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

Received October 26, 2017.

Accepted December 20, 2017.

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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)

Table 1. Chemical composition and fatty acid profile of diet ingredients (% of DM unless stated)

Item	Hay	Wheat	Rye	BBP ¹	RSM ²
DM (% of fresh matter)	92.6	86.0	86.1	92.1	87.5
Ash	8.7	1.7	1.7	3.1	7.2
CP	15.7	12.8	9.6	12.9	37.5
NDF	61.7	11.7	15.2	6.4	24.7
ADF	38.9	4.3	3.6	2.2	20.7
Ether extract	2.7	2.0	1.6	12.2	4.2
Starch	—	67.7	61.6	53.4	—
Sugar	5.3	4.2	7.1	10.7	10.7
NE _L (MJ/kg of DM)	5.11	8.53	8.54	9.32	7.49
Calcium	0.50	0.07	0.08	0.12	0.70
Phosphorus	0.32	0.33	0.34	0.23	1.14
Phytate-phosphorus	—	0.19	0.18	0.07	0.62
Magnesium	0.29	0.10	0.12	0.08	0.49
Potassium	2.38	0.47	0.46	0.33	1.26
Sodium	0.059	0.014	0.014	0.647	0.077
Fatty acid (g/kg of fresh matter)					
SFA	6.366	6.120	10.256	25.681	6.682
MUFA	1.279	3.367	1.938	49.881	14.398
PUFA	6.447	11.958	9.190	27.067	8.658
UFA:SFA	1.21	2.50	1.09	3.00	3.45
C8:0	0.022	0.017	0.025	ND ³	0.023
C10:0	0.106	ND	ND	0.476	ND
C11:0	0.296	0.052	ND	0.081	0.110
C12:0	0.084	0.051	0.054	0.923	0.041
C13:0	ND	1.441	2.860	2.747	ND
C14:0	1.595	0.633	1.275	1.256	1.820
C14:1	0.385	ND	ND	0.061	0.024
C15:0	0.131	ND	ND	0.115	0.033
C16:0	2.860	3.794	2.711	14.170	2.373
C16:1	0.271	1.317	0.020	0.423	0.372
C17:0	0.847	ND	3.233	1.220	1.851
C17:1	ND	ND	ND	ND	0.148
C18:0	0.274	0.131	0.099	4.040	0.334
C18:1n-9 <i>trans</i>	ND	ND	ND	ND	0.165
C18:1n-9 <i>cis</i>	0.395	1.806	1.714	46.493	8.882
C18:1n-7	0.124	0.245	0.204	2.903	4.085
C18:2	ND	ND	ND	ND	0.072
C18:2n-6 <i>cis</i>	1.831	10.979	8.044	24.130	7.229
C20:0	ND	ND	ND	0.257	0.096
C20:1	0.105	ND	ND	ND	ND
C18:3n-3	4.616	0.979	1.146	2.937	1.356
C22:0	0.151	ND	ND	0.396	ND
C22:1	ND	ND	ND	ND	0.721

¹BBP = bakery by-product.²RSM = rapeseed meal.³ND = not detectable.

bags were hand-washed with running cold water until the water was clear and kept at -20°C for chemical composition analysis.

Daily Measurements of Fermentation Characteristics

The pH and redox potential of fermentor fluid samples were immediately determined using a pH meter (Seven Multi, Mettler-Toledo GmbH, Schwerzenbach, Switzerland) equipped with the respective electrodes (InLab Expert Pro-ISM for pH and Pt4805-DPA-SC-S8/120 for redox, respectively; Mettler-Toledo GmbH). The volume of effluent fluid was recorded to control the optimal buffer infusion. Daily gas volume was mea-

sured by the water replacement method (Soliva and Hess, 2007) and gas composition was determined using an infrared detector (ATEX Biogas monitor Check BM 2000, Ansyco, Karlsruhe, Germany).

Concentration and composition of SCFA (acetate, propionate, n-butyrate, isobutyrate, n-valerate, isovalerate, and caproate) were analyzed by GC, as described previously (Qumar et al., 2016). Briefly, incubation fluid samples were thawed and then centrifuged at $20,000 \times g$ for 25 min at 4°C . The supernatant (0.6 mL) was transferred into a fresh tube and 0.2 mL of HCl (1.8 mol/L) was added, followed by 0.2 mL of the internal standard (4-methylvaleric acid, Sigma-Aldrich, St. Louis, MO). After centrifugation of the mixture at $20,000 \times g$ for 25

min at 4°C, the clear supernatant was transferred into the GC vial. Analysis of SCFA concentrations was conducted via a GC apparatus (GC Model 8060 MS 172 DPFC, No. 950713, Fisons, Rodano, Italy), which was equipped with a flame-ionization detector and a 30 m × 0.53 mm ID × 0.53 µm film thickness capillary column (Trace TR Wax, Thermo Fisher Scientific, Waltham, MA). The injector and detector were at temperatures of 170 and 190°C, respectively. Helium was used as carrier gas with a flow rate of 6 mL/min. Stratos Software (Stratos Version 4.5.0.0, Polymer Laboratories, Church Stretton, Shropshire, UK) was used for generation and evaluation of chromatograms.

The concentration of ammonia was determined using the indophenol reaction (Weatherburn, 1967). The fermentor fluid samples were thawed at room temperature and subsequently centrifuged at $15,115 \times g$ for 10 min. The clear supernatant was diluted with distilled water to obtain a concentration within the standard calibration curve. Ammonia and phenol were oxidized by sodium hydroxide in the presence of sodium nitroprusside and dichloroisocyanuric acid. The absorbance was measured at 655 nm using U3000 Spectrophotometer (INULA GmbH, Vienna, Austria) after 90 min of reaction.

