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Substitution of common concentrates with by-products modulated ruminal fermentation, nutrient degradation, and microbial community composition in vitro

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ABSTRACT

A rumen simulation technique was used to evaluate the effects of the complete substitution of a common concentrate mixture (CON) with a mixture consisting solely of by-products from the food industry (BP) at 2 different forage-to-concentrate ratios on ruminal fermentation profile, nutrient degradation, and abundance of rumen microbiota. The experiment was a 2 × 2 factorial arrangement with 2 concentrate types (CON and BP) and 2 concentrate levels (25 and 50% of diet dry matter). The experiment consisted of 2 experimental runs with 12 fermentation vessels each (n = 6 per treatment). Each run lasted for 10 d, with data collection on the last 5 d. The BP diets had lower starch, but higher neutral detergent fiber (NDF) and fat contents compared with CON. Degradation of crude protein was decreased, but NDF and nonfiber carbohydrate degradation were higher for the BP diets. At the 50% concentrate level, organic matter degradation tended to be lower for BP and CH_4 formation per unit of NDF degraded was also lower for BP. The BP mixture led to a higher concentration of propionate and a lower acetate-to-propionate ratio, whereas concentrations of butyrate and caproate decreased. Concentrate type did not affect microbial community composition, except that the abundance of bacteria of the genus *Prevotella* was higher for BP. Increasing the concentrate level resulted in higher degradation of organic matter and crude protein. At the higher concentrate level, total short-chain fatty acid formation increased and concentrations of isobutyrate and valerate decreased. In addition, at the 50% concentrate level, numbers of protozoa increased, whereas numbers of methanogens, anaerobic fungi, and fibrolytic bacteria decreased. No interaction was noted between the 2 dietary factors on most variables, except that at the higher concentrate level the effects of BP on CH_4 and CO_2 formation per unit of NDF degraded, crude protein degradation, and the abundance of *Prevotella* were more prominent. In conclusion, the results of this study suggest that BP in the diet can adequately substitute CON with regard to ruminal fermentation profile and microbiota, showing even favorable fermentation patterns when fed at 50% inclusion rate.

Key words: Rusitec, by-product, concentrate, microbiota

INTRODUCTION

By-products from the food processing industry, such as various brans, middlings, oilseed meals, or beet pulp, have traditionally played an important role in the feeding of dairy cows as a substitute for grains and oil seeds in the diet. This substitution can lead to a highly improved edible feed conversion ratio in dairy cows (Ertl et al., 2015). Due to economic advantages, greater availability, lower human food versus animal feed competition, as well as low suitability for monogastric animals, the role of by-products in dairy cattle nutrition will further increase in the future. According to Bradford (1999), the by-products available worldwide would provide enough energy to support the production of 500 million tons of milk per year. Many studies have covered the topic of by-products as ruminant feeds (Durand et al., 1988; Mowrey et al., 1999; Hall and Chase, 2014), but only few experiments are available on mixtures of by-products as sole supplements in dairy cattle nutrition.

In a previous feeding trial, we tested the effects of a 100% substitution of a common concentrate mixture with a by-product mixture on feed intake, milk performance, blood variables, and the edible feed conversion ratio in organic dairy cow feeding (Ertl et al., 2015).

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In this earlier experiment, however, the consequences of including this by-product mixture in the diet on rumen fermentation and microbial community composition remained unclear. By-products, as opposed to traditional grains, are lower in starch but richer in fat or fiber. Due to these important nutrient shifts in the diet, it is not clear to which extent by-product feeding is reflected in the ruminal fermentation characteristics and abundance of the key microbiota in the rumen. Our hypothesis was that the substitution of common concentrates with a mixture of industrial by-products from the food processing would have substantial effects on the rumen fermentation profile, such as changes in short-chain fatty acid (**SCFA**) concentrations, composition and abundance of rumen microbiota, as well as gas formation. Therefore, the aim of the present study was to evaluate and quantify the effects of this substitution. For this, we chose a semicontinuous rumen simulation technique (**Rusitec**), with standardized similar rumen environmental conditions (i.e., temperature, pH, buffer flow). As it is only a simulation of the rumen, conditions in the Rusitec system differ from in vivo conditions (e.g., lower digestibility, lower total SCFA concentrations, lower presence or lack of protozoa and significant shifts in the microbial population; Prevot et al., 1994; Martínez et al., 2010; Hristov et al., 2012). Thus, results from Rusitec have to be interpreted carefully if applied to in vivo conditions. However, as it is a standardized method, Rusitec allows for the investigation and relative comparisons among various feeding conditions (i.e., concentrate type and proportion in the diet; Khiaosa-Ard et al., 2015).

