Benefits of barley straw as a forage for dairy calves before and after weaning

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ABSTRACT

The aim of this study was to assess the potential consequences on calf intake, performance, behavior, ruminal microbiome, and ruminal epithelium development of combining the inclusion of chopped barley straw and alfalfa hay during the pre- and postweaning periods keeping concentrate to forage ratio constant among dietary treatments. Forty-five Holstein calves (44 ± 5.7 kg of body weight [BW] and 3 ± 1.5 d of age) individually penned were blocked by BW and randomly assigned to a common pellet concentrate fed ad libitum along with one of following forage feeding strategies: barley straw before and after weaning (S-S), barley straw before and alfalfa hay after weaning (S-A), or alfalfa hay before and after weaning (A-A). All calves received the same milk replacer regimen. Forage was supplied in a separated bucket at the rate of 7.5% (preweaning) and 15% (postweaning) of total solid feed intake of the previous day. Feed intake and BW were recorded daily and weekly, respectively. Rumen samples were obtained via a stomach tube at 53, 66, and 87 d and were composite in 3 samples of 5 animals each for subsequent rumen microbiome analysis. A rumen epithelium sample was taken by endoscopy at 90 d to assess gene expression of *OCLN*, *CLDN4*, *SLC9A1*, *SLC9A3*, *SLC16A1*, *SLC16A4*, *IL6*, and *TGFB1*. Data were analyzed with a mixed-effects model accounting for the fixed effects of block, forage, week of study, and their interaction, and calf as a random effect. The type of forage fed did not affect concentrate feed, forage, or total DM intake before weaning. However, S-A and A-A calves consumed less concentrate feed and S-A calves grew at a lower rate after weaning than S-S calves. Expression of the gene coding for *SLC16A1* in the rumen epithelium was greatest in S-S among treatments. Rumen microbiome did not differ among treatments, while the relative abundance of *Acidaminococcus* and *Selenomas* genera increased, while *Alloprevotella*, *Bifidobaterium*, *Olsenella*, and *Succiclasticum* genera decreased with age. In conclusion, feeding barley straw before and after weaning was more effective than feeding alfalfa hay in promoting concentrate feed intake after weaning and fostering an increase in the expression of *SLC16A1* in the rumen epithelium.

Key words: calves, forage, rumen epithelium

INTRODUCTION

Feeding calves forage during the preweaning period has been discouraged when calves are fed restricted amounts of milk (Drackley, 2008). Nevertheless, the use of concentrate as the only solid feed in preweaning calves has been questioned in the last decade (Khan et al., 2011; Khan et al., 2016; Suárez-Mena et al., 2016) because of the potential negative effects on rumen pH (Quigley et al., 1992; Beharka et al., 1998; Khan et al., 2011), and its association with parakeratosis and ruminal papillae agglomeration (Bull et al., 1965). Calf access to forages early in life promotes rumination, reduces non-nutritional oral behaviors (**NNOB**; Phillips, 2004; Castells et al., 2012; Mirzaei et al., 2017), stimulates the development of rumen wall (Tamate et al., 1962), reticulum-rumen anatomical growth (Khan et al., 2011), preserves ruminal epithelium health (Suárez et al., 2007), and its capacity to absorb nutrients (Hinders and Owen, 1965).

Among the forage sources studied for young calves, the use of either alfalfa hay or straw seems controversial. Several authors (Beiranvand et al., 2014; Pazoki et al., 2017; Mojahedi et al., 2018) reported an increase in concentrate feed intake and ADG during the preweaning period in comparison with calves that had no access to alfalfa hay. However, other authors did not observe benefits when feeding alfalfa hay (Jahani-Moghadam et al., 2015; Mirzaei et al., 2017; Mojahedi et al., 2018), and others report a decrease in concentrate feed intake,

ADG, or feed efficiency (Castells et al., 2012; Maktabi et al., 2016; Mirzaei et al., 2017; Movahedi et al., 2017). During the postweaning period, a meta-analysis (Imani et al., 2017) concluded that access to alfalfa hay increases concentrate feed intake compared with other types of forages. More recently, Mitchell and Heinrichs (2020) found that grass hay reduced DMI, ME intake, ADG, empty BW (**EBW**) gain, and tended to reduce final BW compared with corn silage and alfalfa haylage. Also, Castells et al. (2012) compared 6 forage sources under the same management conditions and reported that concentrate feed intake, total DMI, and ADG in the preweaning period increased when the chopped forage offered was oat hay, barley straw, or triticale silage in comparison with calves that had access to chopped rye-grass hay, alfalfa hay, corn silage, or did not have access to forage. In their work, and in several of the studies included in the meta-analysis by Imani et al. (2017), the different forage types were offered ad libitum and separately from the concentrate feed, and it was not possible to isolate the effect of forage source from the effect of forage level in the diet, as the proportion of forage in the final diet differed depending on forage type.

The potential consequences of forage provision on calves' performance, ruminal fermentation level or rumen epithelium macroscopic and microscopic changes have been widely studied (Pazoki et al., 2017). However, few studies address the effect of forage on the rumen epithelium (Castells et al., 2013), or the rumen microbiome (Beharka et al., 1998; Castells et al., 2013). Barley straw and alfalfa hay are the most popular forages evaluated in pre- and postweaning calves (Suárez et al., 2007; Castells et al., 2013); however, the use of the one or the other seems controversial. This study was designed to elucidate the role of barley straw and alfalfa hay during the pre- and postweaning periods in separate buckets, however, limiting the forage offer rate relative to concentrate feed intake. The objective of this study was to assess the potential consequences on calf intake, performance, behavior, ruminal microbiome, and ruminal epithelium development of combining the inclusion of chopped barley straw and alfalfa hay during the pre- and postweaning periods keeping concentrate to forage ratio in dairy calf diets among dietary treatments.

MATERIALS AND METHODS

Forty-five Holstein calves (44 ± 5.7 kg of BW and 3 ± 1.5 d of age) were purchased from a commercial farm and raised at the facilities of IRTA (Torre Marimon, Caldes de Montbui, Spain). In the commercial farm, calves received 3.5 L of colostrum (previously thawed) within the first 6 h of life. At IRTA facilities, calves were managed under the supervision of the IRTA Animal Care Committee (authorization code 9733). In the preweaning period (0 to 56 d of the study) calves were housed in individual pens (2.36 m^2) bedded with sawdust, and during the postweaning period (57 to 91 d of the study) they were moved to a greater (4.72 m^2) pen within the same barn, maintained calves individually separated and also bedded with sawdust.