Chemical Composition Analysis

Analyses of feed and feed residue samples were conducted in duplicate according to VDLUFA (2012). Prior to analyses, feed residue samples were pooled for each fermentor across the last 5 d of each experimental run and oven-dried at 65°C for 48 h and ground, passing through a 0.5-mm screen (Retsch, Haan, Germany). The DM of the feed and pre-dried residue samples was determined by oven drying at 100°C for 24 h. Ash was analyzed by combustion of samples at a temperature of 580°C overnight. The CP content was determined using the Kjeldahl method. The content of NDF and ADF was analyzed separately with the Fiber Therm FT 12 (Gerhardt GmbH & Co. KG, Königswinter, Germany) following Van Soest et al. (1991). Heat-stable α -amylase and sodium sulfite were used in the NDF procedure and both fiber fractions were expressed exclusive residual ash. Ether extract (EE) was analyzed using a Soxhlet extractor (Extraction System B-811, Büchi, Flawil, Switzerland). Starch and the content of phytate-bound phosphorus were determined using commercially available kits (Megazyme, Wicklow, Ireland). Sugar was analyzed via the Luff-Schoorl method according to VDLUFA (2012). The P content was determined photometrically using the vanado-molybdate method, and Ca, Mg, K, and Na were determined by flame atomic absorption spectrophotometry according

to VDLUFA (2012). Apparent nutrient disappearance was calculated from the difference between the nutrient contents in the nylon bags before and after 48 h of incubation.

For the determination of the fatty acid profile, the 1-step methylation method by Sukhija and Palmquist (1988) was applied. Briefly, 0.5 g of feed samples was extracted and converted to methyl esters for 2 h at 70°C with toluene and 5% fresh methanolic HCl using nonadecanoic acid (C19:0, Sigma-Aldrich, Munich, Germany) as an internal standard. Subsequently, 5 mL of 6% K₂CO₃ was added followed by 2 mL of toluene. After centrifugation, 1 µL of the organic layer (split 50:1) was analyzed on a GC (Agilent Technologies 7890A, Waldbronn, Germany) equipped with an Agilent HP-88 capillary column (100 m × 0.25 mm ID × 0.2 µm d_f), and flame-ionization detector. The flow rates of the carrier gases (hydrogen and synthetic air) were 35 and 350 mL·min⁻¹, respectively. The injection port temperature and the detection temperature were 250°C. Oven temperature was gradually increased to 170°C for 1 min followed by an increase to 200°C at 2°C·min⁻¹. After 1 min at 200°C, the temperature was increased by 8°C·min⁻¹ to 230°C, which was held for another 10 min. Commercial standard FAME mixtures (Supelco 37 Component FAME Mix, Supelco, Bellefonte, PA) were used.

Genomic DNA Extraction

All microbial analyses were conducted solely in the liquid phase. Prior to DNA extraction, frozen incubation fluid samples from the last 5 sampling days per fermentor and run, were thawed on ice, vortexed to mix the sample, pooled, and then a 1-mL subsample was taken for DNA isolation. The DNA was first enzymatically and mechanically lysed using a bead beater (sterile ceramic beads, 1.4 mm; FastPrep-24 5G, MP Biomedicals LLC, Santa Ana, CA) according to the previously published protocol (Kong et al., 2010) and then isolated using the QIAamp Fast DNA Stool Mini Kit (Qiagen, Hilden, Germany). Samples were mixed with the supplied buffer and then heated at 95°C for 5 min. Thereafter, PCR inhibitors were removed and purified DNA was eluted following the manufacturer's instructions. The isolated DNA concentration was determined by a Qubit 2.0 Fluorometer (Life Technologies, Carlsbad, CA) using the Qubit dsDNA HS Assay Kit (Life Technologies) and stored at -20°C for further analysis.

Sequencing, Sequence Processing, and Analysis. One 20-µL aliquot of each DNA sample was sent for amplicon sequencing using a MiSeq Illumina sequencing platform and paired-end technology (Microsynth AG,

Balach, Switzerland). Sequencing targeted the V3–V5 hypervariable region of the 16S rRNA gene using the primer set 357F (5'-CCTACGGGAGGCAGCAG-3') and 926R-HMP (5'-CCGTCAATTCMTTTRAGT-3') to produce an amplicon size of approximately 523 bp (Peterson et al., 2009). Libraries were constructed by ligating sequencing adapters and indices onto purified PCR products using the Nextera XT Sample Preparation Kit (Illumina) according to the recommendations of the manufacturer. Equimolar amounts of each of the libraries were pooled and submitted for sequencing on an Illumina MiSeq Personal Sequencer using a 300-bp read length paired-end protocol. After sequencing, paired ends were stitched together by Microsynth, resulting in a total of 3.8 million unfiltered reads with an average of 503 nt in length. Sequence quality control and analyses were performed using the QIIME pipeline (Caporaso et al., 2010). Sequences were first quality filtered following previously published recommendations (Bokulich et al., 2013) and then screened for chimeras using the gold.fa database and USEARCH algorithm, filtered, and then picked using UCLUST (Edgar, 2010). Finally, samples were aligned and clustered to define operational taxonomic units (OTU) using PyNAST (Caporaso et al., 2010) and the SILVA database (v128; accessed August 3, 2017; Quast et al., 2013; Yilmaz et al., 2014). The degree of similarity between sequences was defined as 97% to obtain OTU identity at the species level. Any OTU that clustered with less than 10 reads were manually removed. A total of 2,415,892 sequences clustered into 6,282 OTU for further analysis. All OTU with a relative abundance greater than 0.1% (191 OTU) were taken for statistical analysis. Secondary classification of the OTU was performed using the National Center for Biotechnology Information BLASTN 2.7.0+ (Zhang et al., 2000). For calculation of the nonparametric species, richness estimators Chao 1 and diversity indices Shannon and Simpson at QIIME analysis were used.

