

Rumen Bacterial Populations of Dairy Cows Fed Molasses or Corn with Varying Rumen Degradable Protein



Elif Gunal¹, Mary Beth Hall², Geoff Zanton², Paul Weimer², Garret Suen³, and Kent Weigel¹

¹ Department of Dairy Science, University of Wisconsin-Madison, Madison, WI, USA

² USDA-ARS Dairy Forage Research Center, Madison, WI, USA

³ Department of Bacteriology, University of Wisconsin-Madison, Madison, WI.

Introduction

- The rumen microbiota enables many important metabolic functions in the host dairy cow or other ruminant animal
- The diverse community of microorganisms ferments plant-derived substrates into short-chain fatty acids, which are absorbed by the host to fuel growth, maintenance, and milk production
- Transfaunation studies, in which rumen contents are exchanged between animals, indicate host specificity in composition of the rumen bacterial community (Weimer et al., 2010; Zhou et al., 2018)
- Molasses is a readily available source of sugar, but its effects on rumen fermentation have not been well characterized
- Rumen degradable protein (RDP) affects the way microbes process sugars (Hall et al., 2013), and this may affect the microbial community
- Increasing RDP relative to available carbohydrate can provide a greater yield of microbes per unit of carbohydrate (Hall, 2017)

→ The objective of this study was to evaluate changes induced in the rumen microbial communities of lactating Holstein dairy cows by altering the fractions of dietary carbohydrates and proteins

Materials & Methods

- 12 ruminally cannulated multiparous Holstein cows (parity 2.25 ± 0.62 ; 185 ± 56 days postpartum; 41.3 ± 6.3 kg milk/d) at the USDA-ARS Dairy Forage Research Center experimental farm (Prairie du Sac, WI) were used in this study
- The experimental design was a split-plot 3x3 Latin square, as shown in Table 1, with a whole plot of RDP level (LoRDP or HiRDP) and with molasses substituted for corn grain at three levels (0.0, 5.25, or 10.5% of dry matter), fed sequentially in periods of 28-day duration
- Samples of the liquid and solid fractions of the rumen contents were taken 6 hours after the morning feeding on days 26, 27, and 28 of each period and pooled within cow and fraction (solid or liquid) and period
- 16s ribosomal RNA sequencing was used to identify operating taxonomic units (OTUs), which were summed at genus level
- Sample preparation followed Stevenson and Weimer (2007)
- Richness and diversity metrics were calculated using Mothur v.1.38.1
- The 30 genera with greatest relative abundance were analysed with a mixed linear model with random cow effect and fixed fraction, molasses level, and RDP level effects

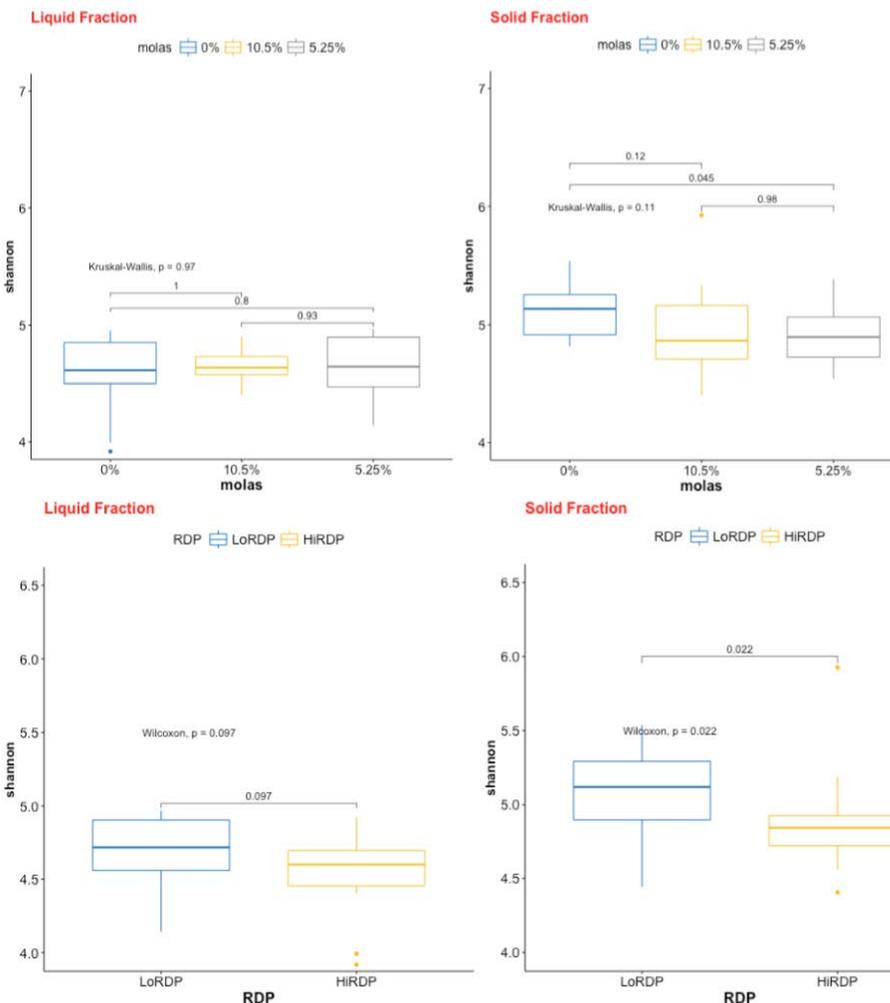
Table 1. Experimental design of the study, where values indicate the proportion of molasses in the diet (%).