Treatments and Feeds

Calves were distributed according to their BW in blocks of arrival date at the research facilities, and they were randomly assigned to an individual pen and into 1 of the 3 treatments (12 males and 3 females; n = 15): calves fed barley straw in pre- and postweaning periods (**S-S**); or barley straw during the preweaning and alfalfa hay during the postweaning period (**S-A**); or alfalfa hay in the pre- and postweaning periods (**A-A**). A power test determined a minimum of 10 and 12 calves per treatment, at 0.05 type I error and 80% power to detect 100 g/d of differences in feed intake with a standard deviation of 75 g/d and 250 g difference on BW with a standard deviation of 200 g among treatments, respectively. In the preweaning period, all calves were fed the same milk replacer (**MR**; Table 1) that was offered in nipple-bottles twice daily at 0800 and 1600 h. Calves received 4 L of MR at 12.5% of DM, 5 L at 12.5%, 6 L at 12.5%, and 6 L at 15.0% during 0 to 3, 4 to 7, 8 to 14, 15 to 49 d of the study, respectively. From 50 to 56 d of the study, calves received 3 L of MR at 15.0% of DM in the morning feeding and they were weaned at 57 d. Calves received a pellet concentrate feed offered ad libitum during the pre- and postweaning periods, however, the ingredient composition differed in each growing period (Table 1).

The amount of forage offered was adjusted every day to represent 7.5 and 15.0% of total solid feed intake on the previous day during the pre- and postweaning periods, respectively. Alfalfa hay and barley straw were chopped using a forage chopper machine (Seko SpA, Curtarolo, Italy). Forage was provided in a separate bucket from the concentrate, and the particle size distribution of forage offered (Table 1) and refused by each calf was determined every week using a 2-screen Penn State Particle Separator (Table 1; Lammers et al., 1996).

Measurement of Feed Intake and Performance

Milk replacer, concentrate feed, and forage intake were recorded daily on an individual basis. Body weight and hip height (**HH**) were measured weekly.

Table 1. Chemical composition of feeds and particle size of forage sources

Item	Milk replacer	Starter feed[1]	Grower feed[1]	Alfalfa hay	Barley straw
Chemical composition, % of DM					
DM	96.6	89.3	89.5	89.8	90.2
CP	24.8	17.9	17.1	18.3	3.3
Ether extract	19.1	3.2	3.2	ND[4]	ND
NDF	ND	15.0	22.9	42.6	80.3
ADF	ND	5.4	13.1	35.2	54.6
Ash	7.5	5.9	6.3	10.8	6.9
ME,[2] Mcal/kg of DM	4.7	3.2	3.1	2.2	1.8
Particle size distribution,[3] % of DM					
Long, >20 mm	ND	0.0	0.0	18.6	69.5
Medium, 8–20 mm	ND	0.0	0.0	21.2	7.3
Short, <8 mm	ND	100.0	100.0	59.5	20.5

[1]From 1 to 56 d of the study calves received starter feed, and from 57 to 91 d grower feed as a concentrate fed.

[2]Metabolizable energy was calculated from NRC (2001).

[3]Particles separated using a Penn State Particle Separator into 3 fractions: long (>20 mm), medium (8− 20 mm), and short (<8 mm).

[4]ND: not determined.

Feed efficiency was calculated weekly by dividing DMI (milk, concentrate, and forage) by total BW gained in that week. Average daily weight gain was calculated considering full calf BW and EBW to calculate EBW daily gain (**EBWg**). The latter was calculated as BW − (BW × gut fill), where gut fill (**GF**; as % of BW) was estimated according to Jahn and Chandler (1976) where GF = 10.4 − [0.39 × CP % from solid feeds] + [0.41 × ADF % from solid feeds].

Feeding Pattern

During one day, on wk 7, 9, and 13 of the study, the feeding rate of forage and concentrate fed were recorded hourly from 0500 to 2100 h on all calves. For forage sorting activity, 6 calves per treatment were randomly chosen according to a power test at 0.05 type I error and a standard deviation of 0.4 g/d to detect a 20% difference among the 3 treatments with greater than 80% power. In these individual calves, daily forage offers and refusals were composited weekly from the 6 to 13 weeks of study, and a 2-screen Penn State Particle Separator was used to determine the proportion of the different forage fractions. To determine sorting activity, DMI of each forage fraction was calculated as the difference between the amount of each forage fraction offered and the amount refused (actual intake). Sorting was calculated as the actual intake of each fraction expressed as a proportion of the theoretically predicted intake of that fraction. Values equal to 1 indicate no sorting, those <1 indicate selective refusals (sorting against), and those >1 indicate preferential consumption (sorting for).

Behavior was monitored by direct observation of 10 animals per treatment for one day on wk 7, 9, and 13 of the study. A power test determined a minimum of 9 calves per treatment at 0.05 type I error and a standard deviation of 1% to detect a 5% difference in rumination relative frequency among treatments with an 80% power. Calves were observed during 4 consecutive hours from the morning solid feed offer using instantaneous scans at 5-min intervals. In total, 12-h observations per calf were recorded split in 3 different weeks. The occurrence of the following behavior events was also recorded: lying (defined as resting with no chewing activity or nonnutritive oral behaviors), standing (defined as erect no chewing activity or nonnutritive oral behaviors), eating concentrate feed (defined as the calf mouth lowered into the concentrate bucket), eating forage (defined as the calf mouth lowered into the forage bucket), ruminating (either lying or standing), and NNOB (when the calf licked any surface or itself, rolled the tongue, or consumed wood shavings).

Ruminal Development

Samples of ruminal content were obtained from each calf on wk 7, 9, and 13 (135 samples in total) using an esophageal tube and a vacuum pump. The pH of the rumen fluid was immediately measured with an electronic pH meter (Crison pH25, Barcelona, Spain), and a 50-mL subsample was immediately frozen at −80°C for subsequent bacteria population analyses. Furthermore, biopsies of ruminal epithelium were obtained by endoscopy, from 5 randomly selected calves in each treatment about 2 h from the morning feeding at 90 d of the experiment to determine the expression of genes coding for monocarboxylate transporter 1 and 4 (*SLC16A1* and *SLC16A4*), sodium/proton exchanger 1 and 3 (*SLC9A1* and *SLC9A3*) transforming growth

factor-β1 (TGFB1), tight junction barrier (*OCLN* and claudins), and proinflammatory cytokines (IL6). The biopsy was done using a CV-170 Optera, Olympus endoscope (Barcelona, Spain) equipped with a 1.68-m probe (CF-Q165L, Olympus) and biopsy disposable fenestrated forceps (EndoJaw FB-214U; Olympus Medical Systems Corp., Tokyo, Japan) according to the procedure described by Bach et al. (2017). About 30 mg of rumen epithelium (capturing at least an entire papilla) from the cranial-dorsal sac was obtained, rinsed by immersion in PBS for about 3 to 5 s, and immediately placed in RNAlater (Invitrogen, Madrid, Spain) for 12 h at 4°C. Then, RNAlater was discarded and the samples were frozen at −80°C until further processing.