Accession Numbers

Sequencing data are available in the BioProject SRA database (<https://www.ncbi.nlm.nih.gov/sra/>) as PRJNA412439 under the accession numbers SAMN07714160 to SAMN07714198.

Statistical Analyses

Statistical analyses were performed by ANOVA using the MIXED procedure of SAS (version 9.2, SAS Institute Inc., Cary, NC). For each variable tested, the model included the fixed effects of dietary treatment, considering the variation between experimental runs as a random effect. For measurements taken daily, the sampling day was included as a fixed effect as well. To

account for the covariation within experimental units, data obtained on the same fermentor at different times within one run were considered as repeated measurements with a first-order autoregressive variance-covariance matrix based on the lowest Akaike information criterion. Degrees of freedom were estimated with the method of Kenward-Roger. The pdiff option was used for each least squares means comparison. Furthermore, for assessing the overall effect of the BBP inclusion, an orthogonal contrast involving the average of the 3 BBP treatments versus CON was tested. Also, contrast analyses were performed to test linear effects of BBP inclusion level. Differences among LSM with $P \leq 0.05$ were considered as representing significant differences and differences among least squares means with $0.05 < P \leq 0.10$ were considered as representing a trend. Correlations between the microbial data and fermentation parameters were analyzed using Pearson correlation coefficient (PROC CORR of SAS).

RESULTS

Chemical Composition of BBP

Nutrient and fatty acid analyses of the feed ingredients used are summarized in Table 1. The BBP showed higher ash content (3.1%) compared with wheat and rye (1.7%). A closer look at macro minerals showed a higher content of Na in the by-product compared with the native cereals (6.5 vs. 0.14 g/kg of DM). Analysis of the proportion of phytate-bound P in relation to total P revealed a decrease from about 56% in wheat and rye to 29% in the BBP. Although the CP content was similar between wheat and BBP, the NDF and ADF fractions decreased by half in the BBP. The content of EE was almost 12.2% in the BBP, and sugar amounted to 10.7%. Besides an increase in the absolute amount of fatty acids in the BBP compared with the cereal grains, a shift in the profile also occurred. The ratio of UFA to SFA increased from 2.5 in wheat and 1.1 in rye, to 3.0 in the BBP, with oleic acid (C18:1n-9 *cis*) being the fatty acid with the highest concentration in the BBP (45% of the total amount of fatty acids compared with 8% in wheat and rye). Finally, a higher content of NE_L was estimated for the BBP (9.3 MJ/kg of DM) compared with wheat and rye (8.5 MJ/kg of DM). The differences between cereals and the BBP are also reflected in the chemical composition of the different experimental diets (Table 2).

Ruminal Fermentation

Results of ruminal fermentation characteristics are shown in Table 3. An overall effect of BBP inclusion

Table 2. Ingredients and chemical composition of diets either containing 45% grains (CON) or varying inclusion levels of bakery by-product (BBP)

Item	Experimental diet ¹			
	CON	15% BBP	30% BBP	45% BBP
Ingredient (% of DM)				
Hay	42	42	42	42
Wheat	33.75	22.50	11.25	—
Rye	11.25	7.5	3.75	—
BBP	—	15	30	45
Rapeseed meal	11	11	11	11
Vitamin-mineral premix ²	1.7	1.7	1.7	1.7
Calcium carbonate	0.3	0.3	0.3	0.3
Chemical composition (% of DM unless stated)				
DM (% of fresh matter)	89.2	90.1	91.0	91.2
Ash	6.9	7.1	7.4	7.6
CP	16.1	16.3	16.4	16.5
NDF	34.3	33.4	32.4	31.5
ADF	20.5	20.2	19.9	19.6
Ether extract	2.5	4.0	5.5	7.1
Starch	29.8	27.9	26.0	24.0
Sugars	5.6	6.5	7.4	8.2
NE _L (MJ/kg of DM)	6.81	6.93	7.05	7.16
Calcium	0.65	0.66	0.67	0.67
Phosphorus	0.49	0.47	0.46	0.44
Phytate-phosphorus	0.15	0.13	0.12	0.10
Magnesium	0.27	0.27	0.27	0.27
Potassium	1.35	1.33	1.31	1.29

¹The diets included 45% cereal grains (CON), 15% BBP and 30% cereal grains (15% BBP), 30% BBP and 15% cereal grains (30% BBP), or 45% BBP (45% BBP).

²Contained: 130 g of calcium, 45 g of phosphorus, 100 g of sodium, 30 g of magnesium, 1,000 mg of copper, 6,600 mg of zinc, 4,500 mg of manganese, 40 mg of selenium, 100 mg of cobalt, 80 mg of iodine, 700,000 IU of vitamin A, 100,000 IU of vitamin D₃, and 3,000 mg of vitamin E.

toward a decrease of the pH of incubation fluid was observed ($P = 0.03$). A more specific comparison revealed only minor differences between CON (6.63) and 45% BBP (6.59, $P = 0.02$). The redox potential and CO₂ formation were not affected by dietary treatment. The methane formation was lowest with 45% BBP ($P = 0.01$); however, no differences among groups were found when methane formation was related to degraded NDF ($P = 0.47$). The concentration of ammonia in the fermentor fluid was affected by the diet ($P < 0.01$). Whereas the concentration was similar in CON and 15% BBP, a significant decrease was found in 30% BBP (−22.5%) and 45% BBP (−33.0%; $P < 0.01$).

