## Characterizing ruminal acidosis risk: A multi-herd, multi-country study

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A multi-center observational study was conducted in early lactation Holstein cows (n = 261; 1<sup>st</sup> to 7<sup>th</sup> parity) from 32 herds in 3 regions (Australia; AU, California; CA, and Canada; CAN) to characterize their acidosis risk status. Diets ranged from pasture supplemented with concentrates to TMR (NFC = 17 to 47 and NDF = 27 to 58 % of DM). Rumen fluid samples were collected <3 h after feeding and analyzed for pH, and ammonia, D- and L-lactate, and VFA concentrations, and bacterial 16S ribosomal DNA sequences. Individual cow milk volume, fat, protein, and SCC were used from the closest herd test to the rumen sampling. Eigenvectors were produced using cluster and discriminant analysis from a combination of all rumen measures, except bacteria, and were used to categorize cows into 3 previously validated acidosis risk groups and calculate the probability of the risk of ruminal acidosis based on proximity to the centroid of the 3 clusters. Rumen bacteria phyla, markers of rumen fermentation, production characteristics, and the probability of acidosis were analyzed by mixed models that included fixed effects of acidosis group, region, and parity, and the random effects of herd nested within region. A total of 26.1% of the cows were classified as acidotic, 47.1% in group 2, and 26.8% in group 3. Acidosis risk differed between regions with AU (37.2%) and CA (39.2%) having similar prevalence of acidotic cows and CAN at 5.2%. The acidotic group had rumen phyla, fermentation, and production characteristics consistent with a model of acidosis that reflected a rapid rate of carbohydrate fermentation. Group 2 possibly represents cattle with a slower rumen fermentation of carbohydrates. Group 3 contains cows that had rumen fermentation profiles that suggest they may be inappetent or had not eaten recently or were in recovery from acidosis. The acidotic group had lower bacterial diversity than the other groups, while CAN had a greater diversity than AU and CA. Rumen fermentation profile, abundance of bacteria phyla, and production characteristics of lactating dairy cattle categorized in 3 acidosis risk states were successfully defined using a cluster and discriminant analysis model.

## Rumen bacterial taxa and dietary nutrient predictors in cows with differing risk of ruminal acidosis

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Early lactation Holstein cows (n = 261; 1<sup>st</sup> to 7<sup>th</sup> parity) from 32 herds in 3 regions (Australia, California, and Canada) were categorized into 3 rumen acidosis risk groups (acidotic = 26.1%). group 2 = 47.1%, or group 3 = 26.8%) using a discriminant analysis model based on eigenvectors produced from a combination of rumen pH, and ammonia, D- and L-lactate, and VFA concentrations from samples collected <3 h after feeding. Diets ranged from pasture supplemented with concentrates to TMR (NFC = 17 to 47 and NDF = 27 to 58 % of DM). Nutrients were determined by feed analysis or estimated in nutrition software (10 herds). Rumen microbiota were characterized by bacterial 16S ribosomal DNA sequence data. The relative abundance of each bacterial phylum and family were center log transformed and subjected to principal components analysis (PCA) for both acidosis group and region. The 20 most influential families from both combined PCA (n = 29) were termed 'influential'. Backward stepwise elimination mixed models were used to determine (1) the bacterial taxa that were collectively associated with each acidosis group and (2) dietary nutrients that were associated with these taxa and the phyla that were most abundant. The fixed effects of herd nested within region were used for (1) and region for (2). Nutrients included (% of DM): NDF, CP, starch, sugar, and crude fat. Five phyla: Firmicutes (OR =  $7.47 \pm 7.43$ ), Spirochaetes (OR  $= 1.28 \pm 0.14$ ), Lentisphaerae (OR = 0.70 \pm 0.07), Planctomycetes (OR = 0.70 \pm 0.09), and Tenericutes (OR =  $0.44 \pm 0.15$ ) and 9 families were associated with acidosis. Of the 5 most abundant phyla and those that were associated with acidosis, (n = 9 phyla of interest), all had  $\geq 1$  nutrient that predicted abundance. Sugar was the most frequently predictive nutrient (in 78% of models), followed by CP (56%), and crude fat (44%). Sugar and CP were most predictive for the influential families and all but 3 families had  $\geq 1$  predictive nutrient. Bacterial taxa can be used to predict acidosis and dietary sugar and CP are predictors of these, and thus of acidosis risk.