Cow	High Rumen Degradable Protein			Low Rumen Degradable Protein			
	Period 1	Period 2	Period 3	Cow	Period 1	Period 2	Period 3
4628	10.5	5.25	0	5046	0	10.5	5.25
5047	5.25	0	10.5	5054	5.25	0	10.5
5017	0	10.5	5.25	5000	10.5	5.25	0
5019	5.25	10.5	0	4668	0	5.25	10.5
4251	10.5	0	5.25	5067	10.5	0	5.25
5048	0	5.25	10.5	5020	5.25	10.5	0

Results

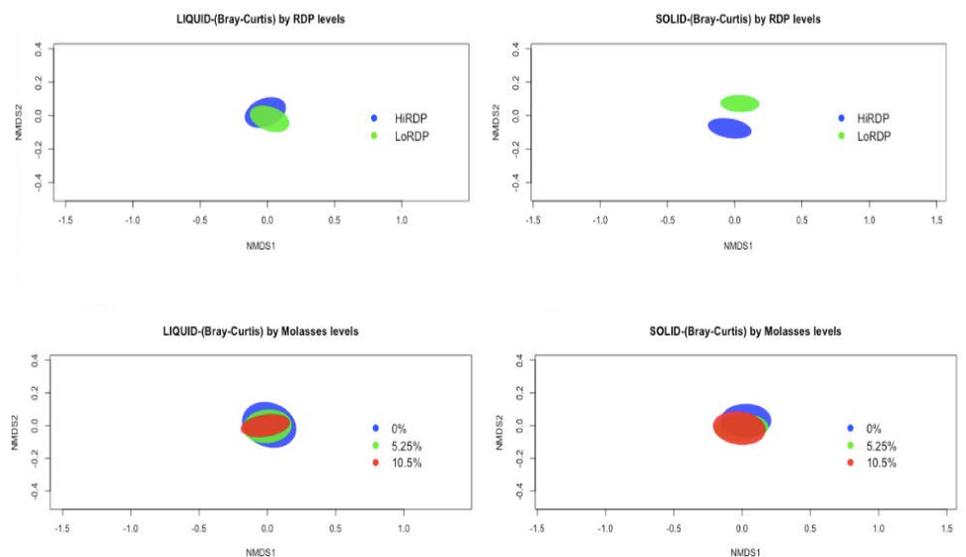
- Alpha diversity was not affected by molasses level in the liquid fraction, but was greater ($P < 0.05$) at 0% molasses in the solid fraction
- Alpha diversity tended to be greater ($P < 0.10$) with LoRDP in the liquid fraction and was significantly greater ($P < 0.05$) with LoRDP in the solid fraction

Figure 1. Alpha diversity of liquid and solid fractions of the rumen contents, by RDP and molasses level.



- Relative abundance of genera represented in the rumen bacterial community did not differ by level of molasses in either the liquid or solid fractions
- Relative abundance of genera represented in the rumen bacterial community differed significantly ($P < 0.05$) between LoRDP and HiRDP, especially in the solid fraction

Figure 2. Beta diversity of liquid and solid fractions of the rumen contents, by RDP and molasses level.