Dietary treatment did not alter total SCFA concentration ($P = 0.92$); however, differences in the SCFA profile were found among treatments (Table 3). Acetate proportion linearly decreased with increasing BBP inclusion levels ($P < 0.01$), with the maximum change in 45% BBP, showing an about 6.2% lower acetate proportion compared with CON ($P < 0.01$). The propionate proportion was linearly increased from 18.6% in CON to 23.7% in 45% BBP ($P < 0.01$). These changes resulted in a strong decrease in the acetate-to-propionate ratio from 2.63 in CON to 1.94 in 45% BBP ($P < 0.01$). Whereas the butyrate proportion remained unaffected

($P = 0.35$), isobutyrate linearly decreased with BBP level ($P < 0.01$), reaching a decrease of about 32% in 45% BBP compared with CON ($P < 0.01$). Enhancing the BBP inclusion level caused an increase in isovalerate ($P < 0.01$), whereas a linear decrease in caproate was found ($P < 0.01$).

Nutrient Disappearance

The disappearance of nutrients, especially starch ($P = 0.01$), was affected by dietary treatment (Table 3). The disappearance of DM was on average 3.1% lower in CON compared with the diets including BBP ($P < 0.01$). A trend toward a linear effect of BBP inclusion level ($P = 0.06$) revealed higher ash disappearance in 45% BBP compared with CON ($P = 0.03$) and 15% BBP ($P = 0.06$). The BBP in the diet decreased the disappearance of CP ($P = 0.01$) by on average 3.1% compared with CON. The disappearance of starch in BBP diets was greater than in the CON diet ($P < 0.01$), showing a strong linear increase from 84.4% (CON) to almost 96.3% (45% BBP). A linear effect of BBP on NDF disappearance was detected ($P < 0.01$), with 45% BBP decreasing this variable by 13.6% compared with CON ($P = 0.01$). However, no differences

among the lower BBP inclusion levels and CON were observed ($P \geq 0.45$). The disappearance of ADF was not affected by the dietary treatment ($P = 0.64$). The EE disappearance was increased by about 20% in the diets containing BBP ($P < 0.01$).

Ruminal Microbiota

Diversity indices and evenness estimators of the rumen community measured in the liquid phase are summarized in Table 4. Numbers of OTU as well as the species richness based on Chao I did not differ between diets. Species diversity based on the Simpson and Shannon indices was lower in 45% BBP compared with CON ($P = 0.04$).

In total, 19 phyla were identified, with *Bacteroidetes* and *Firmicutes* being the most abundant with total relative abundances of 54.9 and 37.3%, respectively (Table 5). No overall effect of the BBP inclusion was found on the phylum level.

According to the OTU assignment, a total of 34.71% of the OTU could be assigned to 83 genera, 15 of them being affected by the inclusion of BBP (Figure 1). Data on the genera that were not affected by the BBP are summarized in Supplemental Table S1 (<https://doi.org/10.3168/jds.2017-14051>). The most abundant genus, *Prevotella* (24.95% total relative abundance), tended to be increased by the BBP inclusion ($P = 0.10$). Furthermore, orthogonal contrast analysis revealed an increase of *Roseburia*, *Megasphaera*, *p-75a-5*, and *Acidaminococcus* ($P \leq 0.05$), as well as a trend toward an increase of *PSB-M-3*, *Atopobium*, and *Syntrophomonas* ($P \leq 0.09$). In contrast, the genera *Butyrivibrio*, *Anaerostipes*, *Shuttleworthia*, and *Azospirillum* were decreased when BBP was used as a substitute for grains ($P \leq 0.09$). A linear effect for *Mogibacterium* ($P = 0.02$) revealed an increase with BBP level. Oppositely, the genus *Agrobacterium* was linearly decreased with the BBP level ($P = 0.02$).

Figure 2 provides a list of the OTU that were affected by the BBP inclusion, within the first most

Table 3. Fermentation characteristics and nutrient degradability as affected by the inclusion level of bakery by-product (BBP)

Item	Experimental diet ¹					P-value ²		
	CON	15% BBP	30% BBP	45% BBP	SEM	Diet	CON vs. BBP	Linear contrast
Fermentation characteristic								
pH	6.63 ^a	6.60 ^{ab}	6.60 ^{ab}	6.59 ^b	0.011	0.14	0.03	0.39
Redox (mV)	-295.1	-290.1	-295.2	-292.3	7.80	0.89	0.67	0.77
CH ₄ (mL/d)	124.7 ^a	135.3 ^a	133.1 ^a	112.8 ^b	6.07	0.01	0.67	<0.01
CH ₄ (mL/g of degraded OM)	20.3 ^a	21.1 ^a	20.2 ^a	17.9 ^b	0.94	0.01	0.49	<0.01
CH ₄ (mL/g of degraded NDF)	121.5	117.1	122.4	131.1	9.83	0.47	0.79	0.13
CO ₂ (mL/d)	933.4	977.1	973.5	934.1	34.0	0.45	0.34	0.23
Ammonia (mM)	10.61 ^a	10.13 ^a	8.23 ^b	7.02 ^c	0.32	<0.01	<0.01	<0.01
SCFA ³ (mM)	120.6	119.2	121.9	125.0	8.15	0.92	0.84	0.50
Individual (mol/100 mol)								
Acetate	48.6 ^a	47.8 ^a	46.8 ^b	45.6 ^c	0.39	<0.01	<0.01	<0.01
Propionate	18.6 ^c	19.6 ^{bc}	20.5 ^b	23.7 ^a	0.89	<0.01	<0.01	<0.01
Butyrate	15.3	15.5	15.8	14.2	0.74	0.35	0.89	0.16
Isobutyrate	0.75 ^a	0.69 ^a	0.59 ^b	0.51 ^b	0.062	<0.01	<0.01	<0.01
Valerate	7.73 ^a	7.37 ^{ab}	7.04 ^b	7.42 ^{ab}	0.32	0.23	0.10	0.87
Isovalerate	3.24 ^c	3.79 ^{bc}	4.24 ^{ab}	4.44 ^a	0.34	0.01	<0.01	0.09
Caproate	5.74 ^a	5.21 ^b	4.74 ^b	4.01 ^c	0.32	<0.01	<0.01	<0.01
Acetate:propionate	2.63 ^a	2.46 ^a	2.26 ^b	1.94 ^c	0.10	<0.01	<0.01	<0.01
Nutrient disappearance (%)								
DM	56.2 ^b	57.6 ^a	58.1 ^a	58.2 ^a	0.54	0.04	<0.01	0.40
OM	55.1 ^b	56.5 ^a	56.9 ^a	57.0 ^a	0.56	0.06	<0.01	0.52
Ash	71.4 ^b	71.6 ^b	72.0 ^{ab}	73.1 ^a	0.71	0.12	0.16	0.06
CP	55.9 ^a	55.6 ^{ab}	54.4 ^b	52.5 ^c	0.60	<0.01	0.01	<0.01
NDF	25.8 ^a	26.6 ^a	24.8 ^a	22.3 ^b	1.17	0.02	0.29	<0.01
ADF	16.6	17.6	15.0	15.7	1.47	0.64	0.76	0.37
Hemicellulose	36.4 ^a	36.1 ^a	35.5 ^a	31.0 ^b	1.61	0.07	0.25	0.03
Ether extract	40.4 ^b	47.3 ^a	48.6 ^a	49.1 ^a	1.32	<0.01	<0.01	0.29
Starch	84.4 ^d	87.6 ^c	91.9 ^b	96.3 ^a	0.96	<0.01	<0.01	<0.01

