

# The coefficient of determination between NEFA and BHBA and the association between their ratio and the development of disease in healthy cows sampled during the post-partum period

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## Introduction

As a consequence of lagging dry matter intake and increased energy demands, nonesterified fatty acid (NEFA) is released into the bloodstream from adipose tissue to be used as a source of energy in dairy cows in early lactation. Nonesterified fatty acid is used for milk fat synthesis, ketone production (e.g., -hydroxybutyrate; BHBA) and liver triglyceride accumulation. Nonesterified fatty acid and BHBA can be used as markers of negative energy balance (NEB) and elevated concentrations in the transition period have been associated with an increased risk of developing disease.

The multiple pathways available for NEFA may result in a non-linear relationship between NEFA and BHBA concentrations. Additionally, the ratio between these two metabolites may differ in sub populations, e.g., subclinically ketotic (SCK) cows, and cows with elevated NEFA. Hence, the ratio of these metabolites may be a useful parameter for further understanding the biological mechanisms of adaptation or maladaptation to NEB. The objectives of this study were to evaluate the R<sup>2</sup> coefficient of determination between NEFA and BHBA in apparently healthy cows three to 14 DIM, and to estimate the association between the ratio of NEFA to BHBA and the subsequent development of displaced abomasum (DA), clinical ketosis (CK), or metritis (MET).

## Materials and Methods

Data from a prospective cohort study in free-stall dairy farms in the Northeast was used for the analysis. Approximately 20 healthy post-partum cows (three to 14 DIM) were sampled cross-sectionally from each farm and NEFA and BHBA concentrations were evaluated. The outcomes of interest were: the development DA, CK, or MET within 30 DIM. The cows were grouped into three categories and analyzed independently: 1) all sampled cows, 2) cows with SCK (BHBA > 1.2 mmol/L); and 3) cows with excessive NEB (NEFA > 0.7mmol/L). The covariates were: NEFA to BHBA ratio, parity, and ME305 calculated at 120 DIM. Statistical analyses were performed with SAS version 9.2. The R<sup>2</sup> were estimated with PROC REG, and risk ratios for the development

of any of the diseases were evaluated with Poisson regression.

## Results

Data from 100 herds with an average of 940 cows were used in the analysis. Results from 1,317 cows in the post-partum period were available; 238 had SCK and 409 had excessive NEB.

### All animals sampled:

Adjusted R<sup>2</sup>: 0.23. While controlling for parity and milk production, cows were 1.6 times more likely to develop CK ( $P = 0.08$ ) as the NEFA:BHBA ratio increased.

### Animals with SCK:

Adjusted R<sup>2</sup>: 0.06. Cows were five times more likely to develop CK ( $P = 0.004$ ) as the NEFA:BHBA ratio increased; however, cows were 0.2 times less likely to develop metritis ( $P = 0.13$ ).

### Animals with excessive NEB:

Adjusted R<sup>2</sup>: 0.09. The ratio was not associated with the risk of disease in this group of cows.

## Significance

NEFA and BHBA concentrations were measured in the same cow at the same time; however, the variability in BHBA concentrations was not well explained by NEFA concentration. Additionally, there was a decrease in the R<sup>2</sup> in the subgroups (i.e., animals with SCK and ENEB) compared with that for the general population. These data demonstrate that NEFA and BHBA concentration are not correlated within cow on the same day, and the correlation is less in cows with elevated concentrations of NEFA or BHBA. In the general population, the lack of correlation may be related to a time lag between increased concentrations of NEFA and then BHBA, but there may be other mechanisms in place in the subgroups. Given that NEFA and BHBA concentrations were not correlated, but NEFA and BHBA concentrations are independently associated with negative downstream outcomes, the association between the ratio of these metabolites and disease was evaluated.

The results indicate that larger ratios (i.e., increased NEFA concentration or decreased BHBA concentration) were associated with increased risk of metabolic diseases; conversely, higher ratios were associated with a decreased risk of infectious metritis.

The low  $R^2$  and minimal association between the ratio and disease outcomes demonstrates that further

evaluation of the relationship between NEFA and BHBA concentrations in the transition period is necessary. This information may be useful in managing cows with excessive NEB and may contribute to the further understanding of the potential causes of maladaptation to NEB (e.g., genetics or other metabolic intermediates like FGF-21).