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Graded substitution of grains with bakery by-products modulates ruminal fermentation, nutrient degradation, and microbial community composition in vitro

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ABSTRACT

A new segment of feed industry based on bakery by-products (BBP) has emerged. Yet, information is lacking regarding the effects of inclusion of BBP in ruminant diets on ruminal fermentation and microbiota. Therefore, the aim of this study was to evaluate the effect of the gradual replacement of grains by BBP on ruminal fermentation, nutrient degradation, and microbial community composition using the rumen-simulation technique. All diets consisted of hay and concentrate mixture with a ratio of 42:58 (dry matter basis), but differed in the concentrate composition with either 45% cereal grains or BBP, whereby 15, 30, or 45% of BBP were used in place of cereal grains. The inclusion of increasing levels of BBP in the diet linearly enhanced ruminal degradation of starch from 84% (control) to 96% (45% BBP), while decreasing degradation of crude protein and fiber. The formation of methane was lowered in the 45% BBP diet compared with all other diets. Whereas the ammonia concentration was similar in the control and 15% BBP, a significant decrease was found in 30% BBP (−23%) and 45% BBP (−33%). Also, BBP feeding shifted fermentation profile toward propionate at the expense of acetate. Moreover, isobutyrate linearly decreased with increasing BBP inclusion. Bacterial 16S rRNA Illumina MiSeq (Microsynth AG, Balach, Switzerland) sequencing revealed a decreased microbial diversity for the 45% BBP diet. Furthermore, the replacement of cereal grains with BBP went along with an increased abundance of the genera *Prevotella*, *Roseburia*, and *Megasphaera*, while decreasing *Butyrivibrio* and several OTU belonging to *Ruminococcaceae*. In conclusion, the inclusion of BBP at up to 30% of the dry matter had no detrimental effects on pH, fiber degradability, and microbial diversity, and enhanced propionate production. However, a higher replacement level (45%) impaired ruminal fermentation traits and fiber degradation and is not recommended.
Key words: bakery by-product, cereal grain, nutrient disappearance, ruminal fermentation, ruminal microbiota

INTRODUCTION

Due to the continuous increase in grain demands, concerns about future food security are rising, which in turn reinforces pressure on livestock systems (Godfray et al., 2010). Although ruminants are able to convert fibrous plant material into high-quality animal products, the high performance levels reached in modern intensive ruminant production systems make it necessary to feed high amounts of energy-rich concentrates (i.e., cereal grains) to ruminants. One strategy to cover the nutrient requirements of high-producing ruminants while reducing the food competition between cattle and humans is the inclusion of energy-rich by-products in the diets. Among the possible by-products fed to ruminants, leftovers from bakeries can serve as energetic food, due to their high concentration of NFC and fat (Arosemena et al., 1995). In many countries, bakery products are frequently delivered to the stores to ensure freshness and those not sold within a day are returned. Although these products are wholesome, their use for human consumption is largely prevented due to consumers' preferences and business policy. Thus, a large volume of bakery by-products (**BBP**) has become available, resulting in the emergence of a whole new segment of the feed industry (Wing, 1964; Franca et al., 2012).

As BBP is characterized by its high energy content, mainly due to the high concentration of NFC (Arosemena et al., 1995), it shows potential as a substitute for cereal grains in ruminant diets. Although BBP is often fed to farm livestock, including lactating dairy cows, little pertinent research is found in the current

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literature (Champe and Church, 1980). Especially research regarding the effects on microbial community structure is missing, and the only report available in dairy cows was conducted half a century ago (Wing, 1964). In general, starch-rich feed ingredients differ largely in the rate and extent of starch degradability (Humer and Zebeli, 2017). Increasing amounts of degradable starch cause a shift in the fermentation profile toward propionate at the expense of acetate, thereby supporting gluconeogenesis and energy supply to the host (Deckardt et al., 2013). However, excessive starch degradation decreases ruminal pH, which enhances the risk of ruminal fermentation disorders. A decrease in ruminal pH is associated with negative effects on fibrolytic bacteria, thereby lowering the ruminal fiber degradability (Humer et al., 2017). Thus, a possible increase in the amount of ruminally degradable NFC in the BBP might enhance ruminal starch fermenters at the expense of cellulolytic microbes. Furthermore, the high fat contents typically found in BBP might impair ruminal fibrolytic bacteria when high amounts are included in the diet (Enjalbert et al., 2017). However, the effects of feeding of BBP on the ruminal nutrient degradability, fermentation profile, and microbial community structure have not been investigated so far. With the help of next-generation sequencing techniques, it is possible to gain a deep understanding of rumen bacterial shifts in response to different diets.

