and 16 DIM. Repeated incidence or prevalence testing is recommended in order to evaluate changes in transition cow management and allow appropriate adjustment of farm SCK testing and treatment protocols. Remember the goal is to not treat many, if any, cows with propylene glycol, but rather have transition cow management strategies in place such that the prevalence of SCK is lower than 15%.

## Conclusion of Intensive Study in High Performing Herds

In well managed TMR fed freestall herds in the northeast and upper Midwest, the incidence and thus prevalence of SCK is high. The SCK is a condition not recognized clinically until it predisposes cows and herds to higher incidences of transition cow diseases, lower milk production, and lower milk production. Thus, it is a costly condition. Work with your management team to develop a testing strategy to assess your level. Treating SCK cows with propylene glycol is cost effective in almost all scenarios until preventive management strategies can be put in place.

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**Table 1.** Receiver operator characteristic **(ROC)** curve determination of critical non-esterified fatty acids **(NEFA)** (mEq/L) and -hydroxybutyrate **(BHB)** (mg/dL) thresholds as predictors of disease and risk ratios of disease based on these critical thresholds.

| Disease[1] | Critical NEFA threshold[2] | AUC[3] | Risk Ratio (RR) | 95% RR CI[4] | P-value |
|---|---|---|---|---|---|
| | Animals sampled pre-partum | | | | |
| DA | 0.27 | 0.6 | 2.0 | 1.1 – 3.7 | 0.03 |
| CK | 0.26 | 0.6 | 1.8 | 1.2 – 2.5 | 0.001 |
| MET and/or RP | 0.37 | 0.6 | 2.2 | 1.6 – 3.0 | <0.0001 |
| Any 3 | 0.29 | 0.6 | 1.8 | 1.4 – 2.2 | <0.0001 |

| Disease | Critical NEFA threshold[1] | AUC[2] | Risk Ratio | 95% RR CI[3] | P-value |
|---|---|---|---|---|---|
| | Animals sampled post-partum | | | | |
| DA | 0.72 | 0.8 | 9.7 | 4.2 – 22 | <0.0001 |
| CK | 0.57 | 0.7 | 5.0 | 2.3 – 11 | <0.0001 |
| MET | 0.36 | 0.6 | 17 | 2.0 – 133 | 0.008 |
| Any 3 | 0.57 | 0.7 | 4.4 | 2.6 – 7.3 | <0.0001 |

| Disease | Critical BHB threshold[1] | AUC[2] | Risk Ratio | 95% RR CI[3] | P-value |
|---|---|---|---|---|---|
| DA | 10 | 0.8 | 6.9 | 3.7 – 12.9 | <0.0001 |
| CK | 10 | 0.7 | 4.9 | 3.2 – 7.3 | <0.0001 |
| MET | 7 | 0.6 | 2.3 | 1.1 – 5.1 | 0.037 |
| Any 3 | 10 | 0.7 | 4.4 | 3.1 – 6.3 | <0.0001 |

[1]DA = Displaced abomasum, CK = clinical ketosis, MET = metritis, and RP = retained placenta.
[2]Highest combined specificity and sensitivity.
[3]Area under the curve.
[4]Risk ratio confidence interval.

**Table 2.** Cox proportional hazard model of the effect of non-esterified fatty acids **(NEFA)** (mEq/L), and/or -hydroxybutyrate **(BHB)** (mg/dL), covariates, and animals clustered within herds on days to conception after voluntary waiting period.

| Sampled Population | Variable | Hazard Ratio | P-value |
|---|---|---|---|
| Pre-partum cohort | NEFA $\geq$ 0.27 | 0.81 | 0.01 |
| | Parity | 0.73 | 0.0004 |
| Post-partum cohort | NEFA $\geq$ 0.72 | 0.84 | 0.05 |
| | BHB $\geq$ 10 | 0.93 | 0.40 |
| | Parity | 0.81 | 0.01 |
| Post-partum cohort | BHB $\geq$ 10 | 0.87 | 0.09 |
| | Parity | 0.80 | 0.01 |

