RUMEN HEALTH CONSEQUENCES OF HIGH-CONCENTRATE FEEDING IN CATTLE: MORE THAN A SIMPLE DROP IN RUMINAL pH

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The intensive feeding of high-yielding milking cows with grain-rich diets causes a drop in ruminal pH and can impact cow health and production with long term consequences, known as subacute ruminal acidosis (SARA). Results from new analytical methods, such as next generation sequencing and gene expression, have shown that feeding high-grain diets is not just a matter of acidosis but of a dysbiosis of the whole rumen ecosystem. We hypothesized that high-concentrate feeding impacts the microbial diversity differently in the rumen digesta compared to the epithelium, shifts fermentation end products, as well as host epithelial metabolism-, and barrier function gene expression.

Eight rumen cannulated Holstein cows were fed a 100 % roughage diet (RD, 1 week), followed by an intermittent 65 %-high-concentrate-diet (HC1 and HC2; 1 and 2 weeks, respectively, interrupted by 1 week roughage only). The feeding protocol was conducted in 2 consecutive runs, with a 3 weeks washout period. Reticular pH was measured continuously; rumen sampling was performed in RD, HC1, and HC2. Ruminal pH, short-chain fatty acids (SCFA), lactate, and ammonia were analysed in the rumen digesta and fluid. Particle-associated rumen microbiota (PaM) and epithelial microbiota (EpM) were analysed using *Illumina MiSeq* sequencing of the 16S rRNA gene, and epithelial gene expression using RT-qPCR to target barrier function-, cellular transport, pH, and metabolism genes. Bioinformatic analysis was performed using *QIIME 1.0*, and statistical analysis using *PROC MIXED of SAS 9.4*.

The drop of reticular pH was more severe in HC1 with 370 min spent <pH 6.0 vs. 164 min <pH 6.0 in HC2. Microbial diversity in PaM decreased the most in HC1 ($P<0.01$), whereas EpM increased in diversity in HC1 and HC2 ($P<0.05$). Distance matrix analysis revealed that PaM in RD clustered more tightly and away from HC samples compared to the EpM. There was a significant decrease of the highest abundant phylum *Firmicutes*, and an increase of *Bacteroidetes*, and *Actinobacteria* in PaM ($P<0.05$), and a decrease of the highest abundant phylum *Proteobacteria* and an increase in *Bacteroidetes* in EpM with HC ($P<0.05$). SCFA shifted with decreasing acetate and increasing propionate in both rumen digesta and fluid ($P<0.01$), lactate increased ($P=0.07$), and ammonia decreased ($P=0.02$) with HC feeding in comparison to RD. Nutrient transport genes such as MCT1 and MCT4, cellular metabolism target BDH1, and barrier function gene CLDN4 were all downregulated in HC1, whereas the barrier function gene DSG1was upregulated in HC1 ($P<0.05$), and cellular pH regulation gene DRA was upregulated in HC2 ($P<0.05$).

In our study HC1 had greater impact on reticular pH and PaM, suggesting that the microbiota that are located in the rumen digesta are more susceptible to all types of nutritive changes. Most targeted genes showed either a significant or numeric decrease in HC1, with recovered levels in HC2, speaking for an adaptation in HC2. EpM also stabilized in HC2, but was generally less impacted by high-concentrate feeding. In summary, our findings show an impact of the high-concentrate feeding not only on pH but both PaM and EpM communities, their metabolic products, and host gene expression.

Keywords: HIGH-GRAIN FEEDING, RUMEN MICROBIOTA, EPITHELIAL-GENE EXPRESSION, RUMEN DYSBIOSIS