Therefore, the aim of this study was to evaluate the effect of the successive replacement of cereal grains by BBP on ruminal fermentation characteristics, nutrient degradation, and microbial community composition using the rumen-simulation technique (**Rusitec**).

MATERIALS AND METHODS

Treatments and Experimental Diets

Four experimental diets were formulated, all containing 42% meadow hay and 58% concentrates (DM basis), with the concentrate mixture differing in its composition among diets (Table 2). The control diet (**CON**) contained 45% cereal grains in diet DM. For the second diet (**15% BBP**), one-third of the cereal grains were replaced with BBP, reaching a level of 15% in total diet DM. The third diet contained 30% BBP and 15% cereal grains (**30% BBP**), thereby substituting the cereal portion by two-thirds. Finally, a diet in which the native grains were completely replaced by BBP (**45% BBP**) was formulated. The cereal proportion in the first 3 diets consisted of 75% wheat and 25% rye, based on the typical composition of the original bakery products. Before use, hay was chopped to about 6 mm in length (Pulverisette 25/19, Fritsch GmBH, Idar-Obersteinn, Germany), whereas the concentrate ingredients were ground to pass a 2-mm sieve, using the same mill. The BBP used was a mixture of leftover materials collected from Viennese bakeries and supermarkets. The analyzed chemical composition of the individual ingredients (Table 1) was used to formulate the diets.

Experimental Design, Rusitec Procedure, and Sample Collection

The experiment was based on a completely randomized arrangement, whereby 4 diets were tested in 3 experimental runs with 3 replicates in each run, resulting in 9 independent measurements per treatment. Each run consisted of 12 fermentors and lasted for 10 d, wherein the final 5 d were used for samplings. For each experimental run, ruminal fluid and solid digesta were obtained from 3 nonlactating rumen-cannulated Holstein cows kept at the Dairy Research Station of the University of Veterinary Medicine Vienna (Pottenstein, Austria). Donor cows were fed with hay and grass silage and were kept according to Austrian guidelines for animal welfare (Federal Ministry of Health, 2004). Prior to inoculation, the ruminal fluid of the cows was mixed and filtered through 4 layers of medical gauze (~1-mm pore size), whereas the solid digesta was mixed and used unprocessed. Each fermentor was inoculated with 600 mL of rumen fluid and 100 mL of artificial saliva. Subsequently, a pair of nylon bags (120 × 65 mm, 150 μm pore size, Fa. Linker Industrie-Technik GmbH, Kassel, Germany) was added to each fermentor, one filled with 12 g of DM of the experimental diet and another bag filled with solid ruminal digesta. The Rusitec apparatus and procedure is explained in detail in a previous study (Khiaosa-Ard et al., 2015), except for a different infusion rate of artificial saliva of 331 ± 16.0 mL/d.

Daily effluent and fermentation gases of each fermentor were collected in effluent bottles kept in an ice tub and gas-tight bags (TecoBag 8 L, Tesseraux Spezialverpackungen GmbH, Bürstadt, Germany), respectively. On the sampling days, directly before feed bag exchange, fermentor fluid samples were collected using a syringe. Part of the fluid samples was immediately analyzed for daily measurement of fermentation characteristics (pH, redox potential), and additional subsamples were stored in separate tubes at −20°C for analysis of short-chain fatty acids (**SCFA**) and ammonia. For microbial analysis, additional fluid subsamples were snap frozen in liquid nitrogen and stored at −80°C until DNA extraction. The incubation feed (residue)