# Resistance or Susceptibility to Production-Related Metabolic Diseases (PRMDs) Can be Predicted with Fecal and Erythrocyte Isotopic Signatures

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## Introduction

The incidence of dairy cow PRMDs (milk fever, fatty liver, ketosis, left-displaced abomasum (LDA), mastitis, +/- infections) is highest the first 60 days-in-milk (DIM), can alter milk composition, and decrease production, conception, life expectancy, and cull value. Although when most PRMDs occur is known, disease incidence has not been altered by transition diets, dietary cation-anion difference (CAD), and over-conditioning avoidance. Increased free fatty acids (FFAs), non-esterified fatty acids (NEFAs), triglycerides (TG), and beta-hydroxybutyric acid (BHBA) serum concentrations and hepatic TG:glycogen correlates with increased PRMD risk. Regulation of hepatic metabolism is dynamic, differing between similarly managed transition cows. Substrate (hair, plasma proteins (PP), RBCs) and metabolic by-product (feces) stable isotopes can imply assimilated diet and metabolism over different periods: days (feces), one to two weeks (PP), weeks to months (hair), and several months (RBCs). Previously, we determined that feces and hair carbon (13C/12C ratio, δ13C) and nitrogen (15N/14N ratio, δ15N) stable isotopes measured 21 days prepartum (P1) and at parturition (P2) predicted 70% of cows at risk for PRMDs during that lactation. Primarily of hepatic origin, PP can approximate liver metabolism. Endogenous lipid and protein stores mobilized for energy should deplete δ13C and enrich δ15N substrate values. The objectives of this study were to determine if δ13C and δ15N RBC or PP signatures improve PRMD risk detection, and correctly classify cows that remain healthy the first five months of lactation.

## Materials and Methods

Randomly chosen, age, lactation, and parity-matched primiparous and multiparous Holstein cows (Brigham Creek Dairy, Elberta, UT) were studied. Feces, hair, plasma, and RBCs were sampled at P1, P2, and 21 days postpartum (P3). Weekly milk production (MP) was recorded. Feces, plasma, and RBC samples were frozen, freeze-dried, ground, and homogenized. Hair samples were cleaned via sonication. Samples of feces, plasma, RBCs, and mm lengths of hair (range of 0.3 to 0.6 mg) were combusted in tin capsules, and analyzed for %C, %N, δ13C, δ15N, and C:N ratios, in duplicate, with an elemental analyzer coupled to a DeltaV isotope ratio mass spectrometer to isolate C and N by atomic weight for stable isotope analysis (SIA). Ratios in parts per million (‰) relative to C and N standards, Pee Dee Belemnite and atmospheric nitrogen, with samples corrected using external standards for C (UCLA Carrera & LSVEC), and N (USGS 25 & 26). Health score (HSC) ranking: 1 (healthy, adequate MP), 2 (illness, recovery, adequate MP), 3 (culled poor MP +/- PRMD), 4 (died), and 5 (culled/died +/- PRMD 1-5 months postpartum). Data were analyzed using SAS. Discriminant analysis, grouping variable HSC, was performed for each pre- and postpartum feces, hair, plasma, and RBC measure. PROC GLM, independent variable HSC, determined if feces, hair, plasma, and RBC measures were different for cows with HSC 3, 4, or 5. Significance level $P<0.05$ for all tests.

## Results

Isotopic differences existed in substrates sampled at P1 and P2 from clinically normal cows consuming the same diet, that remained healthy or later developed PRMDs. Feces, hair, plasma, and RBC isotopic measures were different for 74% of cows that developed PRMD with HSC 3, 4, or 5. The linear discriminant function test, using forward selection to maximize the correct classification for health or risk of PRMD, correctly predicted 3/4 of the outcomes. In order, the best predictive variables for HSC to determine resistance or susceptibility to PRMDs during and after the transition period were P2 feces δ15N, P1 feces δ13C, and P1 RBC δ13C. Pre- and parturient C and N stable isotopes in feces and RBCs correctly identified 75% of cows resistant to PRMDs, but incorrectly classified 25% of HSC 1 cows as HSC 3 (6%), 4 (20%), and 5 (20%), respectively.

## Significance

Feces and RBC isotopic signatures in close-up and parturient cows correctly classified ~75% as healthy or at PRMD risk. Measuring C and N SIA as a management tool during the peripartum period may provide an economical method to identify cow resistance or susceptibility to PRMD, for retention, breeding, early treatment intervention, or culling decisions to increase profit margins.