**Table 3.** Mixed models for the effect of non-esterified fatty acids **(NEFA)** (mEq/L), and/or -hydroxybutyrate **(BHB)** (mg/dL), covariates, and herd as a random effect on milk production measured by 120 DIM mature equivalent **(ME)** 305 (kg).

| Sampled Population | Variable | Hazard Ratio | P-value |
| --- | --- | --- | --- |
| Pre-partum cohort | NEFA $\geq$ 0.33 | -1503 | 0.001 |
| | Parity | -1223 | 0.01 |
| Post-partum cohort - heifers | NEFA $\geq$ 0.57 | -1074 | 0.02 |
| | BHB $\geq$ 10 | -315 | 0.50 |
| Post-partum cohort - cows | NEFA $\geq$ 0.72 | -1423 | 0.001 |
| | BHB $\geq$ 10 | -363 | 0.40 |
| Post-partum cohort - cows | BHB $\geq$ 10 | -865 | 0.04 |

**Table 4.** Herd level effect of elevated nonp-esterified fatty acids **(NEFA)** or -hydroxybutyrate **(BHBA)** concentrations on outcomes.

| Proportion of Animals | Herd Alarm Level Metabolite Level | Effect on Outcomes[1] | P-value |
| --- | --- | --- | --- |
| 15% | Pre-partum NEFA: 0.27 mEq/L | + 3.6% Disease | 0.006 |
| | | - 1.2 % in PR | 0.006 |
| | | - 620 lb of ME 305 | 0.002 |
| 15% | BHBA: 12 mg/dL | + 1.8 % disease | 0.03 |
| | | - 0.8 in PR | 0.03 |
| | BHBA: 10 mg/dL in cows[2] | - 788 lb of ME 305 | 0.0004 |
| 15% | Post-partum NEFA: 0.70 mEq/L | +1.7% disease | 0.04 |
| | 0.60 mEq/L | - 0.9 PR | 0.05 |
| | 0.70 mEq/L in cows[2] | - 1305 lb of ME 305 | <0.001 |

