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Prolonged feeding of high-concentrate diet remodels the hindgut microbiome and modulates nutrient degradation in the rumen and the total gastrointestinal tract of cows

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ABSTRACT

The aims of this research were to evaluate how prolonged feeding of a high-concentrate diet affects the ruminal degradation kinetics of fiber and starch, and to evaluate the effects of the high-concentrate diet on apparent total-tract nutrient digestibility in dairy cows. We also investigated the dysbiotic effects and the remodeling of the hindgut microbiome with prolonged high-concentrate feeding. Nine Holstein cows were used in 2 experimental periods; in each period, cows were first fed a 100% forage diet for 1 wk, followed by stepwise adaptation during one week to a high-concentrate (HC) diet (65% concentrate), which was then fed for 4 consecutive weeks. The kinetics of in situ ruminal degradability of grass silage (DM and NDF), corn grain and wheat grain (DM and starch), as well as the apparent total-tract nutrient digestibility were evaluated in the forage feeding and in wk 4 on the HC diet. Whereas the hindgut microbiome and fermentation profile were evaluated on a weekly basis. Regarding the in situ ruminal degradability due to grain type, the rate of degradation of the potentially degradable fraction and the effective rumen degradability of wheat grain were greater compared with corn grain. The in situ ruminal degradability of NDF decreased with the HC diet. However, the apparent total-tract digestibility of CP, fat, starch, NDF, ADF, and NFC increased with the HC diet compared with forage feeding. In addition, the HC diet increased the concentration of short-chain fatty acids in the hindgut, lowering fecal pH by 0.6 units, which correlated positively with microbial α diversity. This resulted in lower α diversity with the HC diet; however, α diversity (number of amplicon sequence variants) showed recovery in wk 3 and 4 on HC; in addition, microbial β diversity did not change from wk 2 onward on the HC diet. Two microbial enterotypes were identified: one for the forage diet with abundance of *Akkermansia* and *Anaerosporobacter*, and another enterotype for the HC diet with enrichment in *Bifidobacterium* and *Butyrivibrio*. Overall, results show that major microbial shifts and hindgut dysbiosis occurred in wk 1 on the HC diet. However, the hindgut microbial diversity of cows adapted after 3 wk of consuming the starch-rich ration. Thus, feeding the HC diet impaired fiber degradation in the rumen, but increased apparent total-tract nutrient digestibility. Likely, the forage diet contained less digestible NDF than the HC diet due to greater inclusion of forages with lower NDF digestibility and lower inclusion of more digestible nonforage NDF. Results also suggest that the adaptation of the hindgut microbial diversity of cows observed 3 weeks after the diet transition likely contributed to enhance total-tract nutrient digestibility.
Key words: nutrient degradability, apparent total-tract digestibility, hindgut fermentation, microbiome

INTRODUCTION

Dairy cows have evolved in the utilization of fiber-rich diets, thanks to a close relationship with the microbiota in the rumen and hindgut. The reticulorumen is the main metabolic chamber; in addition, the hindgut helps in the utilization of undigested, yet potentially digestible substrates, converting them into short-chain fatty acids (**SCFA**) as a key energy source. Thus, the hindgut serves as an important complementary digestive organ of cows, whose importance increases when passage rate is high and when the rumen function is disturbed, which may lead to increased flow of substrates escaping ruminal degradation (Gressley et al., 2011). Indeed, the extent by which fiber and other nutrients are degraded in the rumen and hindgut depends on multiple factors including the

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diet composition, the substrate structure, and the microbial fermentation both in the rumen and hindgut (Wang and McAllister, 2002; Bach et al., 2005). This is particularly relevant when feeding diets rich in starch, causing shifts in the substrate structure of the diet (starch vs. cellulose), in passage rate, and the microbial composition and activity (Plaizier et al., 2008), mainly stimulating the amylolytic bacteria (Plaizier et al., 2017a,b; Sánchez-Duarte et al., 2019), and reducing the cellulolytic taxa (Li et al., 2014).

Extensive research has been performed on the rumen microbiome, but the dynamics of hindgut ecology has not been deeply explored in dairy cows, especially with prolonged high-starch feeding, despite its crucial relevance in nutrient utilization and health (Gressley et al., 2011). Pieces of evidence indicate that replacement of fiber-rich forages with concentrates increases the starch that reaches the hindgut, influencing the microbiome (Plaizier et al., 2008, 2012), which is composed of less-acid-tolerant bacteria compared with the foregut (Khafipour et al., 2011). In this regard, despite research evaluating changes on hindgut bacteria when feeding fiber-rich diets (Castillo-Lopez et al., 2020) or grain-rich diets (Tafaj et al., 2001; Zebeli et al., 2007; Khafipour et al., 2016), or during the step-up diet transition (Ricci et al., 2022), there is limited information on hindgut microbial evolution due to prolonged high-concentrate (**HC**) feeding after diet transition and the effects on nutrient digestion and fermentation, thus representing an important research gap.

A portion of dietary nutrients can bypass ruminal degradation and be digested in the hindgut; research has demonstrated that up to 14% of ingested starch may be used for SCFA fermentation in the hindgut (Karr et al., 1966; Hoover 1978; Immig, 1996). Thus, understanding the ruminal degradation kinetics of starch-rich grains (i.e., corn and wheat) may contribute to elucidate their potential influence on hindgut ecology when fed to cattle. In addition to starch, it is conceivable that other nutrients (such as fiber) escape the rumen and reach the hindgut as well. In fact, the fate of fiber may be more relevant than the fate of starch, because part of this starch is digested in the small intestine and absorbed as glucose. In contrast, fiber can only be degraded by the microbial enzymes, either in the rumen or in the hindgut, and this is a time-consuming process. Feeding HC diets has been known to reduce ruminal fiber digestibility (Krajcarski-Hunt et al., 2002). However, the effect of HC diets on total-tract nutrient digestibility deserves further attention. A study showed that an HC diet did not affect total-tract digestibility of fiber but increased digestibility of starch in lactating cows consuming an average of 26 kg DM (Sánchez-Duarte et al., 2019). Another study reported no effects of diets containing from 40% to 60% concentrate on total-tract digestibility of starch (Guo et al., 2013). Thus, for a better understanding of the dynamics of nutrient digestion throughout the gastrointestinal tract, there is a need to evaluate both ruminal and apparent total-tract nutrient degradation, as well as its potential association with the hindgut microbiome.

Better knowledge on the extent and site of nutrient digestion is important to prevent dysbiosis, and to estimate the feeding value of diets, because part of the generated nutrients at the hindgut (i.e., microbial protein) cannot be utilized by the animal. Thus, this knowledge would be beneficial for both cattle health and for accurate evaluations of feeding value. Therefore, the aims were to evaluate how prolonged feeding of an HC diet affects the ruminal in situ degradation kinetics of fiber and starch, and to evaluate the effects of the HC diet on apparent total-tract nutrient digestibility in dairy cows. We also investigated the dysbiotic effects and the remodeling of the hindgut microbiome with prolonged HC feeding. We hypothesized that the hindgut microbiome will adapt and remodel with prolonged duration on HC. We also hypothesized that the in situ ruminal fiber degradation will be impaired with HC, but apparent total-tract nutrient digestibility would not be negatively affected.

MATERIALS AND METHODS

Animals, Housing, and Experimental Design

The present report is a continuation of previous studies that are part of a larger project (Ricci et al., 2022; Rivera-Chacon et al., 2022). Specifically, Rivera-Chacon et al. (2022) reported the effects of high-grain feeding on ruminal fermentation and systemic inflammation; Ricci et al. (2022) reported the changes in the ruminal and fecal microbiota during a 6-d step-up diet transition (10% to 60% concentrate). Whereas the present report tests the effects of prolonged duration of high-grain feeding (65% concentrate) on in situ ruminal degradation and hindgut microbiota compared with a baseline diet with 0% concentrate. The project was approved by the ethics and animal welfare committee of University of Veterinary Medicine Vienna (68.205/0003- V/3b/2019). Briefly, 9 rumen-cannulated (Bar Diamond, Parma, ID) nonlactating Holstein cows (992 ± 72 kg) were used at the research dairy farm of University of Veterinary Medicine, Vienna (Pottenstein, Lower Austria). The experiment was a longitudinal design that included 2 periods. In each period, cows were fed a forage-only diet (75% grass silage, 15% corn silage, and 10% grass hay) for 1 week, then were transitioned stepwise during 1 wk to an HC acidogenic diet (26.25% grass silage, 8.75% corn silage, and 65% concentrate, DM basis; Supplemental Table S1, see Notes), which was fed for 4 wk. There was

a 4-wk washout between the 2 periods; during the washout, cows grazed on pasture. In addition, before the start of the experiment, the cows were adapted to the feeders, consuming a forage diet for 2 wk.

