



## Whole-plant flint corn silage inclusion in total mixed rations for pre- and postweaning dairy calves

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### ABSTRACT

Assuming that acetic acid plays a minor role in the development of ruminal epithelium of preweaning dairy calves, the fiber supply for growing calves has been neglected. More research has been done on including starch and nonfibrous carbohydrates in solid feed for preweaning calves. Accordingly, the fiber requirement of these calves is not well known, as diet recommendations vary greatly. Hence, elucidating the effects of including fiber from long particle sizes in the diet may be essential for helping calves overcome the transition challenge during weaning. Forty-five Holstein calves were used in a randomized block design, considering sex, birth date, and weight at 28 d of age, when the supply of the total mixed ration (TMR) with the inclusion of corn silage started. Three TMR with increasing whole-plant flint corn silage content (0, 10, or 20% on a dry matter basis) were compared: 0CS, 10CS, or 20CS, respectively. During the first 28 d of life, the calves were managed homogeneously and were fed 6 L/d of whole milk, a commercial calf starter pelleted, and water *ad libitum*. Next, the solid diet was changed to the respective solid feed treatment. Calves were gradually weaned from 52 to 56 d of age but were evaluated for an additional 14 d postweaning. Feed intake was measured daily, while body weight and metabolic indicators of intermediate metabolism were evaluated weekly. Ruminal fluid was collected at 6, 8, and 10 wk of age. Behavioral analysis was conducted on wk 7 (preweaning) and 10 (postweaning). There was a quadratic effect for dry matter intake from wk 7 to 10, with higher intake for the 10CS diet than the 0CS and 20CS diets. Consequently, the 10CS diet also promoted greater average daily gain at wk 8 and 9 compared with the 0CS and 20CS diets. However, the final body weight was not

affected by the different solid diets. Silage inclusion in calves' diet positively affected time spent ruminating and chewing pre- and postweaning. Including 10% of whole-plant flint corn silage in the diets of young dairy calves is a strategy to increase total solid intake and decrease acidosis risk by increasing pH and ruminating activity around weaning.

**Key words:** corn silage, effective fiber, forage, rumination

### INTRODUCTION

Calf solid feed diets usually contain high levels of NFC to improve efficiency in the preweaning period due to their strong correlation with the maturation of the gastrointestinal tract (Quigley et al., 2019). However, the supply of calf starter with high NFC and starch content combined with small particle size can result in a high fermentation rate and short-chain fatty acids (SCFA) production in the rumen (Laarman et al., 2012). Although this benefits papillae development, it potentially reduces ruminal pH and decreases DMI (Hill et al., 2008; Mojahedi et al., 2018; Toledo et al., 2020). In addition, solid feed intake is variable in calves, and this variation increases with age (Neave et al., 2019), causing negative digestive occurrences, such as diarrhea in response to high fermentation in the intestine (Kehoe et al., 2019; Virgínio Júnior and Bittar, 2021).

Some studies evaluating forage supply during the preweaning period, mainly in the form of chopped grass hay, observed an improvement in DMI and rumen health parameters (Khan et al., 2011; Castells et al., 2012). This effect was observed even when a calf starter presents small particle size and highly available starch, such as diets containing high-processed grains (Beiranvand et al., 2014; Daneshvar et al., 2015; EbnAli et al., 2016). On the other hand, these studies have some limitations on different levels of NDF from forage in the solid diet and the effects on performance and nutritional management compared with calf starters with NDF from nonforage sources.

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Current NASEM (2021) recommendations provided limited advances in fiber sources and inclusion levels for dairy calves, despite the importance and benefits highlighted in recent years.

Including corn silage in the diet would be a possible strategy to provide an effective fiber source to calves with a developing rumen. Although several nutritionists do not recommend it, adopting whole-plant corn silage as the main roughage may be justified by providing high-energy and physically effective NDF from the plant's leaves and stem (Ferraretto et al., 2018).

