J. Dairy Sci. 101:1–15
https://doi.org/10.3168/jds.2017-13565

High-grain diets supplemented with phytogenic compounds or autolyzed yeast modulate ruminal bacterial community and fermentation in dry cows

V. Neubauer,*† R. Petri,* E. Humer,* I. Kröger,* E. Mann,† N. Reisinger,‡ M. Wagner,† and Q. Zebeli*[1]
*Institute of Animal Nutrition and Functional Plant Compounds, and
†Institute for Milk Hygiene, Milk Technology and Food Science, Department for Farm Animals and Veterinary Public Health,
University of Veterinary Medicine, Veterinaerplatz 1, 1210 Vienna, Austria
‡BIOMIN Research Center, BIOMIN Holding GmbH, 3430 Tulln, Austria

ABSTRACT

The feeding of concentrate-rich diets may lead to microbial imbalances and dysfermentation in the rumen. The main objective of this study was to determine the effects of supplementing phytogenic compounds (PHY) or autolyzed yeast (AY) on rumen fermentation and microbial abundance in cows intermittently fed concentrate-rich diets. The experiment was carried out as an incomplete 3 × 4 Latin square design, with 8 nonlactating rumen-fistulated Holstein-Friesian cows. The cows were randomly assigned to a concentrate diet that was either not supplemented (CON), or supplemented with PHY or AY. Each of the 4 consecutive experimental periods was composed of a 1-wk roughage-only diet (RD), 6-d gradual concentrate increase, followed by 1 wk of 65% concentrate (dry matter basis; Conc I), and 1 wk of RD and a final 2-wk 65% concentrate (dry matter basis; Conc II) phase. Digesta samples were collected from the rumen mat for bacterial 16S rRNA gene Illumina MiSeq (Illumina, Balgach, Switzerland) sequencing, and samples of particle-associated rumen liquid were obtained for measuring short-chain fatty acids, lactate, ammonia, and pH during RD (d 6), Conc I (d 19), and Conc II (d 39). The concentrate feeding caused a decrease of overall bacterial diversity indices, especially during Conc I. The genera *Ruminococcus*, *Butyrivibrio*, and *Coprococcus* were decreased, whereas *Prevotella*, *Megasphaera*, *Lachnospira*, and *Bacteroides* were increased in abundance. Supplementation of both feed additives increased the abundance of gram-positive and decreased that of gram-negative bacteria. Supplementation of AY enhanced cellulolytic bacteria such as *Ruminococcus* spp., whereas PHY decreased starch and sugar fermenters including *Bacteroides* spp., *Shuttleworthia* spp., and *Syntrophococcus* spp. Moreover, PHY supplementation increased butyrate percentage in the rumen in both concentrate phases. In conclusion, intermittent high-concentrate feeding altered the digesta-associated rumen bacterial community and rumen fermentation with more significant alterations found in Conc I than in Conc II. The data also showed that both feed additives had the most significant modulatory effects on the bacterial community, and their subsequent fermentation, during periods of low pH.

Key words: feed additive, subacute rumen acidosis, cow-rumen bacteria, short-chain fatty acid

INTRODUCTION

The highly complex microbial community of the rumen enables multiple metabolic functions in the host. This community is evolutionarily adapted to a fiber-rich herbivore diet. Yet, the feeding of large amounts of concentrates, during periods of high energy needs, may lead to rumen bacterial imbalances (McCann et al., 2016). These imbalances may further result in a decline of fiber degradation (Pourazad et al., 2016), shifts in short-chain fatty acid (**SCFA**) profile (Kleen et al., 2003), and accumulation of endotoxins (Plaizier et al., 2012) in the rumen, all of which can further impair cow health and productivity.

With the help of next-generation sequencing techniques, it has been possible to gain a deeper understanding of rumen bacterial shifts in cattle in response to concentrates in the diet. However, digesta-associated microbial responses to concentrate-rich diets were typically investigated in challenge studies with short and continuous feeding periods (Hook et al., 2011; Mao et al., 2013b; Petri et al., 2013), and less commonly evaluated using intermittent feeding studies. The application of an intermittent high-concentrate feeding model provides the opportunity to look at changes in the rumen microbiome and fermentation that may more closely resemble variable DMI patterns and dietary energy intake, and subsequent drops in ruminal pH, such as during transition and peak lactation. In a recent study, we observed that cows experiencing 2 intermittent