Cows were housed in a freestall barn with deep litter cubicles (straw bedding) and free-choice minerals. Water was offered ad libitum; diets were prepared once a day and offered ad libitum in individual feed troughs to each cow at 0800 h. Individual feed intake was recorded with computer-regulated access gates and electronic scales (Insentec B.V., Marknesse, the Netherlands).

The sampling scheme and data collections for hindgut microbiome, hindgut fermentation, ruminal in situ nutrient degradation and apparent total-tract nutrient digestibility are illustrated in Supplemental Figure S1 (see Notes). For each variable evaluated, this experimental set up yielded 18 biological replicates within each corresponding week (9 cows and 2 experimental periods), which contribute to the robustness of the statistical analysis.

Evaluation of In Situ Ruminal Nutrient Degradation

In situ ruminal degradation was performed in the week of forage feeding and in wk 4 on HC diet. Two high-starch substrates (corn and wheat grain) and one high-fiber substrate (grass silage) were evaluated in triplicate. The grains and grass silage were ground to 4 mm and 6 mm, respectively.

The method used was similar to Paz et al. (2014). Briefly, 2 g of corn or wheat grain were placed in nylon bags (5 cm × 5 cm, 50-µm pore size, R55, ANKOM Technology, Macedon, NY), 7 g of grass silage were placed in nylon bags (10 cm × 20 cm, 50-µm pore size, R1020, ANKOM Technology, Macedon, NY). Two hours before feeding, bags were inserted in the rumen, positioned in the ventral sac, and incubated for the corresponding time. Degradation kinetics of the grains were evaluated at 0, 2, 4, 8, 12, and 24 h. Degradation kinetics of grass silage were evaluated at 0, 2, 4, 8, 12, 24, and 48 h.

The degradation kinetics were evaluated with the NLIN procedure of SAS (Version 9.4, SAS Institute Inc.) and according to the Ørskov and McDonald model (Ørskov and McDonald, 1979):

$$\text{For grain: } Dt = a + b \times [1 - \exp(-ct)],$$

$$\text{and for grass silage: } Dt = a + b \times [1 - \exp - c(t - Lt)],$$

where Dt = percentage of substrate degraded in the bag; a = soluble material at 0 h, also known as rapidly degradable fraction; b = potentially degradable fraction over time, also known as slowly degradable fraction; c = constant rate of degradation of fraction b (h^{-1}); t = incubation time (h); Lt = lag time (h), and exp = exponent.

The effective rumen degradability was calculated as follows:

$$a + b \times c/(c + kp),$$

where a, b, and c are degradation constants; kp = passage rate (0.06 h^{-1} for grass, 0.04 h^{-1} for grains).

Determination of Apparent Total-Tract Nutrient Digestibility

Fecal samples were collected from the rectum twice daily at feeding time and 8 h postfeeding (approximately 0.5 kg per sampling point per cow) during 3 consecutive days in the week of Forage feeding and wk 4 on HC, and samples were pooled in a 3-L plastic bucket. To do so, there was one fecal container for cow. The fecal samples were kept in a freezer at −20°C, and the fresh sample was added to the frozen feces after each sampling event. At the end of the experiment, samples were thawed overnight, uniformly mixed the next morning, and freeze-dried (CoolSafe 100–9 Pro, LaboGene, Lillerød, Denmark), and then milled to <500 µm (ZM 200, Retsch GmbH, Haan, Germany). Analyses were performed in triplicates and included CP (method 4.1.1), ether extract (**EE**) (method 5.1.2), NDF (methods 6.5.1), ADF (methods 6.5.2), starch, and ash (method 8.1; all from Association of German Agricultural Analytic and Research Institutes, 2012). Nonfibrous carbohydrates were calculated in fecal samples as 100 − (% CP + % NDF + % EE + % ash). Feed samples were pooled across 3 d within each sampling week, and results for chemical composition of feed and feces were used to determined apparent total-tract nutrient digestibility using acid insoluble ash as an internal marker. Apparent total-tract digestibility of DM, CP, NFC, EE, NDF, ADF, and starch were calculated as: 1 − [(concentration of marker in feed/concentration of marker in feces) × (concentration of nutrient in feces)/concentration of nutrient in feed] according to Bachmann et al. (2019).

Hindgut Fecal Sampling and Evaluation of the Hindgut Microbiome

Hindgut fecal sampling was performed by collecting grab samples of feces rectally using a palpation sleeve for each collection. These samples were taken on a weekly basis 4 h postfeeding. Around 2 mL of the collected feces was placed in cryotubes using a spatula previously sterilized with 70% ethanol, and immediately snap-frozen in liquid nitrogen. Then, samples were stored at

−80°C. Including both experimental periods, a total of 90 fecal samples were collected for hindgut microbiome evaluation (9 cows, 5 sampling weeks per period, and 2 experimental periods). Isolation and purification of genomic DNA was performed using the DNeasy PowerSoil Kit (Qiagen, Hilden, Germany) with minor modifications (Ricci et al., 2022). Amplicon sequencing was performed using Illumina MiSeq paired-ends sequencing (Microsynth AG, Balgach, Switzland). Targeted amplification of the hypervariable region V3-V4 of bacterial 16S rRNA gene (2 × 250 bp) was performed using the primers 341F-ill (5′-CCTACGGGNGGCWGCAG-3′) and 802R-ill (5′-GACTACHVGGGTATCTAATCC-3′). Multiplexed libraries were constructed by ligating sequencing adapters and indices onto purified PCR products using the Nextera XT Sample Preparation Kit (Illumina, Balgach, Switzerland). Primer regions were trimmed by Microsynth (Microsynth AG).

Bioinformatics and Data Analysis

From the 90 fecal samples collected, 7 samples were excluded in the statistical analysis because these did not meet the quality filtering criteria (3 samples for forage diet, 1 sample for wk 2 on HC diet, and 3 samples for wk 3 on HC diet). Specifically, reads were processed using the software package Quantitative Insights into Microbial Ecology (QIIME2 v2020.2; Bolyen et al., 2019). Trimmed reads were imported and read quality was initially inspected using FASTQC v. 0.11.5 (Andrews, 2010). Forward and reverse reads were joined using VSEARCH (Rognes et al., 2016) and quality filtered using the q-score-joined plugin with a minimum acceptable PHRED score of 20 (–p-min-quality 20). Denoising into amplicon sequence variants (**ASV**) was obtained using deblur by trimming all reads to a length of 400 nucleotides and removing low abundance features to a minimum of 10 (Amir et al., 2017). Representative sequences and feature tables were filtered to exclude all features classified as mitochondria or chloroplast sequences, resulting in a total of 4,000 features. Filtered ASV were aligned with mafft (Katoh and Standley, 2013) and phylogeny was constructed with fasttree2 (Price et al., 2009). A classify-sklearn naïve Bayes taxonomy classifier trained with the 341F/802R primer set against the SILVA 132 99% operational taxonomic unit reference sequences (https://www.arb-silva.de, version 132) was used for taxonomy assignment. Rooted tree, taxonomy, and filtered feature table were used as inputs to phyloseq v1.24.2 in R Studio v14.1717 (R Core Team, 2020) and used to perform differential abundance analysis. The DNA sequence reads used in the analysis were submitted in the National Center for Biotechnology Information Sequence Read Archive (https://www.ncbi.nlm.nih.gov/sra; accession number PRJNA802085).