DNA Extraction, 16S rRNA Libraries Preparation, and DNA Sequencing

Samples of ruminal content were thawed and centrifuged at $6,600 \times g$ at 4°C for 15 min. The supernatant was discarded, the pellet homogenized, and 0.20 g were weighed to make 3 composite samples (pooling 5 animals per composite) per treatment for each sampling week. From each composite sample, DNA was extracted by bead-beating in the presence of high concentrations of sodium dodecyl sulfate, salt, and EDTA, and with subsequent DNA purification by QIAmp DNA Stool Mini Kit columns (Qiagen, Hilden, Germany; Yu and Morrison, 2004). The DNA quality was assessed by measuring the 260 nm/280 nm and the 260/230 nm ratios of absorbance. A DNA sample was considered pure if the A260/A280 ratio was within the range of 1.8 to 2.0 and the A260/A230 ratio was within the range of 2.0 to 2.2. The minimum concentration of DNA required for sequencing libraries was 20 ng/μL. All DNA extracts were stored at −20°C and shipped to M_r DNA (Shallowater, Texas) for 16S rRNA gene amplification and sequencing on an Illumina Miseq platform. The 16S rRNA gene V4 variable region PCR primers 515/806 with barcode on the forward primer were used in a 30-cycle PCR using the HotStarTaq Master Mix kit (Qiagen) under the following conditions: 94°C for 3 min, followed by 30–35 cycles of 94°C for 30 s, 53°C for 40 s and 72°C for 1 min, after which a final elongation step at 72°C for 5 min was performed. The resulting PCR products from each sample were visualized by electrophoresis in 2% agarose gels and mixed in equal concentrations of DNA for 16S rRNA gene library preparation. Pooled samples were purified using calibrated AMPure XP beads (Agencourt Bioscience Corporation, Beverly, MA) and paired-end sequence (2x300) on an Illumina Miseq platform following the manufacturer's guidelines.

Sequencing Data Analysis

Sequence data were processed using a proprietary analysis pipeline (MR DNA, Shallowater, TX). In summary, sequences were joined, depleted of barcodes then sequences <150 bp and those with ambiguous base calls were removed. Sequences were denoized, OTU (defined by clustering at 97% similarity) generated, and chimeras removed. Taxonomical classifications of OTU were conducted using Bayesian Classifier RDP, keeping those matches with 80% similarity, and removing singleton OTU.

Rumen Gene Expression

A total reaction volume of 20 μL was used, containing 100 ng of cDNA, 10 μL of SYBR Green (Bio-Rad Laboratories), and the optimized primer concentration for each gene (Table 2). The PCR reactions were conducted using a thermocycler iCycler (Bio-Rad) and cycled as follows: an initial denaturing step of 10 min at 95°C, followed by 40 cycles of 10 s at 95°C, 15 s at optimized annealing temperature for each gene, 30 s at 72°C, and a final extension of 5 min at 72°C. Gene expression values were evaluated using the delta cycle threshold ($\Delta\mathbf{Ct}$) method with β-actin as the housekeeping gene.

Chemical Analyses

Samples of MR, concentrate, and forage were analyzed for DM (method 934.01), ash (method 942.05), CP (N × 6.25; method 990.03), and ether extract (method 920.39) content following AOAC (1990). Neutral detergent fiber was analyzed with sodium sulfite and heat-stable α-amylase (Van Soest et al., 1991), and ADF following the AOAC (1990) method (method 973.18). The ME of each feed was calculated using NRC (2001) equations.

Statistical Analyses

All data were analyzed using SAS software (SAS 9.4 University Edition, SAS Inst. Inc., Cary, NC) unless otherwise specified. Data were analyzed separately for the pre- (from wk 1–8 of the study) and the postweaning periods (from wk 9–13 of the study). Before analyses, all data were screened for normality using the UNIVARIATE procedure of SAS.

Intake of each feed (milk, concentrate, and forage), nutrient intake (DM, CP, NDF, and ME), sorting of forage particle size fractions (long, medium, and short), rate of solid feed consumption (both for concentrate and forage), and performance data (BW, ADG, EBWg,

Table 2. Gene name, sequence (F = forward, R = reverse), annealing temperature (AT), primer concentration (Con.), amplicon size (Amp.), and efficiency (Eff.) of the primers used

Gene	Forward and reverse primer	AT, °C	Con., µM	Amp., bp	Eff., %
SLC16A1	F: CAATGCCACCAGCAGTTG; R: GCAAGCCCAAGACCTCCAAT	50	0.500	375	1.82
SLC16A4	F: AGCGTCTGAGCCCAGGGAGG R: ACCTCGCGGCTTGGCTTCAC	55	0.500	223	1.9
SLC9A1	F: GAAAGACAAGCTCAACCGGTTT R: GGAGCGCTCACCGGCTAT	60	0.500	66	1.9
SLC9A3	F: AGCCTTCGTGCTCCTGACA R: TGACCCCTATGGCCCTGTAC	60	0.500	55	1.9
OCLN	F: ATCAACCCCGGTGCCGGAAG R: GTGGTCTTGCTCTGCCCGCC	57	0.500	162	1.82
CLDN4	F: CATGATCGTGGCCGGCGTG R: AGGGCTTGTCGTTGCGGG	62	0.125	226	1.82
TGFB1	F: TGAGCCAGAGGCGGACTACT R: TGCCGTATTCCACCATTAGCA	60	0.500	61	1.91
IL6	F: GGCGGAGCCTTGCGTTAT R: AACTGCTGTGCTTGCTTCAT	51.5	0.500	117	1.86