The Flint-type corn (*Zea mays ssp. dentata*) is South America's most widely cultivated cereal grain and the main grain used in ruminant diets (Correa et al., 2002). It has a high proportion of vitreous endosperm and a compact and dense protein matrix (Ferraretto et al., 2018). Lower levels of inclusion and supply as a TMR can provide effective fiber and ruminal health without reducing NFC content and ease management for farmers with this forage available instead of hay.

We hypothesized that whole-plant flint corn silage (WPFCS) in the TMR can provide effective fiber from forage to increase DMI and prepare the calves for the weaning process. This study aimed to investigate TMR with increasing levels of WPFCS in the diet of dairy calves and its effects on performance, metabolism, and behavior.

## MATERIALS AND METHODS

This study was conducted between July and November of 2021 at the calf facilities of the Department of Animal Science, "Luiz de Queiroz" College of Agriculture, São Paulo University, located in Piracicaba, São Paulo, Brazil. During this period, the average temperature was 22°C (ranging from 33.7°C to 7.8°C), the mean relative humidity was 64%, and the average rainfall was 63.6 mm/mo. All animal procedures were approved and followed the guidelines recommended by the Animal Care and Use Committee of the Luiz de Queiroz College of Agriculture, University of São Paulo.

### Animals, Experimental Design, and Treatments

Forty-five newborn Holstein calves (36 males and 9 females;  $36.89 \pm 1.21$  kg) from the university dairy herd were used in a randomized complete block design. All calves were weighed and fed high-quality colostrum (10% of BW;  $>50$  mg of IgG/mL) in the first 2 h after birth (Godden et al., 2019). At 48 h of life, blood samples were collected by a jugular puncture to evaluate passive immunity transfer using a Brix refractometer (Deelen et al., 2014). The colostrum protocol resulted

in values between 8.4 and 12.2%, with a mean of  $9.8 \pm 0.21\%$  Brix, suggesting no failure of passive immune transfer (Godden et al., 2019).

The calves were individually housed, initially in suspended pens ( $1.13 \times 140$  cm) until 14 d of age, and then in wood hutches (1.35 m height, 1 m width, and 1.45 m depth), being tethered by chain (2 m long), allowing an area for walking but no physical contact with other calves. Hutches were distributed in a *Paspalum notatum* grass field frequently trimmed to ground level, so no grass was available for grazing. Calves were fed 6 L/d of whole milk by teat buckets until the beginning of the weaning process. Calves had free access to a pelleted commercial calf starter (88.0% DM, 22.0% CP, 16.1% ADF, 28.2% NDF, 3.9% ether extract (EE), 10.2% ash, 35.7% NFC, 22.4% starch), and after 28 d of life, the experimental diets were fed. The solid diet was provided once a day after morning milk feeding and was available until the next day. The calves were managed equally until 28 d and divided into randomized blocks design according to sex, birth date, and weight at 28 d ( $48.40 \pm 1.212$  kg) and distributed in 3 treatments with 15 calves each as follows: (1) TMR with 0% of WPFCS (0CS); (2) TMR with 10% of WPFCS (10CS); and (3) TMR with 20% of WPFCS (20CS). The treatment diets were formulated to be isonitrogenous because we aimed to evaluate and isolate the effects of WPFCS inclusion as a source of forage in the diet. The TMR started to be fed on d 28 due to the reduced intake of forage from wk 1 to 4. The chemical composition and ingredients of the treatment diets are shown in Table 1. Blinding feeders to treatment was not possible because the calf starter and TMR were weighed and fed immediately to the calves.

### Weaning, Measurements, and Sampling

All calves received 6 L/d of whole milk divided into 2 meals (0700 and 1700 h), and refusals were recorded. The solid diet was fed once a day during the morning period, and refusals from the previous day were weighed daily on a digital scale (model 9094, Toledo do Brasil Indústria de Balanças Ltda, São Bernardo do Campo, Brazil) to calculate the daily total DMI. Samples of the solid diet and refusals were collected twice monthly ( $n = 10$ ) to determine chemical composition and calculate nutrient intake.