Hindgut Fecal Sampling and Evaluation of Hindgut Fermentation

Fecal samples were collected from each cow's rectum at the time of feeding, namely 0 h (8:00 a.m.), then at 4, 8, and 12 h postfeeding once a week (on d 7, 21, 28, 35, and 42 of each experimental period; Supplemental Figure S1). These samples were transferred into 8-mL vials and then stored at −20°C. Once at the laboratory, samples were thawed and fecal pH was measured in triplicate using a portable pH meter (Mettler-Toledo, AG; Analytical CH; Schwerzenbach, Switzerland). For SCFA, sample preparation and measurement were conducted in triplicate following Petri et al. (2019). Briefly, samples were thawed overnight at room temperature, subsamples of 1 g of feces were mixed with 1 mL of water, 300 µL of internal standard (4-methylvaleric acid, Sigma-Aldrich), and 200 µL of 25% phosphoric acid. After centrifugation at 20,000 × *g* for 20 min at 4°C, the supernatant was transferred into a fresh tube where the supernatant was again centrifuged at 20,000 × *g* at 4°C for 25 min, this step was repeated until the supernatant was clear. Gas chromatography analysis was performed following previously described protocol (Qumar et al., 2016). The injector and detector had temperatures of 220°C, and helium was used as carrier gas with a flow rate of 6 mL/min. The LabSolution LCGC software (version 5.97, Shimadzu) was used for the generation and evaluation of chromatograms.

Statistical Analyses

Analyses of data for in situ ruminal nutrient degradation, apparent total-tract nutrient digestibility, and hindgut fermentation were performed using the PROC MIXED of SAS (version 9.4; SAS Institute, Cary, NC). For in situ ruminal degradation of grass silage, cow was considered as random effect, and diet was included in the model as fixed effect; for ruminal degradability of corn and wheat grain, diet and grain type were included as fixed effects in the statistical model. Regarding apparent total-tract nutrient digestibility, cow was included as random effect, while diet was included as fixed effect in the statistical model. For fecal pH and SCFA, cow was considered as random effect, and week of sample collection within each time point postfeeding was included as fixed effect in the statistical model. Data from the same cow collected at different times were processed as

repeated measures with first-order variance-covariance structure matrixes (AR(1)) taking into consideration that the variance-covariance decays with time. Outliers were removed based on Cook's D; normal distribution was verified using PROC UNIVARIATE followed by the normal and plot options before the ANOVA. The PDIFF option of SAS was used to generate and compare the LSM. Treatment means are presented as LSM and the largest SEM is reported.

For microbial analysis, the statistical model included the random effect of cow and the fixed effect of week (for example, wk 1 on HC vs. wk 2 on HC). Statistical significance was declared when the Benjamini-Hochberg adjusted $P \leq 0.05$, and tendency is discussed if $0.05 < P \leq 0.10$. Data visualization was conducted in R Studio version 1.3.1093 (R Core Team., 2020), using gplots (v. 3.1.0), dplyr (v. 1.0.2), and ggpubr (v. 0.4.0). Differential abundance of individual taxa was performed in MaAsLin2, ANCOM-BC, and ALDEx2. Network analysis was based on a phyloseq object comprising the fecal ASV, using the weighted UniFrac distance, with a maximum distance between connected nodes of 0.8 and discarding isolates. This igraph-based network was then plotted using the plot_net function in R (R Core Team., 2020).

Using data of apparent total-tract nutrient digestibility and microbial data collected in corresponding weeks, correlation analysis was performed between the hindgut microbiome and nutrient digestibility. To do so, the rcorr() function of the package Hmisc (v 5.1–1) were used in R (R Core Team., 2020).

RESULTS

In Situ Ruminal Nutrient Degradability

Regarding rumen degradability of grass silage, feeding the HC diet increased the in situ 48-h DM degradability, but the rate of degradation of this DM decreased ($P < 0.05$) compared with forage feeding. More specifically, the HC diet decreased in situ ruminal degradability of NDF of the grass silage ($P < 0.01$; Table 1).

Regarding in situ rumen degradability of grains, the rate of degradation and the effective rumen degradability of wheat were greater compared with corn ($P < 0.01$). There was an interaction between diet and grain type on the 24-h degradability of both DM and starch; specifically, this variable was greater for wheat when cows consumed the forage diet ($P < 0.01$; Table 2).

Apparent Total-Tract Nutrient Digestibility

Compared with the forage diet, feeding the HC diet increased intake of DM, CP, EE, NFC, and starch ($P < 0.01$). Considering only the NDF intake proportion in the ration that originated from grass silage, this NDF decreased by 66% ($P < 0.01$) from the forage to the HC feeding (Table 3). Compared with the forage diet, the HC diet resulted in increased apparent total-tract digestibility of all nutrients, including DM ($P < 0.01$), CP ($P < 0.01$), EE ($P < 0.01$), NDF ($P < 0.01$), ADF ($P < 0.05$), NFC ($P < 0.01$), and starch ($P < 0.01$).

Table 1. Effect of a high-concentrate diet[1] after 4 wk of feeding compared with a baseline forage diet on in situ rumen degradation of grass silage in nonlactating Holstein cows

Item[2]	Forage diet	High-concentrate diet	SEM[3]	*P*-value,[4] diet
Grass silage, DM				
a, %	36.7	34.3	0.37	<0.01
b, %	28.2	35.6	0.71	<0.01
c, %/h	18.1	10.0	0.68	<0.01
Effective rumen degradability, %	59.2	59.2	0.52	0.99
48-h degradability, %	63.4	69.0	0.61	<0.01
Grass silage, NDF				
a, %	34.2	35.6	0.56	0.17
b, %	29.9	27.1	0.80	0.07
c, %/h	9.20	5.70	0.51	<0.01
Effective rumen degradability, %	54.3	50.7	0.50	<0.01
48-h degradability, %	63.1	59.6	1.09	<0.01

[1]A diet containing 65% concentrate.

[2]a = rapidly degradable fraction; b = slowly degradable fraction; c = constant rate of degradation of fraction b, effective ruminal degradability: $a + b \times c/(c + kp)$, where kp is the passage rate of forage (0.04 h^{-1}).

[3]The largest standard error of the mean.

[4]*P*-values for the effect of diet.

Table 2. Effect of a high-concentrate diet[1] after 4 wk of feeding compared with a baseline forage diet on in situ rumen degradation of corn and wheat grain in nonlactating Holstein cows

	Forage diet		High-concentrate diet			*P*-value[2]		
Item[3]	Corn	Wheat	Corn	Wheat	SEM[4]	Grain	Diet	I
DM								
a,[5] %	25.0^{c}	39.4^{b}	27.0^{c}	46.3^{a}	1.03	<0.01	<0.01	0.26
b, %	70.1^{a}	38.8^{b}	68.1^{a}	30.9^{c}	1.61	<0.01	<0.01	0.09
c,[5] %/h	9.10^{c}	37.7^{a}	9.50^{c}	30.7^{ab}	1.08	<0.01	0.37	0.15
Effective rumen degradability, %	65.4^{b}	72.0^{a}	68.7^{ab}	72.5^{a}	1.36	<0.01	0.21	0.36
24-h degradability, %	83.7^{b}	89.4^{a}	87.4^{a}	88.7^{a}	0.71	<0.01	<0.05	<0.01
Starch								
a, %	33.3^{d}	61.4^{b}	37.8^{c}	68.7^{a}	1.21	<0.01	<0.01	0.12
b, %	67.2^{a}	32.7^{c}	61.7^{ab}	25.0^{d}	2.28	<0.01	<0.01	0.62
c,[5] %/h	9.50^{c}	47.8^{a}	10.3^{c}	43.0^{ab}	1.09	<0.01	0.88	0.33
Effective rumen degradability, %	69.3^{c}	89.0^{a}	74.3^{b}	89.2^{a}	0.71	<0.01	<0.01	<0.01
24-h degradability, %	89.1^{b}	95.3^{a}	93.8^{a}	95.3^{a}	0.72	<0.01	<0.01	<0.01

$^{a–d}$Within a row, means with different superscripts indicate a statistically significant difference ($P < 0.05$).