HH, HH daily gain, and feed efficiency) were analyzed using a mixed-effects model considering the fixed effect of the block (day of arrival), treatment, week of study (from wk 1–8 or 9–13 of the study), and the interaction between treatment and week, plus the random effect of the calf. Week of study entered the model as a repeated measures using the autoregressive order-1 variance–covariance structure. The initial values of BW were considered covariates for the analysis of BW, ADG, EBWg, and feed efficiency, whereas initial HH values were considered covariates for the analysis of HH and HH gain. The rate of solid feed consumption was analyzed considering hour as a repeated measure and separately for each measurement week (i.e., 7, 9,

Table 3. Feed intake of dairy calves supplemented with barley straw during the pre- and postweaning periods (S-S), barley straw during the pre- and alfalfa hay during postweaning periods (S-A), or alfalfa hay during the pre- and postweaning periods (A-A)

	Treatment				P-value[1]		
Item	S-S	S-A	A-A	SEM	T	W	T × W
Milk, kg DM/d							
Preweaning, 1–8 wk	0.753	0.747	0.753	0.0026	0.124	<0.01	0.421
Concentrate feed, kg DM/d							
Preweaning, 1–8 wk	0.463	0.448	0.392	0.0507	0.581	<0.01	0.148
Postweaning, 9–13 wk	3.417[a]	2.843[b]	2.803[b]	0.162	0.017	<0.01	0.089
Forage, kg DM/d							
Preweaning, 1–8 wk	0.030	0.026	0.020	0.0046	0.293	<0.01	0.234
Postweaning, 9–13 wk	0.201	0.221	0.183	0.0271	0.605	<0.01	<0.01
Forage,[2] %							
Preweaning, 1–8 wk	7.01	6.69	6.60	0.857	0.940	0.013	0.649
Postweaning, 9–13 wk	5.08	6.63	6.38	1.029	0.885	0.011	0.613
Total DM intake, kg/d							
Preweaning, 1–8 wk	1.246	1.221	1.165	0.0543	0.555	<0.01	0.113
Postweaning, 9–13 wk	3.617[a]	3.065[b]	2.986[b]	0.271	0.035	<0.01	0.235
CP intake, kg/d							
Preweaning, 1–8 wk	0.271	0.267	0.261	0.0091	0.746	<0.01	0.313
Postweaning, 9–13 wk	0.592	0.526	0.513	0.0306	0.159	<0.01	0.221
NDF intake, kg/d							
Preweaning, 1–8 wk	0.093	0.088	0.066	0.0107	0.177	<0.01	<0.01
Postweaning, 9–13 wk	0.940[a]	0.746[b]	0.720[b]	0.0470	<0.01	<0.01	0.489
ME,[3] Mcal/d							
Preweaning, 1–8 wk	5.1	5.0	4.9	0.143	0.622	<0.01	0.835
Postweaning, 9–13 wk	11.0[a]	9.3[b]	9.1[b]	0.540	0.036	<0.01	0.178

[a,b]Means within a row with uncommon superscripts differ ($P < 0.05$).

[1]T = effect of treatment; W = effect of week; T × W = interaction between treatment and week.

[2]Forage % = forage as a proportion of the sum of concentrate and forage consumed.

[3]Calculated from NRC (2001).

and 13 wk of the study). Ruminal pH data from wk 7, 9, and 13 of the study were analyzed as repeated measurements adding visual saliva contamination as a Boolean random effect in the model. The frequency of performing each behavior during the time of observation (total fourth hours) in each week was calculated individually and analyzed as a repeated measurement over time using a mixed-effects model. The model considered the fixed effect of treatment, week (7, 9, and 13 wk of the study), and their 2-way interaction, plus the random effect of calf. Week of study entered the model as a repeated measure using the autoregressive order-1 variance–covariance structure.

The potential effect of treatments on gene expression in the ruminal epithelium was analyzed using an ANOVA.

Rumen microbial β diversity analysis was performed with MicrobiomeAnalyst software (Dharawal et al., 2017) using SILVA taxonomy. The OTU table was rarefied to the minimum library size and scaled with the total sum scaling method, only for those OTU detected in at least 10% of the samples. Bray-Curtis index distances were used for the principal coordinate analysis plots performed either for the combination of forages or the week of sampling and analyzed using one-way analysis of similarity. Differences in the composition of bacterial communities were analyzed using the proportion of each OTU assignment within the pooled sample with a mixed-effects logistic regression analysis considering the pool of calf samples as a random effect, and treatment, week of sampling, and their interaction as fixed effects with a false discovery rate correction for multiple hypotheses testing.

To assess the effect of forage source in diversity and richness within forage groups, α diversity indexes (Shannon, the observed number of OTU and Chao) were calculated using MOTHUR and analyzed with a mixed-effects model for repeated measures considering the fixed effects of treatment, week of study (i.e., 7, 9, and 13 wk of the study), and their 2-way interaction, plus the random effect of the pool of calf samples.

Mean separation was conducted with a Tukey's test. Significant differences were declared at $P < 0.05$, and trends were discussed at $0.05 \leq P \leq 0.10$.

RESULTS AND DISCUSSION

Feed Intake and Performance

Both the absolute amount of forage consumed and its proportion of the TS intake before weaning (25 g of DM/d and 6.8%, respectively), and the forage proportion of the TS intake after weaning (6.3%) were similar among treatments (Table 3). During the preweaning
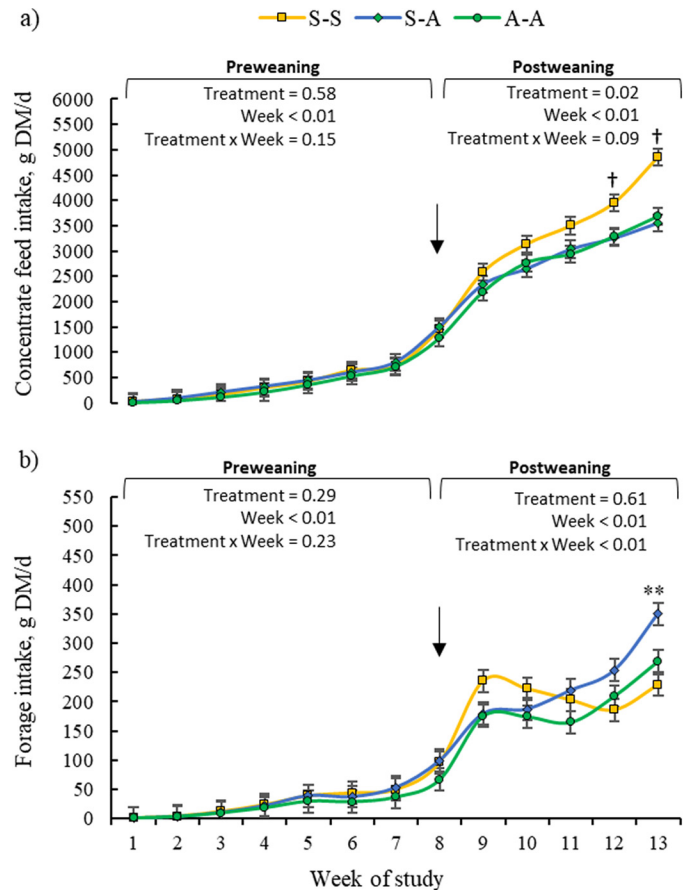

