# Fecal lipidomic biomarkers in production-related metabolic disease (PRMD)-resistant and susceptible dairy cows

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## Introduction

Although it is known that the highest incidence for most production-related metabolic diseases (PRMDs; ie, milk fever, fatty liver, ketosis, LDA, mastitis, and infections) occurs within 60 days-in-milk (DIM), PRMD incidence has not been altered by transition diets, dietary CAD, and avoidance of over-conditioning. Economic returns are significantly affected by PRMDs because of altered milk composition or decreased production, conception, life expectancy, and cull value. The risk for PRMD has been correlated with increased serum FFA, NEFA, TG, and βHBA concentrations and hepatic TG-to-glycogen ratio. Regulation of hepatic metabolism is dynamic and can differ between similarly managed transition cows. The difference in fecal carbon stable isotopes (13C/12C ratio, δ13C) measured 3 weeks prepartum and at parturition predicted 66% of cows resistant to or at risk for PRMDs in the subsequent lactation, with susceptible cows having a more depleted isotopic signature correlated with mobilizing endogenous lipid and protein stores for maintenance needs. Finding these differences prior to PRMD onset prompted investigation of fecal lipids present at the beginning of the transition period and at parturition. The objectives of this study were to determine the fecal lipid profiles for PRMD-resistant and PMRD-susceptible cows, and to determine if PRMD-susceptible cows have unique fecal lipidome biomarkers.

## Materials and Methods

Randomly chosen age-, lactation-, and parity-matched (primiparous [n=124], multiparous, [n=125]) Holstein cows at Brigham Creek Dairy (Elberta, UT) were evaluated. From each cow, fecal samples were collected 21 days prepartum (P1) and at parturition (P2), and health records maintained. Fecal samples were frozen, freeze-dried, ground, and homogenized before being analyzed for %C, %N, δ13C, δ15N, and C:N ratios 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 mil (‰) relative to C and N standards, Pee Dee Belemnite and atmospheric nitrogen, and external standards for C (UCLA Carrera & LSVEC), and N (USGS 25 & 26) were used to ensure adequate precision, with data normalized against standards for accuracy using linear regression and results checked against duplicate samples. Health score ranking (0, healthy; or 1, treated, culled, or died because of PRMD within 60 DIM [HSC]). Data were analyzed with SAS. Discriminant analysis, grouping variable HSC, was performed for each pre- and postpartum fecal variable. PROC GLM, independent variable HSC, was used to determine whether fecal variables were different for cows with PRMD. Electrospray ionization and LTQ Orbitrap mass spectrometry with lipidomic analyses were performed with fecal samples from transition-period matched PRMD-resistant and susceptible cows to detect positive-and negative-charged lipids. Lipids were identified according to structure using LIPID MAPS. Lipids significant to the health score were determined via a step-wise statistical analysis, with the health score being the independent variable and the presence or absence of different lipids being the dependent variables. For all analyses, values of $P < 0.05$ were considered significant.

## Results

Isotopic differences existed in the difference between P1 and P2 feces δ13C sampled from clinically normal cows consuming the same diet that remained healthy or later developed PRMDs. Preliminary lipidomic analysis of 40 fecal samples from cows sampled during P1 and P2 revealed the following 6 significant lipids that can be used as biomarkers to predict PRMD risk with the effect on health score (eHSC): Glycerolipid: DG(18:1(9Z)/20:5(5Z,8Z,11Z,14Z,17Z)/0:0), eHSC=2.08519; Glycerophospholipid: PA(15:0/18:4(6Z,9Z,12Z,15Z)), eHSC =-1.90952; Sphingolipid: Galα1-3Galβ1-4 (Fucα1-3)GlcNAcβ1-3Galβ1-4Glcβ-Cer(d18:1/16:0), eHSC=-1.86382; Prenol Lipid: Siphonaxanthin ester, eHSC=-1.89894; Glycerophospholipid: PC(P-18:0/20:5(5Z,8Z,11Z,14Z,17Z)), eHSC=1.84795; and Polyketide: 2,2,2-Trihydroxy-5,3,5-tribenzylisodiuvaretin, eHSC=1.96826. The presence or absence of each lipid had an estimated effect on the health score. A positive number raised the health score, predicting increased risk for metabolic disease (i.e., the

cow was at increased risk for PRMD), whereas a negative number lowered the risk for metabolic disease (i.e., the cow was likely to remain healthy).

## Significance

Fecal lipidomics in periparturient cows may provide biomarkers indicative of resistance or susceptibility for PRMD. Testing for the presence of specific fecal lipids could be a management tool used during the peripartum period that may provide an economical method to identify cows resistant or susceptible to PRMDs, for retention, breeding, early treatment intervention, or culling decisions to increase profit margins.