[1]A high-concentrate diet with 65% concentrate.

[2]*P*-values for the effect of grain type (Grain), diet type (Diet), and their interaction (I).

[3]a = rapidly degradable fraction; b = slowly degradable fraction; c = constant rate of degradation of fraction b, effective ruminal degradability: a + b × c/(c + kp), where kp is the passage rate of grains (0.06 h^{-1}).

[4]The largest standard error of the mean.

[5]Values were transformed using the log function after checking for normal distribution and were transformed back after the analysis.

Hindgut SCFA and pH

The hindgut total concentration of SCFA as well as the proportion of butyrate were greater with HC ($P < 0.05$). At 8 and 12 h postfeeding during the weeks on HC, the concentration of total SCFA was particularly lower in wk 4 compared with wk 1 ($P < 0.05$). A reduction in the proportion of acetate and an increase in the proportion of propionate was found particularly at 12 h postfeeding in wk 2 on the HC diet compared with their proportions

Table 3. Effect of a high-concentrate diet[1] after 4 wk of feeding compared with a baseline forage diet on apparent total-tract digestibility in nonlactating Holstein cows

Item	Forage diet	High-concentrate diet	SEM[2]	*P*-value,[3] diet
DM				
Intake, kg/d	8.61	13.8	0.76	<0.01
Digestibility, %	69.2	83.8	1.28	<0.01
CP				
Intake, kg/d	1.39	2.44	0.15	<0.01
Digestibility, %	71.6	80.9	1.20	<0.01
Ether extract				
Intake, kg/d	0.23	0.42	0.03	<0.01
Digestibility, %	61.2	84.5	1.77	<0.01
NDF				
Intake, kg/d	4.57	4.41	0.39	0.89
Digestibility, %	69.6	78.5	3.36	<0.01
NDF intake from grass silage, kg/d	3.37	1.15	0.18	<0.01
ADF				
Intake, kg/d	3.24	2.88	0.23	0.24
Digestibility, %	63.7	72.1	1.90	<0.05
ADF intake from grass silage, kg/d	2.38	2.15	0.13	0.23
NFC				
Intake, kg/d	1.52	5.70	0.15	<0.01
Digestibility, %	81.7	94.9	2.13	<0.01
Starch				
Intake, kg/d	0.37	4.01	0.09	<0.01
Digestibility, %	96.1	99.3	0.43	<0.01

[1]A 65% concentrate diet.

[2]The largest standard error of the mean.

[3]*P*-values for the effect of diet.

in the week of forage diet. In addition, an increase in the proportion of butyrate was found throughout the weeks of HC feeding compared with the week of forage feeding. Overall, hindgut pH decreased with the transition from forage and HC diet ($P < 0.05$; Table 4).

Taxonomy of the Hindgut Microbiota

A total of 4,000 features were found across the 83 high-quality samples, representing a total of 15 different bacterial phyla before downstream analysis. The predominant phyla were *Firmicutes* (79%), *Bacteroidetes* (16%), and *Spirochaetes* (1.7%; Supplemental Table S2; see Notes). The remaining 3.4% of the microbiome was composed by *Verrucomicrobia*, *Lentisphaerae*, *Proteobacteria*, *Actinobacteria*, *Patescibacteria*, *Tenericutes*, *Cyanobacteria*, *Fibrobacteres*, *Kiritimatiellaeota*, *Planctomycetes*, *Elusimicrobia*, and *Epsilonbacteraeota*. When evaluating the hindgut microbiota composition at the genus level, the top genera included *Ruminococ-*

Table 4. Hindgut short-chain fatty acids and pH relative to feeding time of nonlactating Holstein cows consuming forage or during 4 wk on a high-concentrate diet[1]

Item	Forage diet	High-concentrate diet, wk 1	High-concentrate diet, wk 2	High-concentrate diet, wk 3	High-concentrate diet, wk 4	SEM[2]	*P*-value,[3] week
Time of feeding							
Total SCFA, m*M*	43.2^{b}	94.6^{a}	93.7^{a}	85.2^{a}	89.6^{a}	5.15	<0.01
% of total SCFA							
Acetate	76.8^{a}	75.7^{a}	75.6^{a}	73.1^{b}	76.2^{a}	0.64	<0.01
Propionate	13.8^{b}	15.1^{ab}	14.3^{b}	16.8^{a}	15.3^{ab}	0.48	<0.01
Butyrate	4.08^{b}	6.20^{a}	7.46^{a}	6.80^{a}	6.10^{a}	0.42	<0.01
Valerate	1.31	1.16	1.21	1.22	1.09	0.07	0.19
Isobutyrate	2.11^{a}	0.99^{b}	0.79^{b}	1.08^{b}	0.77^{b}	0.13	<0.01
Isovalerate	1.77^{a}	0.69^{b}	0.57^{b}	0.89^{b}	0.48^{b}	0.13	<0.01
4 h postfeeding							
Total SCFA, m*M*	42.6^{b}	96.2^{a}	80.6^{a}	102^{a}	83.2^{a}	6.37	<0.01
% of total SCFA							
Acetate	76.6^{x}	76.59^{x}	75.2^{xy}	74.3^{y}	75.7^{xy}	0.60	<0.05
Propionate	14.1^{b}	14.32^{b}	15.43^{ab}	16.2^{a}	15.4^{ab}	0.46	<0.01
Butyrate	4.10^{b}	5.79^{a}	6.42^{a}	6.38^{a}	6.28^{a}	0.34	<0.01
Valerate	1.34^{a}	1.18^{ab}	1.28^{ab}	1.22^{ab}	1.11^{b}	0.06	0.05
Isobutyrate	2.07^{a}	1.29^{b}	0.95^{b}	1.04^{b}	0.98^{b}	0.15	<0.01
Isovalerate	1.77^{a}	0.83^{b}	0.75^{b}	0.87^{b}	0.59^{b}	0.13	<0.01
8 h postfeeding							
Total SCFA, m*M*	48.1^{c}	102^{a}	80.4^{ab}	86.2^{ab}	66.9^{bc}	7.00	<0.01
% of total SCFA							
Acetate	75.3^{ab}	76.5^{a}	74.0^{b}	73.8^{b}	75.3^{ab}	0.68	<0.05
Propionate	14.8^{y}	14.8^{y}	16.6^{x}	16.6^{x}	15.5^{xy}	0.49	<0.05
Butyrate	4.40^{b}	5.50^{ab}	6.16^{a}	6.36^{a}	6.29^{a}	0.29	<0.01
Valerate	1.52^{a}	1.19^{ab}	1.29^{ab}	1.25^{b}	1.13^{b}	0.08	<0.01
Isobutyrate	2.13^{a}	1.18^{b}	1.14^{b}	1.15^{b}	1.10^{b}	0.12	<0.01
Isovalerate	1.85^{a}	0.88^{b}	0.89^{b}	0.89^{b}	0.70^{b}	0.11	<0.01
12 h postfeeding							
Total SCFA, m*M*	48.0^{c}	92.4^{a}	75.7^{ab}	76.4^{ab}	69.8^{b}	4.63	<0.01
% of total SCFA							
Acetate	76.6^{a}	75.8^{ab}	73.7^{b}	75.1^{ab}	75.1^{ab}	0.78	<0.05
Propionate	14.0^{b}	15.4^{ab}	16.5^{a}	15.8^{ab}	16.1^{ab}	0.57	<0.05
Butyrate	4.30^{b}	5.88^{a}	7.06^{a}	6.07^{a}	6.08^{a}	0.41	<0.01
Valerate	1.39^{a}	1.13^{b}	1.23^{ab}	1.13^{b}	1.18^{ab}	0.08	<0.05
Isobutyrate	2.02^{a}	1.05^{b}	0.90^{b}	1.08^{b}	0.96^{b}	0.12	<0.01
Isovalerate	1.77^{a}	0.72^{b}	0.65^{b}	0.81^{b}	0.58^{b}	0.12	<0.01
Hindgut pH relative to feeding time							
At time of feeding	7.60^{a}	6.57^{b}	6.35^{b}	6.53^{b}	6.33^{b}	0.06	<0.01
4 h postfeeding	7.47^{a}	6.51^{b}	6.44^{b}	6.47^{b}	6.37^{b}	0.06	<0.01
8 h postfeeding	7.45^{a}	6.47^{b}	6.50^{b}	6.50^{b}	6.47^{b}	0.06	<0.01
12 h postfeeding	7.43^{a}	6.40^{b}	6.40^{b}	6.62^{b}	6.60^{b}	0.08	<0.01

$^{a–c}$Within corresponding sampling time and variable, means with different superscripts indicate a statistically significant difference ($P < 0.05$).