Figure 1. Evolution (from wk 1 to 13 of age) of concentrate feed (a) and forage (b) intake (g of DM/d) of dairy calves supplemented with barley straw before and after weaning (S-S), barley straw before and alfalfa hay after weaning (S-A), or alfalfa hay before and after weaning (A-A). † Denotes tendency ($P < 0.1$) between S-S versus S-A and A-A. ** Denotes differences ($P < 0.01$) between S-A and S-S. Arrow indicates weaning. Error bars indicate SEM for each time point.

period, no differences among treatments were observed for intake of MR, concentrate, total DM, CP, NDF, or ME (Table 3). Thus, it seems that the proportion of forage in the diet could be one of the main factors explaining differences reported in previous studies during the preweaning period (Suárez et al., 2007; Castells et al., 2013; Imani et al., 2017). After weaning, calves that had access to alfalfa hay consumed less ($P < 0.05$) concentrate and total solid feed than calves that had access to straw (Table 3). Concentrate feed intake in S-S calves tended ($P = 0.09$) to be greater than in S-A and A-A calves during wk 12 and 13 of the study, and forage intake was greater ($P < 0.01$) in S-A than in S-S calves on wk 13 (Figure 1). Changes in nutrient intake also differed (Table 3) and followed the same trend as concentrate and forage feed intake (Figure 1). These results indicated that straw provision was more beneficial than the provision of alfalfa hay during the post-

Table 4. Performance of dairy calves supplemented with barley straw during the pre- and postweaning periods (S-S), barley straw during the pre- and alfalfa hay during postweaning period (S-A), or alfalfa hay during the pre- and postweaning periods (A-A)

Item	Treatment				P-value[1]		
	S-S	S-A	A-A	SEM	T	W	T × W
BW, kg							
Initial BW, kg	45.7	44.2	42.7	1.48	0.362	—	—
Weaning BW, kg	88.7	88.9	86.5	1.32	0.391	—	—
Final BW, kg	134.7	128.0	129.1	2.96	0.233	—	—
Preweaning, 1–8wk	64.0	63.7	63.3	0.85	0.843	<0.01	0.502
Postweaning, 9–13 wk	117.0	111.0	111.4	2.75	0.190	<0.01	0.326
ADG, g/d							
Preweaning, 1–8 wk	812	782	756	33.9	0.548	<0.01	0.400
Postweaning, 9–13 wk	1,310[x]	1,102[y]	1,219[xy]	72.8	0.097	0.051	0.728
EBWg, g/d[2]							
Preweaning, 1–8 wk	646	622	597	30.3	0.590	<0.01	0.345
Postweaning, 9–13 wk	1,175[x]	988[y]	1,094[xy]	65.3	0.097	0.051	0.726
Hip height, cm							
Preweaning, 1–8 wk	88.3	88.5	87.8	0.68	0.766	<0.01	0.829
Postweaning, 9–13 wk	98.9	98.4	97.9	0.76	0.710	<0.01	0.062
Hip height gain, cm/d							
Preweaning, 1–8 wk	0.190	0.193	0.190	0.0209	0.989	0.477	0.781
Postweaning, 9–13 wk	0.229	0.218	0.203	0.0240	0.765	0.238	0.017
Feed efficiency, kg BW/ kg DM							
Preweaning, 1–8 wk	0.65	0.62	0.64	0.025	0.722	<0.01	0.195
Postweaning, 9–13 wk	0.37	0.36	0.40	0.014	0.116	<0.01	0.681

[x,y]Means within a row with uncommon superscripts tend to differ at $P < 0.10$.

[1]T = effect of treatment; W = effect of week; T × W = interaction between treatment and week.

[2]EBWg = average daily weight gain corrected by gut fill according to Jahn and Chandler (1976).

weaning period, and they are consistent with previous studies that report lower concentrate feed intake and ADG of calves around 8 to 10 wk of age, when alfalfa hay was fed instead of wheat straw (Movahedi et al., 2017), barley straw, oat hay, and triticale silage (Castells et al., 2012), or when alfalfa haylage was compared against grass hay (Mitchell et al., 2020). The rate of body growth (ADG and HH gain) during the preweaning period did not differ among treatments, but ADG (either considering or not GF) tended ($P = 0.097$) to

be greater in S-S compared with S-A calves after weaning. Body weight at weaning and final BW did not differ among the forage feeding programs (Table 4). Differences in ADG in calves that had access to forage have been, on some occasions, attributed to the longer retention time of forages in the gastrointestinal tract (Castells et al., 2013; van Gastelen et al., 2021) that, in some cases, may increase GF (Jahn and Chandler, 1976; Hill et al., 2008; Imani et al., 2017). Because calves that had access to straw after weaning tended

Table 5. Proportion of time devoted to performing different behaviors for the first 4 h after the morning feeding of dairy calves supplemented with barley straw during the pre- and postweaning periods (S-S), barley straw during the pre- and alfalfa hay during postweaning period (S-A), or alfalfa hay during the pre- and postweaning periods (A-A)

Item	Treatment				P-value[1]		
	S-S	S-A	A-A	SEM	T	W	T × W
Ruminating	0.132[x]	0.096[xy]	0.080[y]	0.0148	0.054	0.056	0.887
Eating concentrate	0.064	0.057	0.051	0.0074	0.497	0.180	0.629
Eating forage	0.053	0.074	0.049	0.0101	0.191	0.444	0.673
Lying	0.403[b]	0.450[b]	0.550[a]	0.0212	<0.001	0.447	0.645
Standing	0.233[x]	0.204[xy]	0.170[y]	0.0174	0.058	0.044	0.081
Drinking water	0.032[a]	0.030[a]	0.018[b]	0.0056	0.024	0.098	0.250
NNOB[2]	0.084	0.090	0.077	0.0157	0.854	0.766	0.094

[a,b]Means within a row with uncommon superscripts differ ($P < 0.05$).