[1]Disease = incidence of displaced abomasum or clinical ketosis in sampled animals; PR = herd level pregnancy rate; and ME 305 = Estimated mature milk equivalent 305 at 120 DIM (4 test days).
[2]Cows = parity $\geq$ 2.
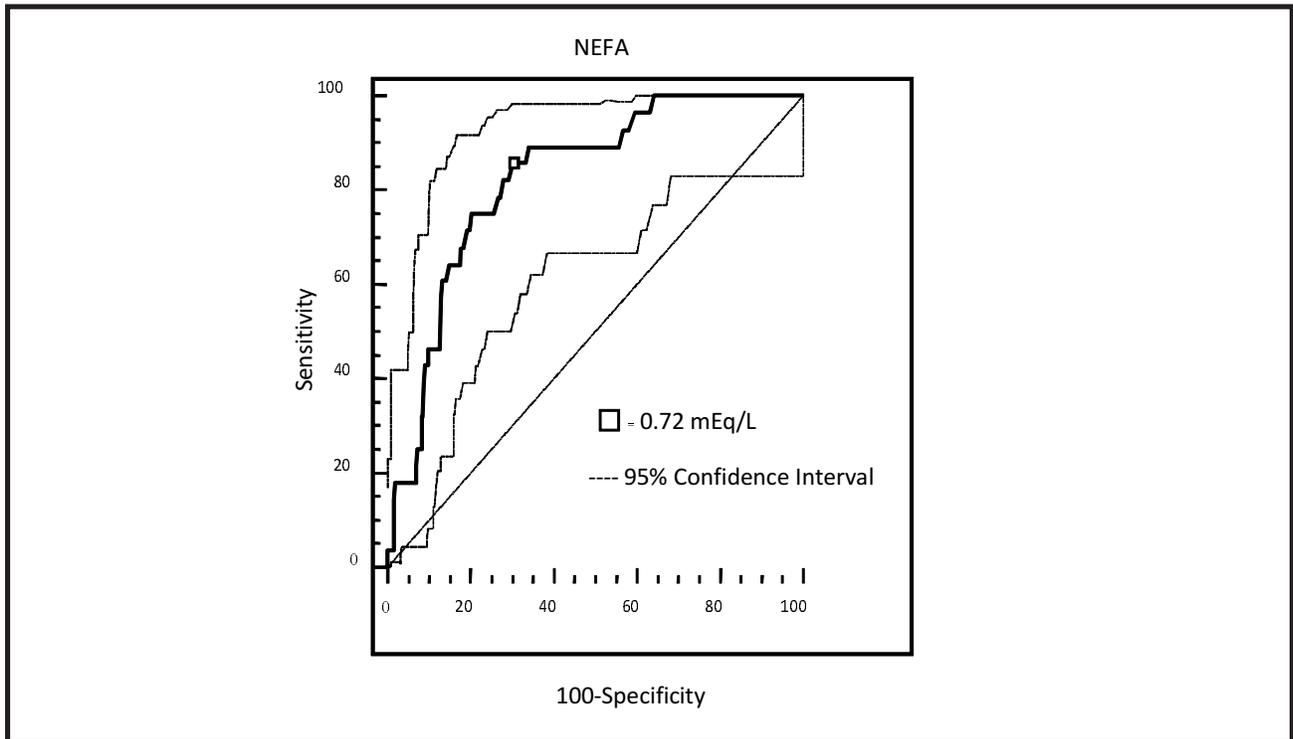
**Figure 1**. Receiver operator characteristic **(ROC)** curve determination of critical threshold (upper most left hand corner) for NEFA concentrations predicting displaced abomasum in animals sampled post-partum.
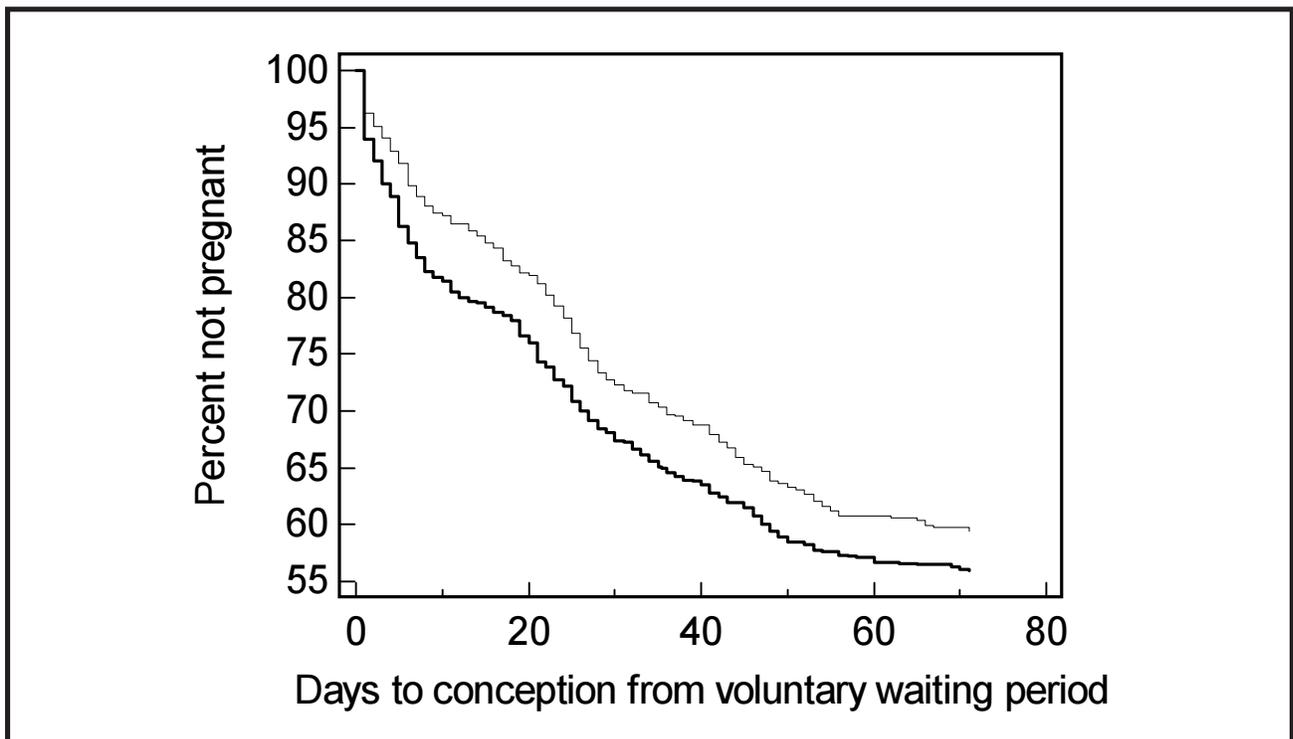