^{a-d}Different superscripts indicate differences among LSM in the same row at $P \leq 0.05$.

¹The diets included 45% cereal grains (CON), 15% BBP and 30% cereal grains (15% BBP), 30% BBP and 15% cereal grains (30% BBP), or 45% BBP (45% BBP).

²Effect of experimental diet (Diet), overall effect of BBP inclusion (CON vs. BBP), and linear effect of BBP inclusion level (linear contrast).

³SCFA = short-chain fatty acids.

Table 4. Diversity indices and evenness estimators of the rumen community measured in the liquid phase as affected by the inclusion level of bakery by-product (BBP)

Diversity	Experimental diet ¹					P-value ²		
	CON	15% BBP	30% BBP	45% BBP	SEM	Diet	CON vs. BBP	Linear contrast
Chao I	4,417.42	4,075.27	4,370.64	4,083.80	238	0.54	0.34	0.98
Shannon	9.26 ^a	8.92 ^{ab}	9.10 ^{ab}	8.69 ^b	0.291	0.20	0.11	0.41
Simpson	0.9942 ^a	0.9920 ^{ab}	0.9928 ^{ab}	0.9898 ^b	0.002	0.19	0.11	0.29
Observed OTU ³	3,412	3,069	3,242	2,976	190	0.39	0.16	0.73

^{a,b}Different superscripts indicate differences among LSM in the same row at $P \leq 0.05$.

¹The diets included 45% cereal grains (CON), 15% BBP and 30% cereal grains (15% BBP), 30% BBP and 15% cereal grains (30% BBP), or 45% BBP (45% BBP).

²Effect of experimental diet (Diet), overall effect of BBP inclusion (CON vs. BBP), and linear effect of BBP inclusion level (linear contrast).

³OTU = operational taxonomic unit.

abundant 191 OTU, representing those with a total relative abundance of $\geq 0.10\%$. Overall, 22 OTU were affected by the inclusion of the BBP. Similar to the genera data, the most abundant OTU, OTU_1 (*Prevotella ruminicola*), was increased when BBP was included in the diets ($P = 0.05$), whereby the increase was especially pronounced in 30% BBP (+27%, $P = 0.06$) and 45% BBP (+32% $P = 0.03$). Similarly, *Roseburia faecis* (OTU_20) was increased by on average 67% ($P = 0.02$) in the diets containing BBP, again with only 30% BBP and 45% BBP differing significantly from CON ($P = 0.03$). Also, *Megasphaera* (OTU_63) was

enhanced by on average 69% in the BBP diets ($P = 0.05$). A significant effect of BBP toward an increase was also noticed for OTU_36 (*Clostridiales*), OTU_69 (*Christensenellaceae*), OTU_112 (*Coprococcus*), and OTU_113 (*Acidaminococcus fermentans*), whereas the orthogonal contrast analysis revealed a decrease of OTU_67 (*Bacteroidales*), OTU_96 (*Clostridiales*), and OTU_125 and OTU_172 (*Ruminococcaceae*). A linear effect for OTU_4 (*Clostridiales*) found an increase with BBP level. Also for OTU_37, OTU_82, and OTU_120 (*Bacteroidales*), as well as for the OTU_115 (*Prevotella*) and OTU_157 (CF231), a linear increase with the

Table 5. Phyla measured in the liquid phase as affected by the inclusion level of bakery by-product (BBP)