The calves were gradually weaned (reducing 1 L/d), regardless of the solid diet intake, starting at 52 d until the end of 56 d. After weaning, the calves were evaluated for a further 14 d. Data were divided between preweaning (28–56 d) and postweaning (57–70 d) periods, considering that the preweaning period included the

**Table 1.** Ingredients and chemical composition of experimental solid feed diets<sup>1</sup>

Item	0CS	10CS	20CS	Silage
Ingredient, % DM				
Ground corn	54.69	50.89	47.87	—
Corn silage	0.00	10.18	20.21	—
Soybean meal	23.70	26.72	28.72	—
Wheat meal	18.23	8.90	0.00	—
Mineral and vitamin supplement <sup>2</sup>	3.38	3.31	3.20	—
Chemical composition of diets				
DM, %	87.07	78.27	72.53	39.18
CP, % DM	19.13	19.10	19.30	6.13
ADF, % DM	7.07	9.34	11.70	29.83
NDF, % DM	20.63	22.10	25.37	50.55
Ash, % DM	7.61	6.93	6.86	3.65
Ether extract, % DM	3.30	3.03	5.74	3.51
Starch, % DM	43.20	41.00	39.40	25.20
NFC, % DM	49.33	48.84	42.73	36.16
In vitro digestibility, %				
DM	76.60	72.01	67.70	56.06
NDF	55.89	53.78	51.94	49.58
Particle size distribution, %				
19 mm	0.00	0.83	1.41	11.63
8 mm	0.03	5.14	8.89	56.05
4 mm	1.73	5.95	8.74	20.96
1.18 mm	49.99	44.91	45.67	10.53
Bottom pan	48.25	43.17	35.29	0.83
peNDF <sup>3</sup> >4 mm, %	0.34	2.63	4.85	44.89
peNDF <sup>3</sup> >1.18 mm, %	10.62	12.52	16.40	50.15

<sup>1</sup>0CS = 0% inclusion of corn silage on the diet; 10CS = 10% inclusion of corn silage on the diet; 20CS = 20% inclusion of corn silage on the diet; Silage = whole-plant flint corn silage.

<sup>2</sup>Composition: Ca 20%; P 6.5%; F 650 mg/kg; In 4%; K 1%; Mg 7%; S 0.7%; Co 25 mg/kg; Cu 800 mg/kg; Cr 20 mg/kg; I 40 mg/kg; Fe 1,400 mg/kg; Mn 1,500 mg/kg; Si 18 mg/kg; Zn 3,200 mg/kg; vitamin A 140,000 IU/kg; vitamin D<sub>3</sub> 50,000 IU/kg; vitamin E 1,500 IU/kg; vitamin B<sub>1</sub> 250, vitamin B<sub>2</sub> 250,000 mg/kg; vitamin B<sub>6</sub> 250 mg/kg; vitamin B<sub>12</sub> 250 mg/kg; niacin 400 mg/kg; B.C. pantothenic 500 mg/kg; folic acid 20 mg/kg; biotin 10 mg/kg; butylated hydroxytoluene 800 mg/kg, sodium monensin 900 mg/kg (Trouw Nutrition Brasil Ltd.).

<sup>3</sup>Physically effective neutral detergent fiber.

weaning process. All calves were weighed weekly on a mechanical scale (ICS-300, Coimma Ltda., Dracena, SP, Brazil) before the morning feeding of the liquid diet.

Blood samples were collected 2 h after morning feeding, beginning at the fourth week of life, using 3 different vacuum blood tubes (Vacuette do Brasil, Campinas, SP, Brazil) containing sodium fluoride as antiglycolytic agent or potassium EDTA as anticoagulant to obtain plasma; and a clot activator to obtain serum. Plasma and serum were obtained after centrifugation (Universal 320R, Hettich, Tuttlingen, Germany) at  $2,000 \times g$  for 20 min at 4°C.