**Figure 2**. Kaplan-Meier curves of time to pregnancy of cows and heifers with non-esterified fatty acids **(NEFA)** $\geq 0.27$ mEq/L or $< 0.27$ mEq/L measured in serum 14 to 2 days prepartum.
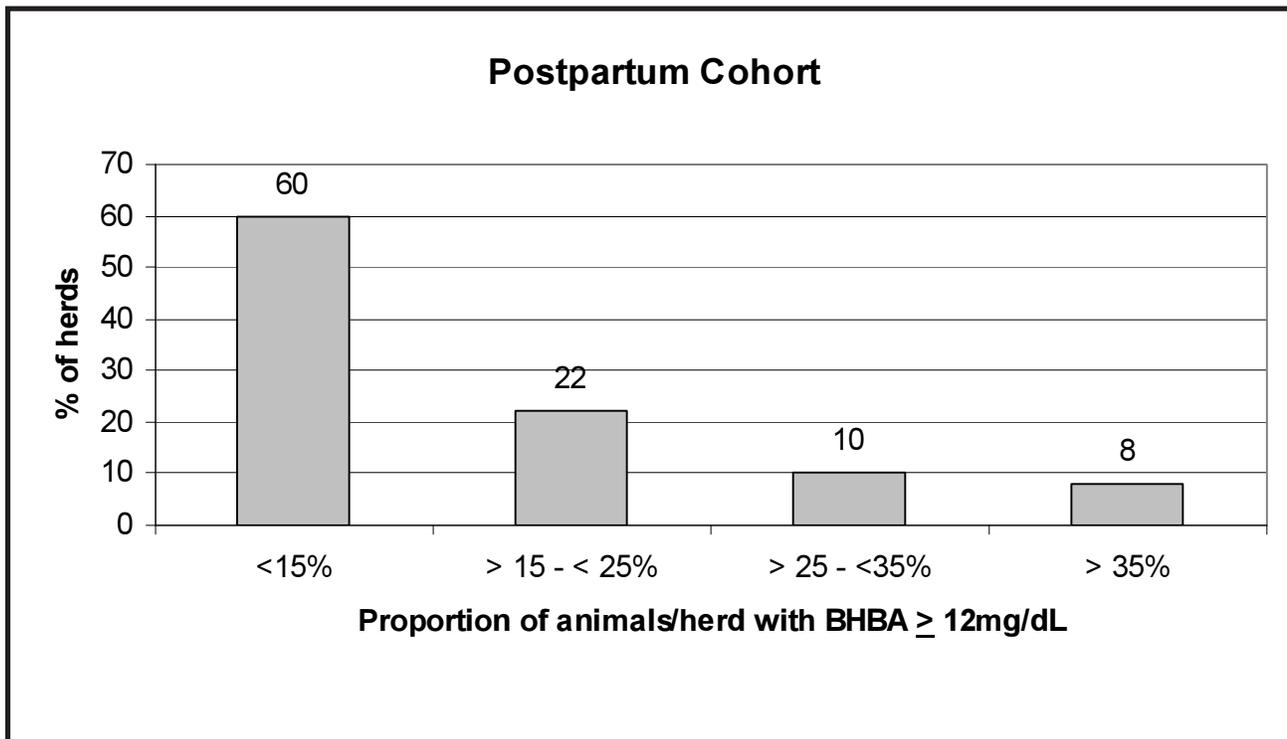
**Figure 3**. Prevalence of herds showing the percent of sampled animals within