Phylum	Total relative abundance (%)	Experimental diet ¹					P-value ²		
		CON	15% BBP	30% BBP	45% BBP	SEM	Diet	CON vs. BBP	Linear contrast
<i>Bacteroidetes</i>	54.877	54.20	54.97	54.73	55.61	1.347	0.79	0.44	0.65
<i>Firmicutes</i>	37.258	37.40	36.57	37.67	37.40	0.9897	0.81	0.85	0.49
<i>Spirochaetes</i>	1.909	1.950 ^{yz}	1.729 ^z	2.140 ^y	1.816 ^{yz}	0.4444	0.22	0.73	0.67
<i>Proteobacteria</i>	0.762	0.865	0.953	0.573	0.658	0.1919	0.35	0.48	0.22
<i>Cyanobacteria</i>	0.757	1.207	0.883	0.410	0.529	0.0059	0.53	0.22	0.55
<i>Lentisphaerae</i>	0.716	0.642	0.702	0.800	0.721	0.1995	0.66	0.34	0.88
<i>Synergistetes</i>	0.614	0.441	0.899	0.499	0.616	0.2458	0.38	0.32	0.32
<i>Verrucomicrobia</i>	0.465	0.461	0.437	0.509	0.455	0.1035	0.80	0.92	0.81
<i>Tenericutes</i>	0.463	0.687	0.491	0.401	0.271	0.2261	0.57	0.23	0.47
<i>Elusimicrobia</i>	0.350	0.379 ^{ab}	0.276 ^b	0.460 ^a	0.283 ^b	0.0011	0.03	0.47	0.91
<i>TM7</i>	0.273	0.315 ^a	0.198 ^b	0.326 ^a	0.254 ^{ab}	0.0896	0.12	0.25	0.34
<i>Actinobacteria</i>	0.226	0.221	0.225	0.213	0.244	0.0295	0.64	0.74	0.44
<i>Fibrobacteres</i>	0.205	0.217	0.336	0.156	0.110	0.0013	0.63	0.91	0.22
<i>WPS-2</i>	0.032	0.036	0.038	0.021	0.035	0.0172	0.28	0.56	0.73
<i>Fusobacteria</i>	0.030	0.024 ^z	0.049 ^y	0.023 ^z	0.025 ^z	0.0098	0.16	0.41	0.07
<i>Chloroflexi</i>	0.017	0.017 ^{yz}	0.015 ^z	0.023 ^y	0.014 ^z	0.0049	0.16	0.81	0.84
<i>SR1</i>	0.016	0.027	0.025	0.005	0.005	0.0155	0.50	0.34	0.30
<i>Planctomycetes</i>	0.008	0.006	0.006	0.01	0.009	0.0021	0.46	0.50	0.30
<i>Armatimonadetes</i>	0.003	0.002	0.003	0.005	0.003	0.0012	0.13	0.27	0.77

^{a,b}Different superscripts indicate differences among LSM in the same row at $P \leq 0.05$.

^{yz}Different superscripts indicate differences among LSM in the same row at $P \leq 0.10$.

¹The diets included 45% cereal grains (CON), 15% BBP and 30% cereal grains (15% BBP), 30% BBP and 15% cereal grains (30% BBP), or 45% BBP (45% BBP).

²Effect of experimental diet (Diet), overall effect of BBP inclusion (CON vs. BBP), and linear effect of BBP inclusion level (linear contrast).

Genus	total relative abundance (%)	Experimental diets ¹				SEM	P-value ²			relative abundance	high
		CON	15% BBP	30% BBP	45% BBP		Diet	CON vs. BBP	Linear Contrast		
<i>Prevotella</i>	24.95		†		†	1.3010	0.15	0.10	0.97	>25	
<i>Roseburia</i>	0.681			*	*	0.1499	0.10	0.02	0.36	20-25	
<i>Butyrivibrio</i>	0.560					0.1250	0.40	0.09	0.80	0.5-1	
<i>Megasphaera</i>	0.252		†		†	0.0493	0.19	0.05	0.98	0.4-0.5	
<i>p-75a-5</i>	0.212		*		*	0.0329	0.02	0.03	0.50	0.3-0.4	
<i>Acidaminococcus</i>	0.172			†	*	0.0389	0.05	0.02	0.14	0.2-0.3	
<i>Paludibacter</i>	0.109		*	†	*	0.0226	0.06	0.67	0.49	0.1-0.2	
<i>Anaerostipes</i>	0.0573		†		*	0.0207	0.09	0.08	0.65	0.05-0.1	
<i>PSB-M-3</i>	0.0282			†		0.0039	0.34	0.07	0.96	0.025-0.05	
<i>Mogibacterium</i>	0.0276				*	0.0037	0.06	0.48	0.02	0.01-0.025	
<i>Shuttleworthia</i>	0.0229				†	0.0090	0.35	0.09	0.62	<0.01	
<i>Atopobium</i>	0.0115		*			0.0018	0.20	0.09	0.41		low
<i>Agrobacterium</i>	0.0031		*			0.00001	<0.01	0.12	0.04		
<i>Syntrophomonas</i>	0.0026			†		0.00001	0.36	0.09	0.81	*	P ≤ 0.05
<i>Azospirillum</i>	0.0025		*		†	0.00001	0.19	0.04	0.70	†	P ≤ 0.10

Figure 1. Genera measured in the liquid phase as affected by the inclusion level of bakery by-product (BBP). *Differences of the BBP diets compared with CON at $P \leq 0.05$. †Differences at $P \leq 0.10$. ¹The diets included 45% cereal grains (CON), 15% BBP and 30% cereal grains (15% BBP), 30% BBP and 15% cereal grains (30% BBP), or 45% BBP (45% BBP). ²Effect of experimental diet (Diet), overall effect of BBP inclusion (CON vs. BBP), and linear effect of BBP inclusion level (linear contrast). Color version available online.

BBP inclusion was found ($P \leq 0.04$). On the contrary, OTU_185 (*Bacteroidales*) was linearly decreased, with the 45% BBP tending to differ from CON ($P = 0.06$).