^{x,y}Within corresponding sampling time and variable, means with different superscripts indicate a tendency for a statistical difference ($0.05 < P \leq 0.10$).

[1]A 65% concentrate diet.

[2]The largest standard error of the mean.

[3]*P*-values of the effect of sampling week.

caceae UCG-005, *Christensenellaceae* R-7, *Ruminococcaceae* UCG-010, *Romboutsia*, *Rikenellaceae* RC9 gut group, *Eubacterium* coprostanoligenes group, *Lachnospiraceae* NK3A20 group, *Paeniclostridium*, *Alistipes*, and *Bacteroides* (Figure 1).

Microbial Alpha- and Beta-Diversity and Most influential ASV

Alpha-Diversity. Most of the α-diversity indices were influenced by the change from forage to HC feeding. The

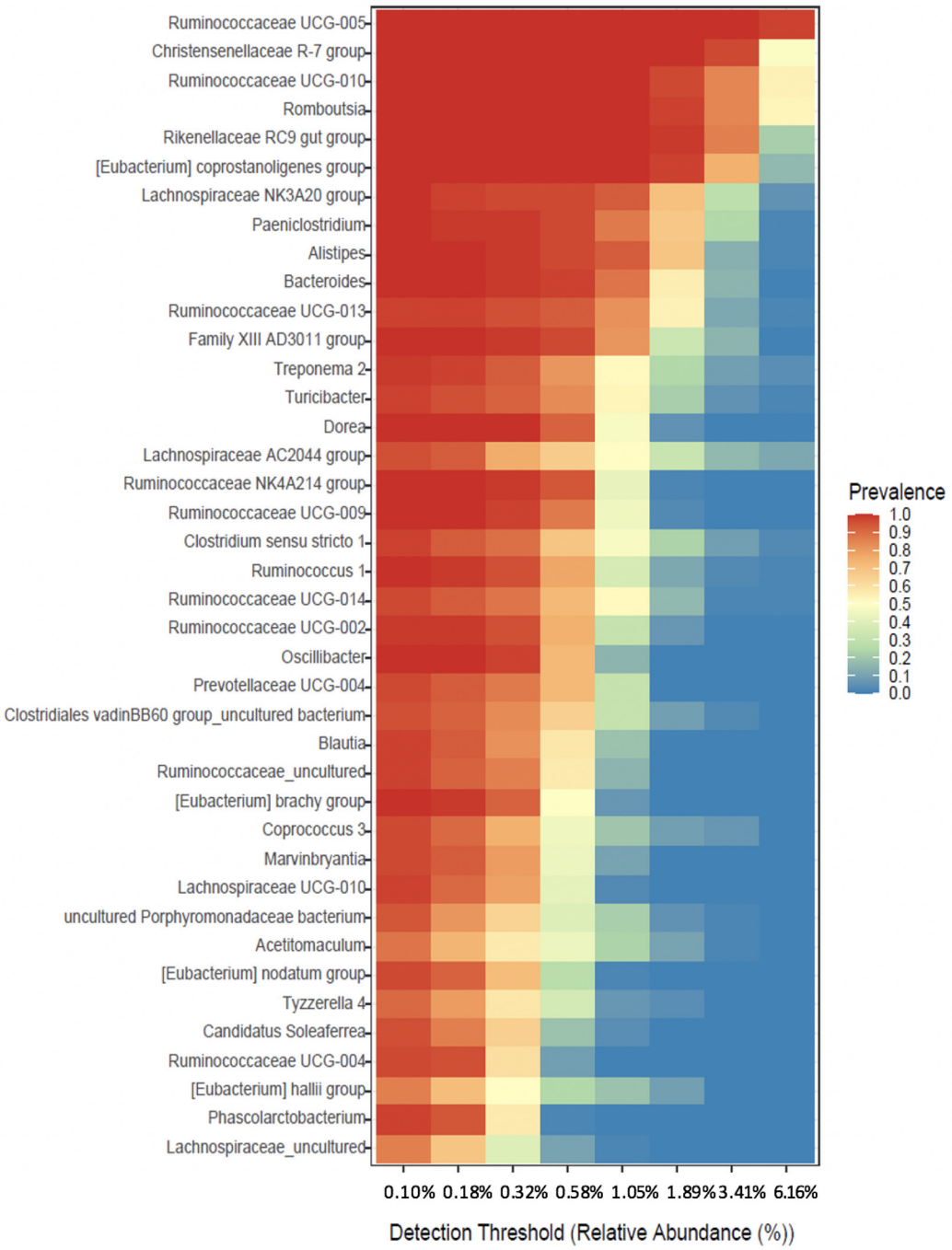

Figure 1. Heatmap showing the core microbiota composition of hindgut samples of nonlactating Holstein cows. The top 40 genera were identified based on prevalence with a detection threshold >0.1% across all samples (i.e., at the minimum detection threshold of 0.1% relative abundance, the highest prevalence was observed for *Ruminococcaceae* UCG-005, with at least 6% across samples).

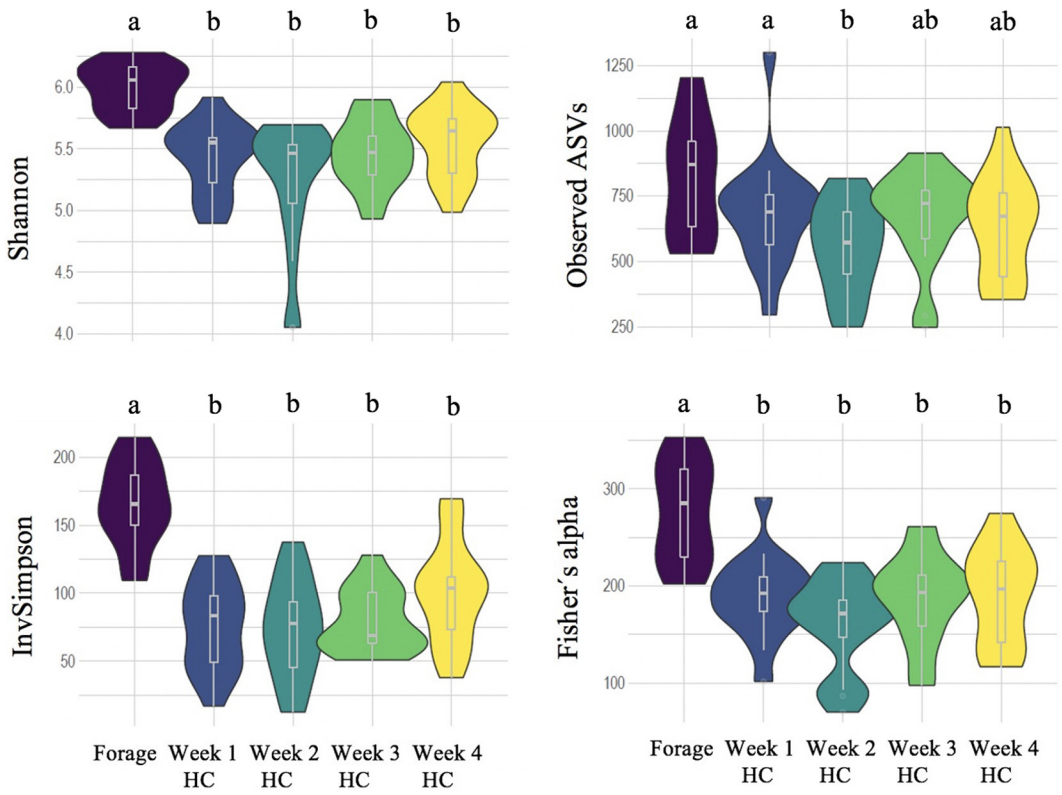

Figure 2. Alpha-diversity indices of the hindgut microbiome of nonlactating Holstein cows consuming forage and during 4 wk of high-concentrate feeding (HC, wk 1–4). Purple: week of forage diet; blue: wk 1 HC diet; turquoise: wk 2 HC diet; light green: wk 3 HC diet; yellow: wk 4 HC diet. Box limits indicate the range for 50% of the data, with the central line marking the median value. The upper and lower whiskers represent the 25% upper and 25% lower values, respectively. Within corresponding variable, means with different letters (a, b) indicate a statistically significant difference ($P < 0.05$).