herd above -hydroxybutyrate **(BHB)** $\geq 1.2$ mmol/L (~12 mg/dl). About 40% of herds were above the herd alarm level of 15% of sampled animals.
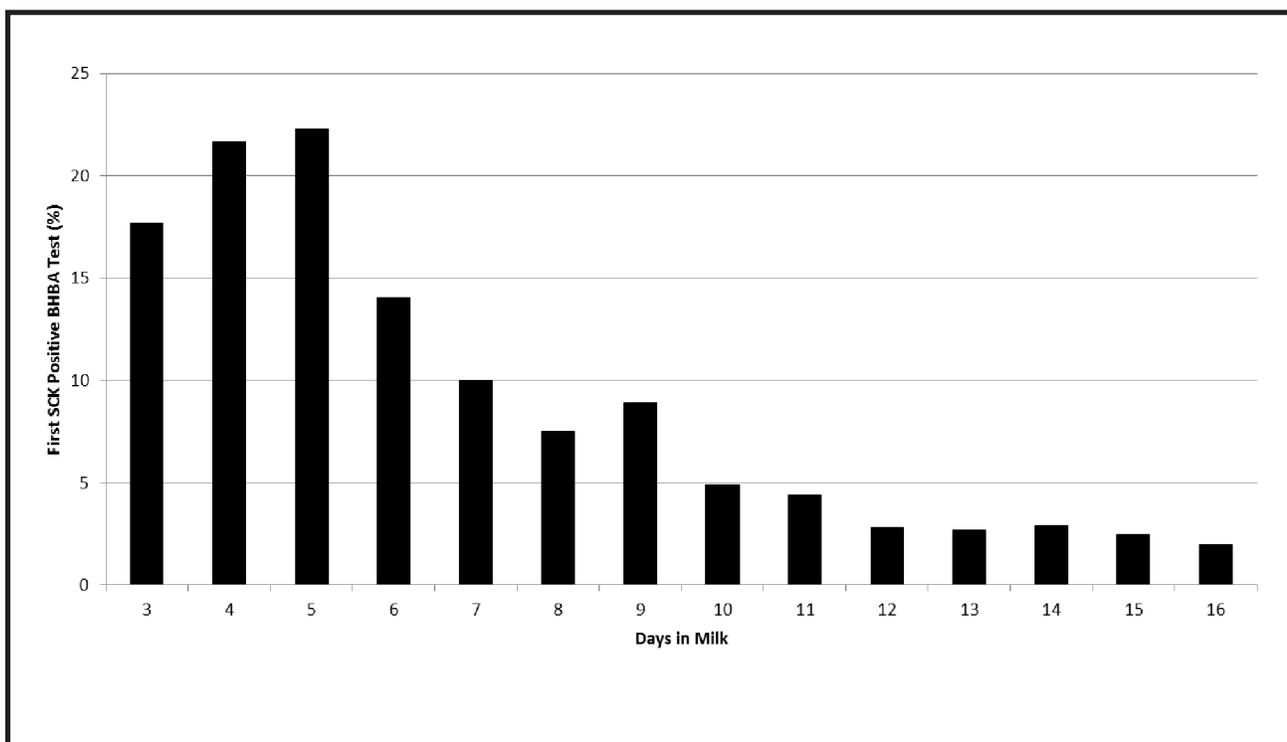


**Figure 4**. Incidence of subclinical ketosis **(SCK)** ( -hydroxybutyrate **(BHB)** $\geq 1.2$ mmol/L) by days in milk of test.