### Analytical Methodology

Feed samples were oven-dried (MA035, Marconi, Piracicaba, São Paulo, Brazil) at 55°C for 24 h and ground in a 1-mm Wiley mill (Marconi, Piracicaba, Brazil). Dry matter was determined by drying samples in an oven at 105°C for 24 h (AOAC International, 2002; method 925.40) and ash by incineration of samples in a muffle furnace at 550°C for 4 h (AOAC International, 2002; method 942.05). Total nitrogen

concentration was determined using the Leco TruMac N apparatus (Leco Corporation, St. Joseph, MI; AOAC International, 2002; method 968.06), and CP was calculated by multiplying the total nitrogen by 6.25. The EE concentration was determined using petroleum ether (AOAC International, 2002; method 920.39). Starch was determined using the commercial kit Total Starch Assay Kit AA/AMG – Megazyme (AOAC International, 2002; method 996.11). Sequential detergent fiber analyses were used to determine the concentration of NDF (Van Soest et al., 1991) and ADF (Goering and Van Soest, 1970) on an Ankom 2000 fiber analyzer (Ankom Tech. Corp., Fairport, NY). Heat stable  $\alpha$ -amylase and sodium sulfite were included in the NDF analysis. The NFC content of the diets was estimated according to Mertens (1997), with the following equation:  $\text{NFC (\%)} = 100\% - (\% \text{ NDF} + \% \text{ CP} + \% \text{ fat} + \% \text{ ash})$ .

Samples of TMR and WPFCS were separated using a Penn State 4-screen particle size separator (long: >19 mm; medium: between 8 and 19 mm; short: between 8 and 4 mm; and fine: <1.18 mm), and average particle size was calculated. Physically effective NDF (**peNDF**) was calculated considering particles >4 mm,

which were retained on the top 3 screens of the Penn State Particle Separator (Yang and Beauchemin, 2006). The kernel processing score (**KPS**) was determined as described by Ferreira and Mertens (2005). The *in vitro* true digestibility over 72 h was determined according to Goering and Van Soest (1970).

Milk samples were collected monthly during the overall period of the experiment for milk composition determination ( $12.6 \pm 0.13\%$  solids,  $3.96 \pm 0.10\%$  fat,  $3.30 \pm 0.05\%$  protein, and  $4.44 \pm 0.04\%$  lactose) by Fourier transform infrared spectroscopy (Lefier et al., 1996). The concentration of total milk solids was used to calculate total DMI and feed efficiency.

The blood parameters determinations were performed using commercial kits of glucose, urea, lactate (LABTEST Diagnóstica S.A., Lagoa Santa, MG, Brazil), and BHB (Ref. RB100; RANDOX Laboratories–Life Science Ltd., Crumlin, UK). All assays were performed using an Automatic System for Biochemistry (Model SBA 200, CELM, Barueri, SP, Brazil).

### Ruminal and Fecal Variables

Ruminal fluid samples were collected 2 h after feeding at wk 6, 8, and 10. The sampling was performed oropharygeally using a flexible hose (150 cm long, 1.3-cm internal diameter, and 0.2-cm wall thickness) connected to a vacuum pump (Model TE-0581, Tecnal Ltda., Piracicaba, SP, Brazil). The initial 50-mL portion was discarded, and the visual evaluation of the ruminal fluid was performed to detect saliva contamination according to the method described by Terré et al. (2013a). After filtration on cotton cloth, an aliquot was used for pH measurement by a digital potentiometer (Model tec-5, Tecnal Ltda, Piracicaba-SP, Brazil), and another aliquot was pipetted in plastic tubes and stored in a freezer ( $-10^{\circ}\text{C}$ ) for subsequent determination of SCFA and ammonia-nitrogen according to de Paula et al. (2017).

The fecal score was evaluated daily for its consistency according to Larson et al. (1977), considering (1) normal and firm, (2) consistent and mushy, (3) mushy and slightly liquid, and (4) watery. Cases of diarrhea were considered when the fecal score was  $\geq 3$ , when the rehydration protocol based on oral fluid administration (dextrose, salt, and bicarbonate) was provided 6 h after morning feeding through a bottle. Antibiotic therapy was performed according to a veterinarian recommendation.