MATERIALS AND METHODS

Diets

Two different concentrate mixtures were tested at 2 different forage-to-concentrate ratios (75:25 and 50:50 on DM basis) with Rusitec. Concentrate and forage mixtures were obtained from our previous in vivo feeding trial (Ertl et al., 2015). The control concentrate mixture (**CON**) contained feeds commonly used in Austrian organic dairy cow feeding, whereas the experimental concentrate mixture (**BP**) consisted solely of industrial by-products, abundantly available in organic quality. Daily supply of feeds and the chemical composition of the different diets are shown in Table 1. The forage mixture was kept at 4 to 6°C after air-drying until the start of the experiment. Forage and concentrate mixtures were ground to pass through a 3-mm sieve before diet preparation. All experimental diets (10 g of DM) were prepared before the start of the first experimental run and stored at 4 to 6°C.

Experimental Procedure and Sampling

The experiment, based on a 2 × 2 factorial arrangement with 2 concentrate types (CON and BP) and 2 concentrate levels (25 and 50%), consisted of 2 experimental runs, including all dietary treatments each time. Both runs comprised 12 fermenters (n = 6 per treatment) and lasted for 10 d, with the final 5 d for data collection. For each run, ruminal fluid and solid digesta were collected randomly from 2 out of 8 nonlactating rumen-fistulated Holstein cows, housed at the Teaching and Research Farm Kremesberg of the University of Veterinary Medicine, Vienna, Austria. The ruminal fluid was filtered through 4 layers of medicinal gauze (~1-mm pore size) prior to inoculation, whereas the solid digesta of the same cows was collected and used unprocessed to inoculate the system (Khiaosa-Ard et al., 2015). Cows were fed hay ad libitum and were kept according to the Austrian guidelines for animal welfare (Federal Ministry of Health, 2004). The Rusitec apparatus and the experimental procedure were as described in Klevenhusen et al. (2015), except for a different infusion rate of artificial saliva of 328 mL/d (±6.9).

Fermenter fluid samples were collected daily from the open fermenters directly before exchanging of the feed bags, using a syringe equipped with a plastic tube. Part of the fluid samples was immediately analyzed for pH, redox potential, and NH_3 (Klevenhusen et al., 2015), whereas the other part was stored in separate tubes at −20°C for determination of SCFA concentrations and analysis of microbiota composition. Daily fermentation gases of each fermenter were collected in gas-tight aluminum bags (Tecobag 8 L, Tesseraux Spezialverpackungen, Bürstadt, Germany).

Laboratory Analyses

Nutrient degradation was calculated from the difference between nutrient contents in the nylon bags before and after 48 h of incubation (feed residues from bags that were removed on sampling d 6–10 were pooled for each fermenter). After 48 h of incubation, feed bags were prepared and analyzed for DM, OM, CP, ether extract, and NDF corrected for ash (**aNDFom**) according to the methods and equipment presented in Klevenhusen et al. (2015). Nonfiber carbohydrates content was calculated as NFC = OM – (CP + ether extract + aNDFom). Determination of concentrations of SCFA (acetate, propionate, butyrate, isobutyrate, valerate, isovalerate, and caproate) in fermenter fluid was conducted via gas chromatography as described in detail in Klevenhusen et al. (2015). The CH_4 and CO_2 concentrations were measured using an infrared detector (ATEX Biogas Monitor Check BM 2000, Ansyco,