[x,y]Means within a row with uncommon superscripts tend to differ at $P < 0.10$.

[1]T = effect of treatment; W = effect of week; T × W = interaction between treatment and week.

[2]NNOB: non-nutritive oral behavior (when the animal licked any surface, tongue rolled, or consumed wood shavings).
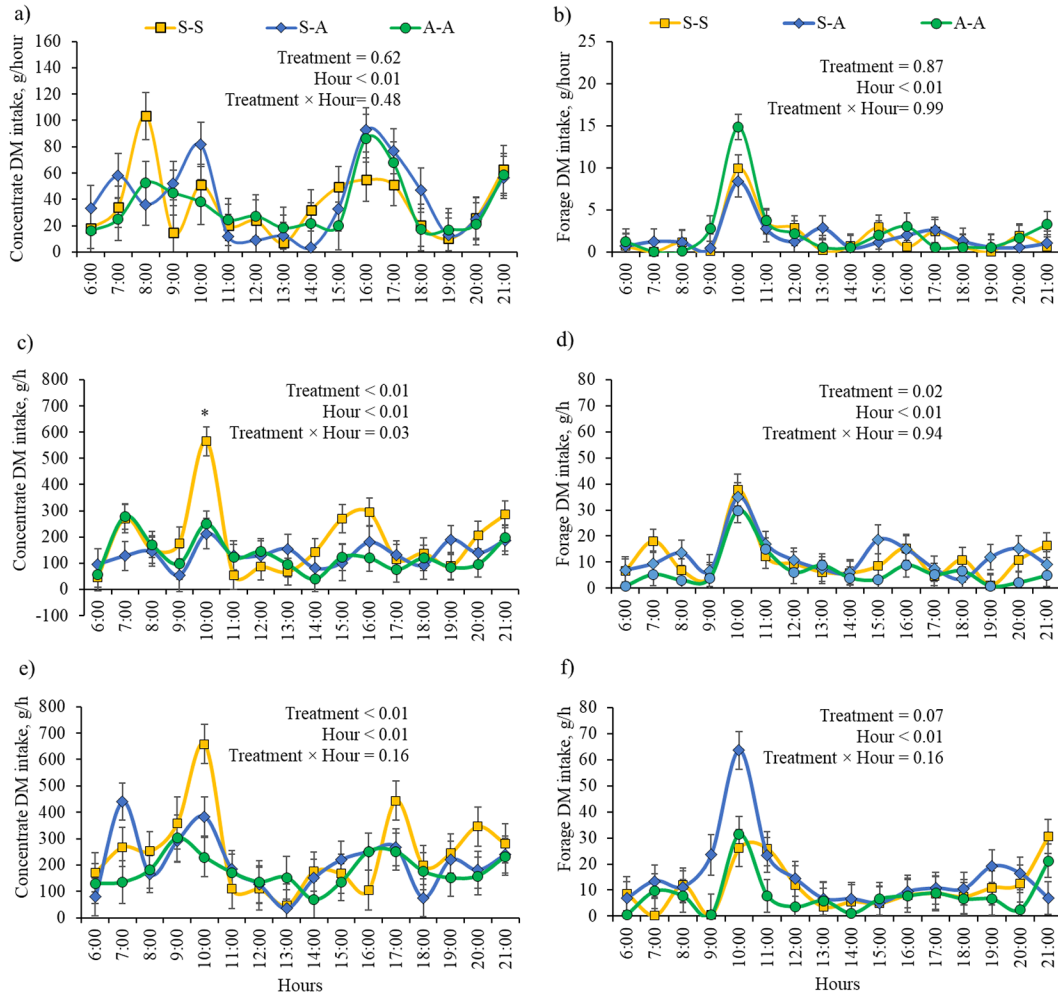
Figure 2. Intake rate of concentrate feed and forage on wk 7 (a and b, respectively), 9 (c and d, respectively), and 13 (e and f, respectively) of study. Calves on S-S treatment received chop barley straw before and after weaning; S-A calves received barley straw before weaning and alfalfa hay after weaning; A-A calves received alfalfa hay before and after weaning. Error bars indicate SEM for each time point. *Denotes differences ($P < 0.05$) between S-A and A-A versus S-S.

to have a greater ADG corrected for GF, we attribute differences in growth performance herein to greater ME and CP intakes rather than to an increased GF.

Feed Intake Pattern and Behavior

Before weaning (wk 7), forage and concentrate feed intake rates and their daily evolution throughout time did not show differences among the 3 feeding strategies (Figures 2a and b). During wk 7, the rate of concentrate feed intake had 3 peaks around the morning (0800 h) and evening (1600 h) milk feedings, and another at 2100 h (Figure 2a). However, the rate of forage intake was greatest ($P < 0.01$) 2 h after the morning milk feeding in all treatments (Figure 2b). At wk 9 (postweaning), the rate of concentrate feed intake was greater ($P < 0.01$) in S-S calves than in A-A, and tended

($P = 0.06$) to be greater than S-A ($P = 0.06$), and these differences were mainly attributed to the greater feed intake rate 2 h after the morning offer of solid feed (Figure 2c). However, the rate of forage intake was greater ($P < 0.01$) in S-A than in A-A calves, but the rate of forage intake followed a similar pattern among treatments throughout the measured hours (Figure 2d). At wk 13, either rate of concentrate feed or forage intake followed a similar pattern among feeding strategies (Figure 2e and 2f). However, S-A calves tended ($P = 0.07$) to have a greater rate of forage intake than A-A and S-S calves (Figure 2f) and the rate of concentrate feed intake was greater ($P < 0.01$) in S-S than in S-A and A-A calves (Figure 2e). In the literature, Montoro et al. (2012) also observed 2 marked peaks of concentrate feed intake as observed in the present study (Figure 2). Hourly differences in concentrate feed intake between calves fed

Table 6. Ruminal pH and relative mRNA expression of selected genes in the rumen epithelium of dairy calves supplemented with barley straw during the pre- and postweaning periods (S-S), barley straw during the pre- and alfalfa hay during postweaning periods (S-A), or alfalfa hay during the pre- and postweaning periods (A-A)

	Treatment				P-value[1]		
Item	S-S	S-A	A-A	SEM	T	W	T × W
Rumen pH[2]	5.91	5.86	5.73	0.101	0.400	0.025	0.751
Expression of selected genes[3] at wk 13 of study							
SLC16A1	1.028[a]	0.537[b]	0.636[b]	0.1116	0.021	—	—
SLC16A4	0.912	0.939	1.090	0.1324	0.494	—	—
SLC9A1	1.010	0.673	0.951	0.1419	0.239	—	—
SLC9A3	1.074	0.600	0.806	0.2011	0.286	—	—
OCLN	1.028	0.726	0.832	0.1552	0.406	—	—
CLDN4	0.997	0.996	0.873	0.1716	0.844	—	—
TGFB1	0.992	1.161	1.588	0.2775	0.173	—	—
IL6	1.098	1.115	1.530	0.2675	0.403	—	—

[a,b]Means within a row with uncommon superscripts tend to differ at $P < 0.10$.