Shannon, inverse Simpson, and Fishers' α were reduced from wk 1 on the HC diet until wk 4 on the HC diet ($P < 0.05$) indicating a loss of microbial diversity when the HC diet was introduced. However, the number of observed ASV recovered in wk 3 and 4 on the HC diet (Figure 2).

Beta-Diversity and Bacterial Network Analysis. The principal coordinate analysis plot using the weighted UniFrac distance showed separate clusters, with a differentiation between week of the forage diet, wk 1 of the HC diet, and the following weeks together (Figure 3a). Microbial network analysis strengthens the previous observations, with the week of forage feeding being clearly separated from the remaining weeks. Again, there was evident separation between wk 1 of HC feeding and the remaining weeks (Figure 3b).

PERMANOVA was used to investigate the effect of diet and week on HC diet on the microbial community structure on both the weighted UniFrac and Aitchison distances; Betadisper was also used. With the Aitchison distance, significant effects of diet ($P = 0.001$), $R^2 = 0.767$ was observed, although week of feeding ($P = 0.001$) had a low R^2 (0.057). Forage was always different from any week where the HC diet was fed; wk 1 of HC feeding had a stronger impact on the microbial communities than the remaining weeks. However, no differences were found among wk 2, 3, and 4 of HC feeding (Table 5).

Most influential ASV belonged to *Firmicutes* (Supplemental Table S3; see Notes), and were mainly uncultured species belonging to *Ruminococcaceae*, *Lachnospiraceae*, *Romboutsia*, *Paeniclostridium*, *Turicibacter*, and *Clostridium* sensu stricto 1, highlighting the importance of these microbes in the transition from a forage to an HC diet.

Microbial Enterotypes

Hindgut enterotyping (Figure 4) revealed the existence of 2 clusters separated based on diet. Cluster 2 includes samples taken only in week of forage feeding, whereas cluster 1 includes wk 1 to 4 (HC diet). Interestingly, no separation between the first week of HC and the remaining weeks is visible with this method, showing that even though major changes occurred in wk 1 from the microbial diversity perspective, there was no generation

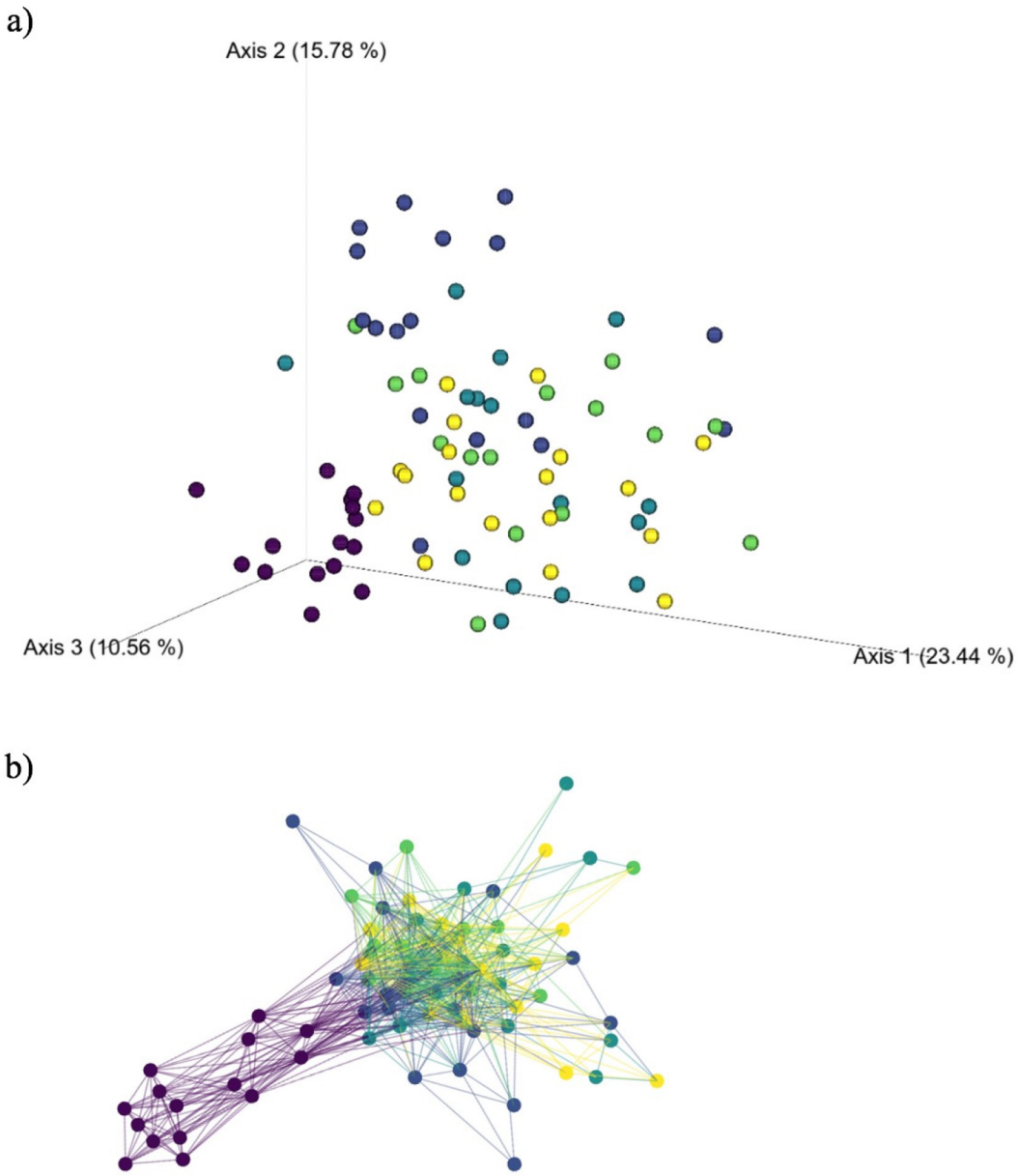

Figure 3. Weighted UniFrac Plot for the hindgut microbiome (a), and network analysis built for the hindgut microbiome in samples of nonlactating Holsteins cows (b). Purple: week of forage diet, blue: wk 1 of high-concentrate (HC) diet, turquoise: wk 2 HC diet, light green: wk 3 HC diet, and yellow: wk 4 HC diet.

of new enterotypes once cows were already consuming the HC diet.

Differential Abundance of Bacterial Genera Between Clusters

To identify differentially abundant genera between cluster 1 (HC feeding) and cluster 2 (forage diet), ANCOM-BC, MaAsLin2, and ALDEx2 were used (Table 6). Although ANCOM-BC identified 126 genera differing between cluster 1 and cluster 2, ALDEx2 identified 74, and MaAsLin2 identified 95 differential abundant bacterial genera. A total of 28 overlapping bacteria between these 3 methods were considered as being differentially abundant. For example, *Roseburia*, *Bacteroides*, and *Faecalibacterium* were more abundant in the forage diet; while *Bifidobacterium*, *Acetitomaculum*, and *Butyrivibrio* were more abundant in the HC diet.