Fecal samples were collected from all calves by rectal palpation 2 h after morning feeding at wk 6, 8, and 10, and 4 g of fecal matter was added to 4 mL of de-ionized water for pH reading. Fecal pH was measured

in a digital potentiometer (Model tec-5, Tecnal Ltda, Piracicaba-SP, Brazil) according to the methodology described by Channon et al. (2004).

### Feeding Behavior

The behavioral data were obtained by direct observations of all calves by 4 trained observers. A scanning observation was performed for standing, lying down, sleeping, suckling liquid diet, eating the solid diet, drinking water, defecating, vocalizing, non-nutritive oral behaviors, exploring the environment, standing or lying idle, and ruminating. The chewing variable was created by adding the observations of ruminating and eating solid diet behaviors. The variables number of meals and eating time were created by adding the number of times the animal went to the trough and the time it remained during the visits. Observations were performed every 5 min, as suggested by Miller-Cushon and DeVries (2015), starting at the morning feeding (0700 h) for 10 h at wk 7 and 10 for a total observation time of 20 h per calf.

### Statistical Analysis

The calculation of the sample number was performed using PROC POWER with 90% test power and a significance level of  $P \leq 0.05$ , obtaining results from 15 animals per treatment. Statistical analysis was performed using the MIXED procedure of SAS (2002; SAS Institute Inc.). The  $P$ -value  $\leq 0.05$  was adopted as a significant effect, and we considered trends at  $0.05 > P < 0.10$ . The following statistical model was used for the variables analyzed as repeated measures over time:  $Y_{ijk} = \mu + D_i + b_j + e_{ij} + I_k + (D_i)I_k + e_{ijk}$ , where  $\mu$  = overall mean;  $D_i$  = fixed effect of diet;  $b_j$  = random effect of block;  $e_{ij}$  = residual error (A);  $I_k$  = fixed effect of age;  $(D_i)I_k$  = fixed effect of diet  $\times$  age interaction, and  $e_{ijk}$  = residual error (B). Covariance matrices were tested and defined according to the lowest value obtained for Akaike's information criterion corrected. Fixed variables were evaluated using the following statistical model:  $Y_{ij} = \mu + D_i + b_j + e_{ij}$ , where  $\mu$  = overall mean;  $D_i$  = diet effect;  $b_j$  = random effect of block; and  $e_{ij}$  = residual error. For all response variables, the means were obtained using the LSMEANS command. The model included the effects of treatment, week (age of calves), and the interaction between treatment and week as fixed effects. The block effect was included in the model as a random effect. The subject of the repeated measures was animal within treatment. The effect of corn silage inclusion (0% CS, 10% CS, 20% CS) was evaluated using the



linear orthogonal polynomials and linearity deviation. In the case of variables analyzed as repeated measures in time, the effect of week (age) and diet interaction was defined based on the F-test.