---

Received July 25, 2017.
Accepted November 20, 2017.
[1] Corresponding author: qendrim.zebeli@vetmeduni.ac.at

concentrate-rich challenges had a more severe drop in ruminal pH and rumination behavior during the first than during the second challenge (Kröger et al., 2017), despite feeding the same diets. These findings suggest a differing response of the ruminal microbiome during the 2 concentrate phases. More interestingly, the same experiment revealed enhancement effects on rumination behavior and ruminal pH in cows supplemented with phytogenic compounds (**PHY**) or autolyzed yeast (**AY**), with effects evolving especially during the first challenge phase. In vitro studies have reported PHY to have a direct bacteriostatic effect and therefore alter fermentation end products (Cardozo et al., 2005; Calsamiglia et al., 2007), whereas AY has an indirect effect by either acting as ligand for gram-negative bacteria (**GNB**; Nocek et al., 2011; Ganner and Schatzmayr, 2012) or as a substrate for cellulolytic bacteria (Harrison et al., 1988; Mao et al., 2013a) and so increases SCFA load (Kesselring et al., 2017). However, if the effects of intermittent concentrate feeding are related to alterations of ruminal microbiome, and whether PHY and AY supplementation modulate rumen microbiome and fermentation in an in vivo model, have not yet been evaluated.

Therefore, one aim of this study was to determine the changes of the rumen bacterial community and fermentation products, such as SCFA, ammonia, and lactate, when feeding an intermittent concentrate-rich diet to cattle. Another aim was to determine the effect of supplementation of PHY or AY feed additives on rumen bacteria shifts and the fermentation products during that intermittent high-concentrate feeding. Our hypothesis was that the increase of concentrate in the diet will decrease bacterial diversity and mediate rumen bacteria community composition and the end products of its metabolism in the rumen. Further, we hypothesized that PHY and AY will modulate the bacterial community structure in the rumen, likely resulting in a reduction in starch-fermenting bacteria and an increase in cellulolytic bacteria, leading to favorable changes in the fermentation products, such as SCFA profile. Effects are expected to be more pronounced during the first feeding phase because the time cows spent under pH 6.0 was longer during this phase (Kröger et al., 2017).

MATERIALS AND METHODS

Animals, Diets, and Experimental Setup

All procedures involving animal handling and treatment were approved by the institutional ethics committee of the University of Veterinary Medicine (Vetmeduni) Vienna and the national authority according to §26 of the Law for Animal Experiments, Tierversuchsgesetz 2012- TVG (GZ: BMWFW-68.205/0023-WF/V/3b/2015).

Detailed information about cows, feeding, and experimental setup is given in our companion paper (Kröger et al., 2017). In brief, the experiment used 8 nonlactating rumen-fistulated Holstein-Frisian cows (863 ± 65 kg of BW ± SD) in an incomplete 3 × 4 Latin square design, balanced for carry-over effects. Cows were randomly assigned to 1 of the 3 feeding groups: control (**CON**, no feed additive), PHY (Digestarom; phytogenic product, containing herbs, spices, essential oils, and nonvolatile extracts, with major active compounds derived from mint, thyme, rosemary, and clove; BIOMIN Holding GmbH, Getzersdorf, Austria; 3 g per cow per day), or AY (Levabon, Rumen E; containing yeast cell components from autolysis, as mannan oligosaccharides, glucans, chitin, nucleotides, peptides, and AA; BIOMIN Holding GmbH; 15 g per cow per day). Each of the 4 periods included 1 wk of baseline feeding (100% roughage diet, **RD**), 6 d of gradual concentrate adaptation, 1 wk of high-concentrate challenge (65% concentrates, on a DM basis, **Conc I**), 1 wk of recovery with roughage only, followed by a second 65% concentrate challenge lasting 2 wk (**Conc II**). This feeding model was used to reflect the dietary challenge milking cows undergo during the transition period, and by using dry, nonpregnant cows, excluding any lactation-cycle-dependent influences. Feed additives were provided in the concentrate mix except during the recovery phase, where the daily doses of PHY and AY were given via the rumen cannula. Each experimental period was followed by a 3-wk washout phase to avoid carry-over effects.

Exact protocols of feeding and feed sampling, as well as chemical composition of dietary ingredients, were described in the companion paper (Kröger et al., 2017). In brief, the RD consisted of grass silage and hay in 50:50 ratio (DM basis) and the concentrate mixture contained barley grain (33%), wheat (30%), rapeseed meal (16%), corn (15%), beet pulp (3.2%), a mineral-vitamin premix (1%; containing 13.5% Ca, 9% Mg, 5% P, 1.5% Na, 2,100,000 IU of vitamin A/kg, 300,000 IU of vitamin D/kg, and 7,500 mg of vitamin E/kg), beet molasses (1%), calcium carbonate (0.5%), and NaCl (0.3%) on a DM basis. Diets were offered separately via individual feeding troughs equipped with computer-controlled electronic scales. Access was regulated electronically with transponder access gates (Insentec B.V., Marknesse, the Netherlands) and daily feed intake was recorded for each individual cow.