- Relative abundance of specific genera tended to differ ($P < 0.10$) in liquid and solid fractions with changes in molasses and RDP levels

Figure 3. Changes in relative abundance of top 30 genera in liquid solid fractions of the rumen contents, by RDP and molasses level (red = decreased abundance; green = increased abundance).

Liquid - molasses affected genera	Liquid - RDP affected genera	RDP - molasses interaction	Solid - molasses affected genera	Solid - RDP affected genera
<ul style="list-style-type: none"> <i>Succiniclasticum</i> <i>Ruminococcus</i> <i>Anaerovibrio</i> <i>Schwartzia</i> <i>Shuttleworthia</i> <i>Anaeroplasm</i> 	<ul style="list-style-type: none"> <i>Prevotella</i> <i>Coprococcus</i> <i>Succinivibrio</i> 	<ul style="list-style-type: none"> <i>Butyrivibrio</i> <i>Anaerovibrio</i> <i>Selenomonas</i> <i>Anaerovibrio</i> 	<ul style="list-style-type: none"> <i>CF231</i> <i>YRC22</i> <i>Clostridium</i> <i>Desulfovibrio</i> <i>BF311</i> <i>Oscillospira</i> <i>Schwartzia</i> 	<ul style="list-style-type: none"> <i>Succiniclasticum</i> <i>YRC22</i> <i>Pseudobutyrvibrio</i> <i>Succinivibrio</i>

Conclusions

- Replacing sucrose-rich molasses with starch-rich corn grain at different levels of RDP caused significant alterations in the ruminal bacterial community composition in the liquid and solid fractions on all taxonomic levels, presumably by changing the substrate source and availability
- Ruminal bacterial community composition in the liquid and solid fractions was not affected by supplementation of molasses
- Dietary RDP level changed ruminal BCC significantly, most notably in the solid fraction
- Greater stratification of *Fibrobacter* in the solid phase was not observed, which was contrary to expectations
- Prevotella* was the predominant genus, but it was more abundant in the liquid fraction than the solid fraction, which was contrary to expectations
- Some of the most active proteolytic genera, including *Ruminobacter* and *Butyrivibrio*, were not affected by dietary treatments
- Some new genera showed a more significant presence than expected, such as *Succiniclasticum*, which was second in abundance in both the liquid and solid fractions, with unclear characteristics of protein degradability (particularly in solid digesta)
- Negative dietary effects of more degradable protein were seen on many carbohydrate utilizers, such as *Clostridium*, *Succinivibrio*, *Pseudobutyrvibrio*, and *Coprococcus*
- Findings of the present study are of high interest for ruminant microbiologists, particularly those who are exploring new rumen functions and interactions with health parameters, as well as options for breeding or inoculating the microbiome of the animal

References

- Hall, M. B. 2013. Dietary starch source and protein degradability in diets containing sucrose: Effects on ruminal measures and proposed mechanism for degradable protein effects. *Journal of Dairy Science* 96:7093-7109
- Hall, M. B. 2017. Nitrogen source and concentration affect utilization of glucose by mixed ruminal microbes in vitro. *Journal of Dairy Science* 100:2739-2750
- Stevenson, D. M., and P. J. Weimer. 2007. Dominance of *Prevotella* and low abundance of classical ruminal bacterial species in the bovine rumen revealed by relative quantification real-time PCR. *Applied Microbiology and Biotechnology* 75:165-174
- Weimer, P. J., D. M. Stevenson, H. C. Mantovani, and S. L. Man. 2010. Host specificity of the rumen bacterial community in the dairy cow following near-total exchange of ruminal contents. *Journal of Dairy Science* 93:5902-5912
- Zhou, M., Y.-J. Peng, Y. Chen, C. M. Klinger, M. Oba, J.-X. Liu, and L. L. Guan. 2018. Assessment of microbiome changes after rumen transfaunation: implications on improving feed efficiency in beef cattle. *Microbiome* 6:62-75

Acknowledgments

- The authors acknowledge Westway Feed Products (Tomball, TX) for funding the original nutrition experiment
- The authors also acknowledge Andrew Steinberger for assistance and troubleshooting in the 16S rRNA sequencing and subsequent statistical analyses