Correlation Analysis

Figure 3a shows significant correlations among OTU and the disappearance of starch ($P \leq 0.05$). The highest positive associations were found for *A. fermentans* (OTU_113), *Prevotella* sp.-associated OTU (OTU_1, OTU_115, OTU_78, OTU_191, and OTU_182), and *Roseburia faecis* (OTU_20). Among the OTU being negatively associated with the pH (Figure 3b), mainly *P. ruminicola* (OTU_1, OTU_182, and OTU_162) showed significant correlations ($P \leq 0.05$). Also, *Christensenellaceae* (OTU_69), *Clostridiales* (OTU_36), *Coproccoccus* (OTU_112), and *Roseburia faecis* (OTU_20) were negatively correlated with the ruminal pH ($P \leq 0.07$). In contrast, mainly OTU belonging to *Bacteroidales* (OTU_139, OTU_67, OTU_151, OTU_158 and OTU_133) and *Ruminococcaceae* (OTU_125 and OTU_181) showed a positive association with pH ($P \leq 0.07$).

DISCUSSION

The present study was performed to evaluate the effect of the gradual replacement of cereal grains by BBP on ruminal fermentation characteristics, nutrient degradation, and microbial community composition in vitro.

One main finding of the study was that substitution of common cereals such as wheat and rye up to two-thirds did not impair ruminal pH, nutrient degradation, or microbial diversity. Indeed, whereas fiber degradation remained unchanged up to an inclusion rate of 30% BBP in the diet, DM and starch degradation linearly increased.

Although total SCFA concentration was not affected, decreased acetate, isobutyrate, valerate, and caproate, and increased propionate and isovalerate levels clearly showed that inclusion of BBP modulated microbial metabolic activity when compared with the control treatment. However, one has to consider that the SCFA analysis gives no information regarding the production of SCFA except concentration. Thus, it is possible that the higher provision of rapidly degradable carbohydrates in the diets containing BBP might have enhanced the total production of SCFA, as supported by the overall reducing effect on ruminal pH. Moreover, it is also possible that the time of sampling (23 h after incubation) might be a reason for the insignificant differences among groups. Thus, a more frequent sampling, especially in the early hours after the incubation of fresh feed, should be considered in further in vivo studies.

The replacement of cereal grains by BBP increased the proportion of easily degradable NFC, most importantly starch, in the diets. Moreover, higher levels of sugars were present in the BBP compared with the native grains. Therefore, the decrease in the ratio of

acetate to propionate reflects these changes in the nutrient composition as well as in the enhanced degradation of starch.

Our study revealed a decreased methane formation when cereal grains were fully replaced by the BBP. This is likely due to the enhanced fat content or the reduced content of degradable NDF of the diet (or both), as both diet components have been reported to have a profound effect on methane emission in cattle. Although NDF is generally known to promote the formation of

methane (Jayanegara et al., 2010), fat has an opposite effect (Hünerberg et al., 2013). Thus, when taking the reduced NDF degradability of the 45% BBP diet into account, the reducing effect on methane diminished. Therefore, the methane-mitigating effect of the 45% BBP diet seems to be of limited benefit, as it happened at the expense of fiber degradability.

So far only one study has investigated the effect of the substitution of cereals with BBP on ruminal fermentation parameters (Franca et al., 2012). In line with

Figure 2. Operational taxonomic units (OTU) measured in the liquid phase as affected by the inclusion level of bakery by-product (BBP). *Differences of the BBP diets compared with CON at $P \leq 0.05$. †Differences at $P \leq 0.10$. ¹The diets included 45% cereal grains (CON), 15% BBP and 30% cereal grains (15% BBP), 30% BBP and 15% cereal grains (30% BBP), or 45% BBP (45% BBP). ²Effect of experimental diet (Diet), overall effect of BBP inclusion (CON vs. BBP), and linear effect of BBP inclusion level (linear contrast). Color version available online.

Figure 3. Bar graphs showing the operational taxonomic units (OTU) measured in the liquid phase correlating with the starch disappearance (A) and the pH (B). The OTU affected by the inclusion of the bakery by-product are shown in bold. The x-axis indicates Pearson correlation coefficients.

our results, an inclusion level of BBP up to 30% did not affect ruminal pH and concentration of SCFA, whereas a decreased ammonia level was observed. Thus, it is likely that besides a decrease in protein degradability, the increased energy availability (mainly due to the higher content of readily degradable carbohydrates in the BBP diets) for microbial protein synthesis caused the linear decrease in ruminal ammonia concentration. In accordance, the proportion of isobutyrate, the end product of ruminal deamination of dietary AA (Wallace, 1994), decreased. However, a linear increase in the molar proportion of isovalerate with increasing BBP level was noticed. A possible reason might be that this branched-chain fatty acid is linked to varying bacterial crossfeeding and changes in the microbial community. More specifically, recent studies suggest that the common rumen fibrolytic bacteria require branched-chain SCFA for the synthesis of branched-chain lipids (Suen et al., 2011a,b). Therefore, the higher abundance of fiber-fermenting bacteria (i.e., the genus *Butyrivibrio* and several OTU belonging to *Ruminococcaceae* in the CON group) might have caused an enhanced utilization of isovalerate.

Enhanced DM degradation is in line with a previous in situ degradation study conducted in fistulated sheep fed increasing BBP levels as a substitute for barley (Afzalzadeh et al., 2007). To the authors' knowledge, no further studies regarding the ruminal disappearance of diets including BBP are available. However, based on measurements on the total-tract level, contradictory effects of the inclusion of BBP on the digestibility have been reported, showing either no effect of the substitution of BBP for cereal grains (Franca et al., 2012; Obeidat et al., 2012) or a decrease in the digestibility of CP (Haddad and Ereifej, 2004).