Table 5. Summary of pairwise PERMANOVA test on weighted UniFrac and Aitchinson distances for the hindgut microbiome of nonlactating Holstein cows consuming forage or during 4 wk of high-concentrate (HC) feeding[1]

Pairwise comparison		Weighted UniFrac		Aitchinson	
Diet or week	Diet or week	Pseudo-F	Q-values	Pseudo-F	Q-values
Forage	1 on HC	14.639	***0.002**	41.338	***0.001**
Forage	2 on HC	12.509	***0.002**	53.356	***0.001**
Forage	3 on HC	12.796	***0.002**	44.323	***0.001**
Forage	4 on HC	14.076	***0.002**	52.101	***0.001**
1 on HC	2 on HC	3.935	***0.004**	12.555	***0.001**
1 on HC	3 on HC	3.228	***0.004**	14.899	***0.001**
1 on HC	4 on HC	4.641	***0.002**	13.595	***0.001**
2 on HC	3 on HC	1.104	0.369	0.997	0.348
2 on HC	4 on HC	1.398	0.174	0.520	0.600
3 on HC	4 on HC	0.655	0.778	0.442	0.646

[1]Asterisks indicate significant difference ($P < 0.01$). Metadata categories (week) with significant P-values and F-values >0.1 are shown in bold fonts.

Correlations Among the Microbiome, Hindgut Fermentation, and Nutrient Digestibility

The 3 main SCFA, as well as total SCFA, were positively correlated ($P < 0.05$) with *Bifidobacterium* and *Blautia*. However, these variables were negatively correlated with microbial α diversity indices and with *Oscillospira*, *Mailhella*, and *Akkermansia* ($P < 0.05$). Additionally, positive correlations were found between α diversity indices and hindgut pH. Furthermore, acetate, propionate, and butyrate were positively correlated with DM, CP, NFC, starch, and NDF digestibility ($P < 0.05$). Most of the products of hindgut fermentation including acetate, propionate, butyrate, valerate, and total SCFA were negatively correlated with hindgut pH (r = 0.75, $P < 0.05$; Supplemental Figure S2; see Notes).

DISCUSSION

The first hypothesis of this study was that the hindgut microbiome of dairy cows will adapt and remodel with prolonged duration on the HC diet. In agreement with the stated hypothesis, results show that the HC diet resulted in strong dysbiosis in the hindgut microbiota. However,

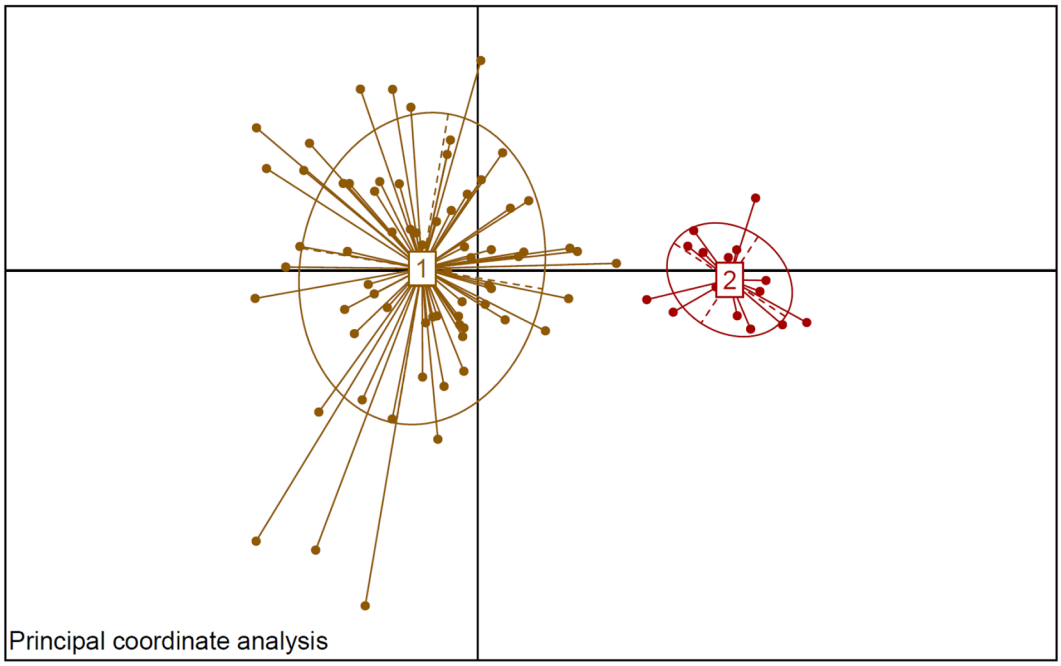

Figure 4. Principal coordinate analysis plot enterotyping of the hindgut microbiome of nonlactating Holstein cows in week of forage diet (cluster 2) and wk 1 to 4 of high-concentrate diet (cluster 1).

Table 6. Bacterial genera found to be differentially abundant between the hindgut microbial cluster of forage feeding and the cluster of high-concentrate (HC) feeding evaluated with different approaches in nonlactating Holstein cows

	Relative abundance (%)		Statistical approach and *P*-values[1]		
Item	Forage	HC	ALDEx2	ANCOM-BC	MaAsLin2
Genera enriched in forage diet					
Roseburia	0.924	0.171	0.001	0.005	0.001
Bacteroides	3.620	1.883	0.005	0.001	0.005
Dysgonomonadaceae uncultured	0.089	0.037	0.005	0.001	0.005
Oceanobacillus	0.072	0.001	0.005	0.001	0.001
Christensenellaceae uncultured	6.306	5.937	0.001	0.001	0.001
Mogibacterium	0.124	0.023	0.001	0.001	0.001
Faecalibacterium	0.074	0.021	0.005	0.001	0.001
Anaerosporobacter	0.249	0.082	0.001	0.001	0.001
Oscillospira	0.222	0.308	0.001	0.001	0.001
Ruminiclostridium	0.306	0.089	0.005	0.001	0.001
Victivallis	0.094	0.016	0.005	0.001	0.001
Mailhella	0.477	0.093	0.001	0.001	0.001
Akkermansia	2.102	0.348	0.001	0.001	0.001
Genera enriched in high-concentrate diet					
Bifidobacterium	0	0.378	0.001	0.001	0.001
Acetitomaculum	0.155	0.922	0.001	0.001	0.001
Blautia	0.275	0.823	0.001	0.001	0.001
Butyrivibrio	0.024	0.292	0.001	0.001	0.001
Eisenbergiella	0.009	0.049	0.001	0.001	0.001
Lachnospira	0	0.054	0.005	0.001	0.005
Marvinbryantia	0.270	0.623	0.001	0.001	0.001
Erysipelatoclostridium	0.014	0.057	0.005	0.001	0.001
Intestinibacter	0	0.150	0.001	0.001	0.001
Paeniclostridium	1.376	2.709	0.001	0.001	0.001
Romboutsia	4.732	6.820	0.001	0.005	0.005
Turicibacter	0.746	1.617	0.001	0.005	0.001
Negativibacillus	0.105	0.220	0.005	0.005	0.001
Oscillibacter	0.625	0.824	0.001	0.005	0.005
Pygmaiobacter	0.098	0.197	0.005	0.005	0.005

[1]Benjamini-Hochberg adjusted *P*-values.

after the second week of feeding the HC diet, this microbiome adapted, as indicated by the microbial α and β diversity indices.

More specifically, the α microbial diversity indices reached lowest values in wk 1 and 2 on HC, but most of these diversity indices did not show further changes in wk 3 and 4. This suggests that bacterial diversity, albeit affected at the start of HC feeding, eventually remodels. Additionally, the β diversity demonstrated 3 clusters consisting of forage feeding, wk 1 on the HC diet, and wk 2, 3, and 4 on HC. Therefore, the changes observed in the first week on the HC diet were particularly abrupt, while in the following weeks, the microbiota gradually adapted, without further changes in wk 3 and 4 on the HC diet. The hindgut microbiota has been shown to respond rapidly during transition to high-starch diet (Ricci et al., 2022). In this regard, the present study shows that this microbiome needs additional time after diet adaptation to fully adjust in terms of diversity.