[1]T = effect of treatment; W = effect of week; T × W = interaction between treatment and week.

[2]Rumen pH derived including the effect of visual saliva contamination as a random factor in the statistical analysis.

[3]MCT1: Monocarboxylate transporter 1; MCT4: Monocarboxylate transporter 4; NHE1: Sodium/proton exchanger 1; NHE3: Sodium/proton exchanger 3; OCLN: Occludin; CLDN4: Claudin-4; TGFB1: Transforming growth factor-β; IL6: Interleukin-6.

straw or alfalfa hay occurred during these 2 main peaks of feed intake. This might suggest that satiety signals, induced by feed consumption, may have taken place later in calves fed straw than in those fed alfalfa hay, allowing the former to consume more feed during these preferred eating periods.

Both before and after weaning, calves sorted for the fraction containing long-size forage particles without differences among treatments and sorted against the short-size forage fraction. The short-size fraction was refused the most ($P < 0.05$) by S-S calves after weaning (Supplemental Table S1; https://doi.org/10.34810/data789; Antúnez Tort and Terré Trullà, 2023). Before weaning, calves on treatments S-S and S-A sorted ($P < 0.01$) against the medium-size forage fraction, but A-A calves sorted for the medium-size forage fraction. After weaning, calves in all treatments sorted for the long and medium-size forage fraction, independently of treatment. In our study, calves' rumen pH was below 6 (see in rumen parameters subsection), and this may have influenced their long particle choice as occurred in dairy cows that were challenged to decrease rumen pH from 6.02 to 5.77 and they increased the preference for long in contrast to a short-forage-particle-size diet (Maulfair et al., 2013).

The behavior of calves during the morning feeding evolved in a different ($P < 0.05$) manner across treatments throughout the 3 observation weeks (Table 5). Calves in the S-S treatment tended ($P = 0.054$) to ruminate more than A-A calves throughout the study. In general, calves in A-A treatment spent more time lying

($P < 0.01$) than calves in S-S and S-A treatments (Table 5). Calves in S-S and S-A treatments spent more time drinking water ($P = 0.02$) than A-A calves throughout the 3 observation weeks. Lastly, no difference among treatments was detected in the frequency of performing NNOB (Table 5). The reason for the increase in concentrate feed intake may be related to changes in the proportion of time spent ruminating, and differences in forage particle size between alfalfa and straw-fed calves. More rumination and longer particle size in straw-fed calves may have influenced ruminal pH and ultimately, concentrate feed intake pattern, allowing them to increase concentrate feed intake at peak hours.

Rumen Parameters

Expression of *SLC16A1* in the rumen epithelium was greater ($P < 0.05$) in S-S than in A-A and S-A calves, but no differences in the expression of genes that encode to short-chain fatty acids (**SCFA**) transporters, remodeling of the ruminal epithelium or inflammatory were found (Table 6). Ruminal pH did not differ across wk 7, 9, and 13 of the study among treatments (Table 6). However, ruminal pH was greater ($P < 0.05$) on wk 13 than on wk 7 and 9 of the study (6.01 vs. 5.74 and 5.76 ± 0.088, respectively). The cotransporter of SCFA and protons, *SLC16A1* contribute to removing protons from the rumen lumen to the bloodstream (Graham et al., 2007; Aschenbach et al., 2011; Yohe et al., 2019). Previously, some studies reported an increase in the expression of *SLC16A1* in the rumen of calves fed
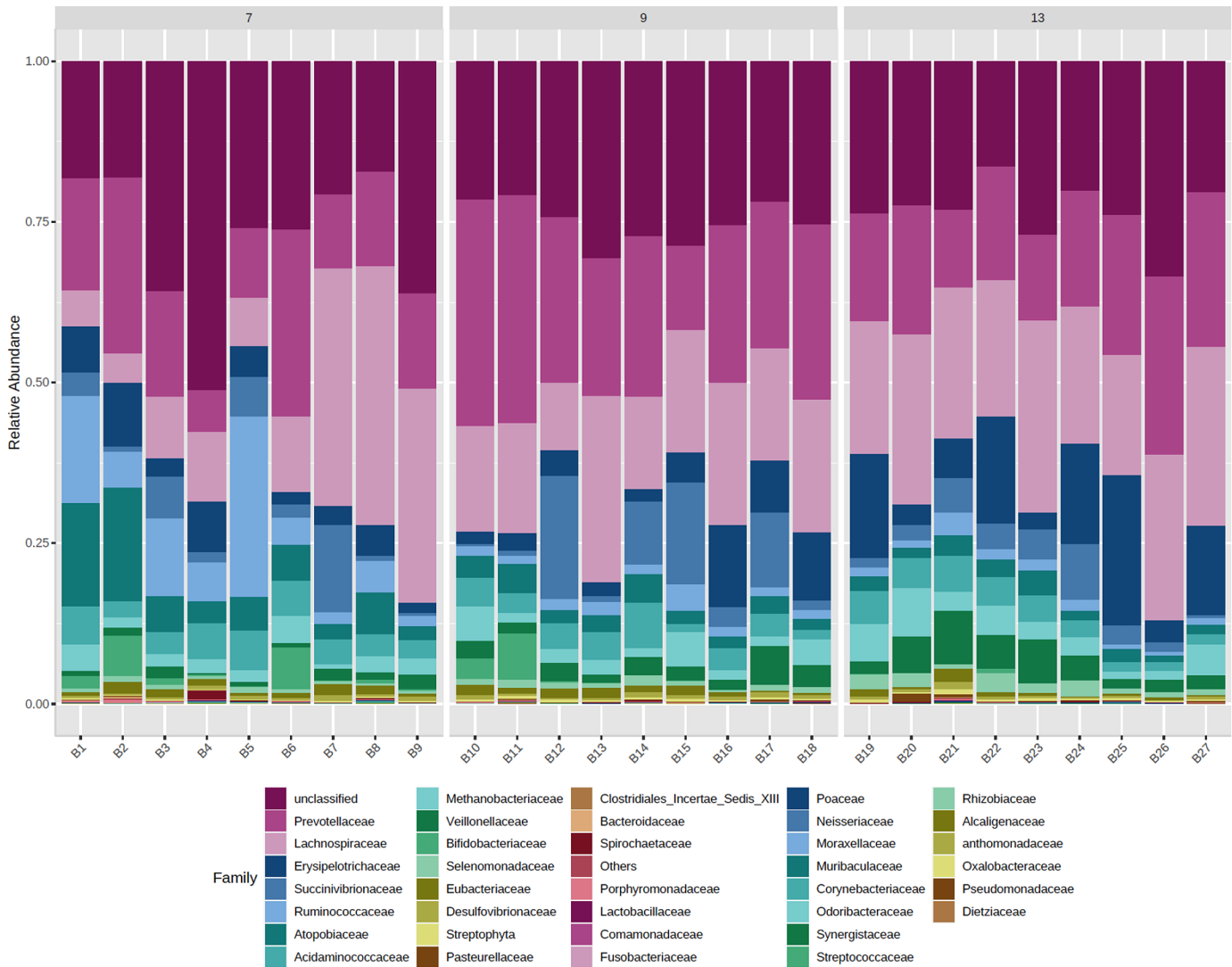
Figure 3. Mean relative abundance of bacteria at the family level of 3 composite samples per treatment before weaning (wk 7), at weaning (wk 9), and after weaning, (wk 13) of dairy calves supplemented with barley straw before and after weaning (S-S), barley straw before and alfalfa hay after weaning (S-A), or alfalfa hay before and after weaning (A-A). Composite samples B1-3, B10-12, and B19-21 correspond to S-S calves; samples B4-6, B13-15, and B22-24 correspond to S-A calves; and samples B7-9, B16-18, and B25-27 correspond to A-A calves.