One of the main aims of this study was to determine the effect of BBP on rumen bacterial community structure. We found that the full replacement of cereal grains with BBP decreased rumen bacterial diversity, whereas the lower inclusion levels caused no changes in bacterial diversity. However, a lowered diversity does not necessarily imply a negative effect on the cow. Shabat et al. (2016) revealed that a lower richness of the rumen microbiome was tightly linked to higher feed efficiency, stating the hypothesis that lowered diversity may result in improved bacterial efficiency due to reduced competition for substrates.

The main effects on the bacterial community were an increase in *Prevotella*, *Roseburia*, and *Megasphaera* when BBP was included in the diet, mirroring the different substrate availability and improved starch digestibility of the BBP. The increased abundance of *Prevotella* likely explains the increased proportion of propionate in the diets containing BBP, as this versatile genus is a

major propionate producer (Mickdam et al., 2017). An increase in *Prevotella* is typically observed when cows are fed increasing concentrate levels, such as during the transition from low- to high-concentrate diets, as they are amylolytic and thereby thrive on starch, but also due to their ability to grow under low pH conditions (Purushe et al., 2010). Due to the higher digestibility of starch in the BBP as well as the decrease in pH, the increasing effect on *Prevotella* was expected and this is supported by the negative correlation between several OTU belonging to the genus *Prevotella* and the pH. Also the increase in *Megasphaera* was expected, as this bacteria is a major soluble sugar fermenter and also well known to be boosted by feeding diets high in digestible starch (Pitta et al., 2014), due to their ability to convert the lactic acid that is produced by ruminal bacteria to acetic acid and propionic acid, thereby being able to attenuate lactic acidosis (Henning et al., 2010). Interestingly, a strong increase in *Roseburia* was found. Overall, *Roseburia* is able to use starch and a dominant role of this group of bacteria in the formation of butyrate has been reported (Louis et al., 2007; Lee, 2013), and is supported by the positive correlation with starch disappearance ($r = 0.38$, $P = 0.02$) and butyrate ($r = 0.43$, $P = 0.01$) production. However, other bacteria that are also able to produce butyrate, such as *Butyrivibrio*, decreased with the BBP inclusion. Overall, the 28% decrease in the abundance of the genus *Butyrivibrio* with the BBP inclusion, is likely derived from the lower availability of fiber or the increased content of EE in these diets (or both), as this genus is considered a major fibrolytic rumen microorganism (Derakhshani et al., 2017). This might be one reason for the unchanged butyrate concentration, as even an enhanced molar proportion in the BBP groups could have been expected, due to their higher amounts of sugars (Oba, 2011). Also, the decrease of some OTU belonging to the family of *Ruminococcaceae* pinpoints the negative effect of the BBP on fibrolytic bacteria, which is further supported by the decreased proportion of acetate, the main fermentation product of these taxa (Esquivel-Elizondo et al., 2017). Indeed, the lowest acetate proportion was found in the 45% BBP diet, which went along with the lowest NDF disappearance measured in the respective group. Also, several OTU belonging to the order of *Bacteroidales* were decreased in the BBP diets. In general, the order of *Bacteroidales* comprises amylolytic as well as cellulolytic taxa with diverse metabolic capacities (van Houtert, 1993; Henderson et al., 2015). It seems that several OTU of this order that were decreased by the BBP belong to fibrolytic bacteria, as supported by the positive association with the ruminal pH found especially for OTU belonging to the order of *Bacteroidales*, whereas these OTU

were negatively associated with the disappearance of starch. Also, several OTU belonging to the family of *Ruminococcaceae* showed a positive association with the ruminal pH, thereby supporting the general knowledge that especially fibrolytic microorganisms are sensitive to low pH values (Russell and Wilson, 1996). The decreased abundance of fibrolytic bacteria with the BBP inclusion might be related to the high fat content and higher concentration of UFA of the BBP, as especially elevated contents of UFA, such as oleic acid, have negative effects on microbial growth of fibrolytic bacteria in particular (Enjalbert et al., 2017).

Furthermore, this study also showed that the proportions of unclassified *Clostridiales* were enhanced in the BBP groups, which is consistent with previous reports that the bacterial populations in cattle fed high-grain diets contained more species belonging to the unclassified *Clostridiaceae* (Fernando et al., 2010; Mao et al., 2013). Therefore, these taxa likely play a significant role in the digestion of high-concentrate diets.

CONCLUSIONS

Replacing typical grains with BBP increased starch degradation and propionate proportion showing a potential for increased gluconeogenesis in cattle. However, feeding of 45% BBP resulted in decreased fiber degradation and diversity of microbes and therefore seems less recommendable in cattle diets. Further in vivo research is needed to validate our findings and to clarify the effects of the inclusion of BBP on animal health, productivity and product quality.

ACKNOWLEDGMENTS

We thank A. Dockner, S. Leiner, A. Sener, S. Sharma, and M. Wild (Institute of Animal Nutrition and Functional Plant Compounds, Vetmeduni Vienna) for their assistance with laboratory analyses and experimental material preparation. The authors acknowledge I. Kröger (Institute of Animal Nutrition and Functional Plant Compounds, Vetmeduni Vienna) for her help and technical assistance with the Rusitec trial. The authors are very grateful to the Institute of Animal Nutrition, Livestock Products and Nutrition Physiology, University of Natural Resources and Life Sciences Vienna, especially to A. Busch-Petersen, for the analysis of the fatty acid profile of the feeds. The bakery by-product was provided by Königshofer Futtermittel, Königshofer GmbH, Ebergassing, Austria. S. Aditya thanks the Austrian Agency for International Cooperation in Education and Research (ASEA UNINET, Vienna) for the PhD program financial support in Austria. We gratefully acknowledge the Viennese Chamber of Commerce

for the support of this research (Wirtschaftskammerpreis 2016).

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