Comparison between Individual and Pooled Samples of Non-esterified Fatty Acids (NEFA) and B-hydroxybutyrate (BHBA) in Transition Dairy Cows to Determine Herd Alarm Level Status

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Introduction

As energy demands surpass dry matter intake during the transition period, elevated non-esterified fatty acids (NEFA) and B-hydroxybutyrate (BHBA) concentrations can be used as markers of the extent of negative energy balance both at the individual cow and herd level. Previous studies have demonstrated that there is a threshold above which elevated NEFA and BHBA concentrations are associated with increased risk of disease and decreased reproductive and milk production performance in individual cows. Recently, the herd alarm level, i.e., the proportion of sampled animals with metabolite concentrations above the threshold which was associated with detrimental herd-level outcomes, was determined. The objective of this study was to compare the interpretation of the proportion of individual samples above the metabolite threshold versus pooled samples to evaluate the herd alarm level status.

Materials and Methods

Herds from a prospective cohort study which evaluated NEFA and BHBA as predictors of clinical disease were randomly selected to participate in this study. For inclusion, each herd required a minimum of 12 samples per cohort. Within each herd, two cohorts of cows were simultaneously evaluated, those 14 to three days prepartum and those three to 14 days postpartum. The metabolite NEFA was measured in cows sampled prepartum and both NEFA and BHBA were measured from cows sampled postpartum. For each herd, the metabolites were measured in 12 individual cow serum samples and in a pooled sample, derived from equal volumes (200 µL) of each serum sample. Based on our previous studies, herds were defined as being above the herd alarm level if the proportion of animals with metabolite concentration above the threshold was more than 15% ($\geq$ 2 animals from 12). The metabolite thresholds were 0.3 mEq/L for prepartum NEFA, 0.6 mEq/L for postpartum NEFA, and 12 mg/dL for BHBA. To assess whether a herd was above the herd alarm level, and therefore at increased risk for detrimental downstream outcomes, the proportion of sampled animals above the threshold and the pooled concentration were compared. Two methods were used for this comparison: 1) the proportion of animals above the threshold was used as the reference test, and the sensitivity and specificity of the pooled concentration was evaluated; 2) the Kappa statistic was evaluated to test the agreement beyond chance between the proportion and the pooled concentration. All analyses were performed using SAS v. 9.1 (SAS Inst., Inc., Cary, NC).

Results

Sensitivity of the pooled metabolite concentration as a single test was 58% (95% Confidence Intervals: 28 to 85) for prepartum NEFA; 55% (95% CI 24 to 83) for postpartum NEFA; and 36% (95% CI 11 to 69) for BHBA. Specificity of the pooled sample was 100% (95% CI: 17 to 100, 19 to 100, and 19 to 100) for prepartum NEFA, postpartum NEFA and BHBA, respectively. The Kappa statistics were: 0.2 (95% CI: -0.1 to 0.5), 0.3 (95% CI: -0.07 to 0.6), and 0.2 (95% CI: -0.07 to 0.4) for prepartum NEFA, postpartum NEFA, and BHBA, respectively. The McNemar's *P*-values for the Kappa statistics were 0.02, 0.02, and 0.008, respectively.

Significance

These data demonstrate that pooled samples have low sensitivity and there is only poor to fair agreement between this and the proportion of animals above the threshold test. Although pooled samples may seem desirable due to lower laboratory cost, the low sensitivity and lack of agreement between pooled samples and the proportion can lead to an increased number of false negatives. An increased number of false negatives may result in missed opportunities for herds looking to improve transition cow negative energy balance.