milk and concentrate feed compared with calves only fed milk (Laarman et al., 2012), but also when forage (either alfalfa or oat hay) was supplemented in calves fed milk and concentrate feed (Castells et al., 2013). However, the mechanism explaining why barley straw contributes to increase $SLC16A1$ expression could not be elucidated in the current study. This increase might be related to the long particle size of barley straw compared with alfalfa hay (despite both forages being chopped using the same machine and theoretical length of cut). Also, it might reflect the need to regulate the intracellular pH of the rumen epithelium cells because of greater consumption of concentrate feed after weaning, as observed in goats fed medium–compared with

low-concentrate diets (Yan et al., 2014). In our study, there were no differences in the relative expression of genes that encode for tight junction proteins ($CLDN4$ and $OCLN$), remodeling of the ruminal epithelium (TGFB1), and inflammatory factors (IL6; Table 6). This is consistent with the study of van Niekerk et al. (2021), in which despite observing prolonged depressions on ruminal pH content (<5.6), did not find evidence of damage on the ruminal epithelium of calves. In an ex vivo experiment (Meissner et al., 2017), the relative expression of $CLDN4$ and $OCLN$ in the rumen epithelium decreased due to a combined effect of low ruminal pH (~5.1) and high SCFA concentrations (100 mM). However, the relative expression of IL6 in

the rumen epithelium seems to respond more to the concentration of LPS than to ruminal pH (Zhang et al., 2016). Although ruminal epithelium damage herein cannot be discarded, results suggest that providing different combinations of alfalfa hay and barley straw before and after weaning exerts a similar effect on the expression of genes related to the ruminal damage.

Alpha diversity was similar among treatments. However, the Shannon index increased ($P < 0.01$) from wk 7 to wk 9 and wk 13 of the study (from 3.91 to 4.15 and 4.30 ± 0.065, respectively). There were no differences in the ruminal microbiota population among the forage combination treatments. However, the ruminal microbiome changed over sampling weeks. At the kingdom level, a greater ($P < 0.05$) relative abundance (**RA**) of Archaea was found with calf age, to detriment of RA of the rest of the prokaryote. At the phylum level, the RA of Actinobacteria decreased ($P < 0.05$) with calf age, and at the order level, the RA of Bifidobacteriales tended ($P = 0.07$) to decrease with calf age, whereas RA of Coriobacteriales and Mycobacteriales decreased ($P < 0.05$) and that of Selenomonadales ($P < 0.05$) increased with calf age. Similarly, at the family level (Figure 3), the RA of Bifidobacteriacea tended ($P = 0.08$) to decrease with calf age, the RA of Atopobiaceae and Ruminococcaceae decreased ($P < 0.01$) and that of Selenomonadaceae increased ($P < 0.01$) with calf age. At the class level, differences were observed in the RA of Actinobacteria and Coriobacteriia, which both decreased ($P < 0.05$) with calf age. Lastly, at the genus level, the RA of *Alloprevotella*, *Bifidobacterium*, *Olsenella*, *Sharpea*, and *Succiniclasticum* decreased ($P < 0.05$), and that of *Acidaminococcus* and *Selenomonas* increased ($P < 0.05$) with calf age. The lack of differences in ruminal microbiome among treatments agrees with the lack of differences in ruminal pH observed before and after weaning among treatments. It appears that the consumption of rations containing 5.1 to 7.0% of forage is not sufficient to generate detectable changes in pre- and postweaning ruminal microbiome. Changes in bacterial populations before weaning seem to be mainly associated with the nature of the substrate fermented rather than the amount ingested of each feed (Rey et al., 2014; Malmuthuge et al., 2015; Dias et al., 2017). In our experiment, the forage to concentrate ratio, and CP and NFC dietary concentrations were similar among treatments, which would explain the close similarity of the rumen microbiota that we observed herein.

To sum up, this work confirms the benefits of supplying limited amounts of straw before and after weaning. Barley straw improved feed intake and performance in the postweaning period without adverse effects on feed efficiency. In addition, a small amount of straw on the pre- and postweaning diets promoted the expression of *SLC16A1* in the rumen epithelium, but it did not generate changes in the ruminal microbiome. The question arises to elucidate how feeding straw can modify the expression of *SLC16A1* in the rumen.

CONCLUSIONS

Feeding barley straw before and after weaning is more effective than feeding alfalfa hay in promoting concentrate feed intake after weaning, and fostering an increase in the expression of the gene coding for *SLC16A1* in the rumen epithelium.

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