The adaptation of the hindgut microbiome to prolonged HC feeding likely played a key role on nutrient digestibility, as shown by the increment in apparent total-tract nutrient digestibility with HC compared with forage feeding. This increase in nutrient digestibility may also be due to increased availability of nutrients for microbial degradation, resulting from greater content of readily available carbohydrates in the HC diet, which enhances the ME supply to the cow. In addition, the smaller particle size of the HC diets possibly resulted in greater surface area available for microbial enzymatic degradation (McAllister et al., 1994).

In this study, we also hypothesized that the in situ ruminal fiber degradation will be impaired by the HC diet, but apparent total-tract nutrient digestibility would not be negatively affected. In agreement with our hypothesis, the degradation of fiber from grass silage was impaired by HC in the rumen, but the apparent total-tract fiber digestibility increased with HC diet. Likely, the forage diet contained less digestible NDF than the HC diet due to greater inclusion of forages with lower NDF digestibility and lower inclusion of more digestible nonforage NDF. Understanding the kinetics of nutrient degradation in the rumen is very important because of its influence on the hindgut environment. For example, greater availabil-

ity of undegraded carbohydrates in the rumen increases the bypass of this nutrient to the lower gut, where it is fermented. In general, nutrient degradation in the rumen followed a similar pattern from other reports (Plaizier et al., 2001; Krajcarski-Hunt et al., 2002; Li et al., 2014), with lower degradation of fiber and greater degradation of readily fermentable carbohydrates. Additionally, it is relevant to highlight that a greater proportion of NDF intake in the forage diet originated from grass silage, which differs from NDF of the HC diet. This may have contributed to enhance NDF apparent total-tract digestibility, because the NDF from concentrates may be more digestible than NDF from forages due to small particle size and greater surface area in concentrates, facilitating microbial attachment and digestion (McAllister et al., 1994). In addition, pioneering research has demonstrated that NDF from grains have a higher potential for digestion than NDF from fibrous feed (Van Soest, 1982; Urias, 1986; Poore et al., 1990).

The increased total-tract starch digestibility suggests increased activity of amylolytic bacteria with HC diet, likely due to the lower gastrointestinal pH when feeding starch-rich rations, which allows starch digesting bacteria to thrive (Nagaraja and Titgemeyer, 2007). In addition, we found differences in the extent of starch degradation due to grain type in the rumen, which supports previous reports. For example, degradation of wheat grain was greater compared with the degradation of corn grain in the rumen, which agrees with previous reports (Xu et al., 2018), and this may be explained by the differences in the starch–protein matrix and starch granules in the wheat and corn endosperm mixture (Xu et al., 2018), factors that play an important role in the regulation of rumen fermentation (Patton et al., 2012). Therefore, findings from this study show that the rate of ruminal degradation, as well as the effective ruminal degradability of corn grain, is lower than degradation of wheat. Thus, when corn-based diets are fed to cattle, a greater proportion of starch may escape ruminal degradation and reach the hindgut, potentially affecting the hindgut environment. The results on ruminal degradation kinetics of grass silage and grains support other reports (Moody et al., 2007), where a HC diet tended to increase corn silage DM degradation rate and increased degradation rate of a 67% concentrate mix. These observations highlight the relevance of the dietary concentrate inclusion in the modulation of rumen degradability of substrates.

The greater hindgut fermentation observed in this study with HC diet lowered pH, stimulating proliferation of acidophilic bacteria, as demonstrated by the different enterotypes found between forage and HC diets. Interestingly, we also found a positive correlation between α diversity indices and hindgut pH, indicating that a less acidic hindgut pH translates to a more diverse ecosystem. Similar results were reported by other researchers (Mao et al., 2012; Plaizier et al., 2017a,b; Qiu et al., 2019) when feeding dairy rations ranging from 31.8% to 33.7% starch or beef cattle rations with 25.9% to 50.8% starch. Similarly, Mao et al. (2012) reported that most of the bacterial community was affected by diet, and they also reported increased abundance of *Turicibacter* with a high-grain diet. Thus, a less diverse hindgut microbiome with HC diet may reflect hindgut dysbiosis (Neubauer et al., 2020) due to greater starch availability in the hindgut compared with a fiber-rich environment (Mao et al., 2012). In this regard, ruminants' hindgut is normally equipped for fiber degradation (Hoover, 1978), as shown by our observations on most influential ASV with high abundance of *Ruminococcaceae*, known to include bacterial members that digest fiber (Russell, 2002).

Additionally, our results for the differentially abundant taxa between forage and HC diets indicate proliferation of amylolytic bacteria and decrease in fibrolytic bacteria. For example, *Roseburia* (abundant in the forage diet) is known to digest fibers (Nie et al., 2021) and starch (Aminov et al., 2006; Ramsay et al., 2006; Lin et al., 2021), whereas *Lachnospiraceae* (abundant in HC diet) is known to digest sugars (Russell, 2002). This implies that sugar degradation, rather than fiber, is enhanced by gut microbes following the HC group. Supporting our findings, Mao et al. (2012) reported increasing levels of *Lachnospiraceae* and lower levels of *Roseburia* during high-grain feeding. Furthermore, Zhang et al. (2022), reported increased *Lachnospiraceae* when feeding a 70% grain diet compared with a 40% grain diet. Altogether, these adjustments in the microbial community profile likely contributed to the increased apparent total-tract digestibility of nutrients. Our findings also suggest the key roles of certain microbial taxa on nutrient fermentation in the hindgut of cattle, as demonstrated by the positive correlations between major SCFA and the genera *Bifidobacterium* and with *Blautia*.

In general, the lower hindgut pH observed during the weeks of HC feeding agrees with the high levels of total SCFA, emphasizing the effect of this feeding regimen on SCFA buildup. Other researchers have also reported greater propionate with HC diets (Li et al., 2014), reflecting the increased starch degradation in the hindgut. It is worth noting that although hindgut pH decreased with the HC diet, daily average pH remained above 6.40; thus, more research is warranted to evaluate how further reductions in hindgut pH (i.e., hindgut acidosis) may influence nutrient degradation. In this regard, it is important to note that measurements of hindgut fermentation and fecal pH represent a less invasive option that should be further explored to evaluate gut acidification in dairy cattle when feeding HC diets.

Overall, this study contributes to improve the knowledge on nutrient utilization in cattle when feeding either forage or high-grain diet, coupled with understanding how the hindgut microbiota copes with duration on high-grain. In addition, results contribute to improve our understanding on the time needed by cattle to adapt to a diet change; specifically, despite the 1-week step wise diet transition, cows needed at least 3 additional weeks to adjust in terms of hindgut microbial diversity; this may be particularly important when designing and implementing studies that involve a diet change in cattle, such as change-over experimental designs.

CONCLUSIONS

Despite the initial decrease in microbial diversity, the hindgut microbiota of dairy cows adapted well to prolonged HC feeding. In wk 3 and 4 on HC, no further changes in microbial diversity were observed. The HC diet resulted in greater digestibility of nutrients in the total-tract, reflecting greater availability of nutrients for degradation, partly because the forage diet contained less digestible NDF than the HC diet. Results also showed that the extent of rumen degradation of starch-rich substrates differs due to grain type. However, apparent total-tract starch digestibility is not negatively affected, possibly due to lower gastrointestinal pH when feeding starch-rich rations, which allows starch digesting bacteria to thrive. Future research is warranted to evaluate whether and to what extent the decrease of ruminal fiber degradation affects the utilization efficiency of other nutrients in the rumen, and to evaluate how a further reduction in hindgut pH (hindgut acidosis) may influence the microbiota in the lower gut.

NOTES

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Nonstandard abbreviations used: ASV = amplicon sequence variants; EE = ether extract; HC = high-concentrate; SCFA = short-chain fatty acids.

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