## RESULTS

### Chemical Composition of Experimental Diets

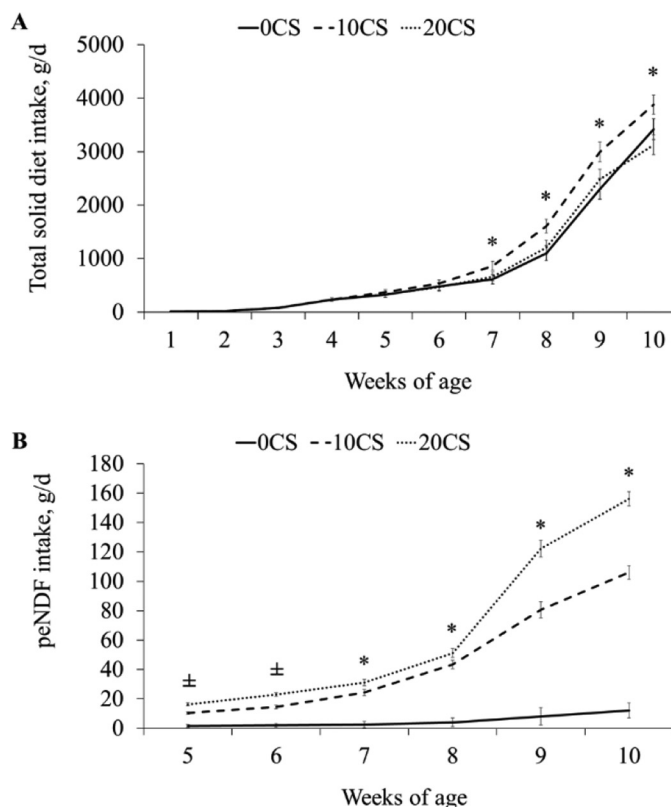
The WPFCS used in the experimental diets followed the average chemical composition commonly observed in Brazil, with 25% starch and 50% NDF (Table 1). The silage in vitro NDF digestibility indicates that diet fiber quality is above expectation compared with other roughages commonly used to feed calves. The KPS was 70%, considered an excellent level, leaving the starch more available (Ferreira and Mertens, 2005). Also, the percentage of long particles (8- and 19-mm sieves) was lower than 10%, reducing the probability of feed sorting by the calves. As the inclusion of silage in the diet increased the DM, starch, and NFC content. In contrast, the levels of NDF and peNDF from the sieve above 4 mm increased linearly with the inclusion of silage. In addition, the protein of the experimental diets remained the same as expected.

### Intake and Growth Performance

During the preweaning period, a significant interaction was observed between diet and age for total solid feed DMI (Figure 1A; Table 2). There was no diet effect at wk 5 and 6; however, a quadratic effect was observed for wk 7 and 8, with the highest total solid feed DMI for calves fed the 10CS diet ( $P < 0.05$ ). The same interaction effect was observed postweaning at wk 9 and 10. A similar response was observed for total solid feed DMI expressed as a percentage BW pre- and postweaning.

There was an interaction between diet and age for NDF intake during preweaning and peNDF >4.00 mm intake during the pre- and postweaning period (Figure 1B). There was no solid feed diet effect at wk 5 for NDF intake; however, at wk 6, we observed a trend for a linear increasing effect ( $P = 0.09$ ) with the inclusion of silage in the diet. From wk 7 to 8, we observed a quadratic effect, and calves fed 10CS and 20CS diets had higher NDF intake ( $P = 0.05$ ). The same effect was observed postweaning at wk 9 and 10 ( $P = 0.04$ ).

At wk 5 and 6, there was a linear increasing effect for peNDF intake with the inclusion of silage in the solid feed ( $P < 0.01$ , Figure 1B). We observed a quadratic effect from wk 7 to 8, and calves fed 20CS and 10CS diets



**Figure 1.** (A) Total solid diet intake (g/d) of calves during the whole period. \*Denotes quadratic effect. Calves that received the 10CS diet had a higher total solid diet intake compared with the 0CS and 20CS diets ( $P < 0.05$ ). (B) Physically effective neutral detergent fiber intake (peNDF; g/d) of calves during the whole period. ±Denotes increasing linear effect for peNDF intake of the calves with the inclusion of silage in the diet ( $P < 0.01$ ). \*Denotes quadratic effect. Calves that received the 20CS diet had a greater peNDF intake than the 10CS and 0CS diets ( $P < 0.03$ ). Calves ( $n = 15/\text{treatment}$ ) were fed with 0CS = 0% inclusion of corn silage on the diet; 10CS = inclusion of 10% corn silage on the diet; 20CS = inclusion of 20% corn silage on the diet. Error bars represent SEM.

had the highest peNDF >4.00 mm intake ( $P = 0.05$ ). The same effect was observed postweaning ( $P = 0.03$ ).

There was an interaction between diet and age for NDF intake expressed as the percentage of BW (NDF%BW) during the preweaning period. There was no effect at wk 5 and 6; however, we observed a quadratic trend effect at wk 7 and 8 ( $P < 0.06$ ). A quadratic trend effect was also observed postweaning ( $P < 0.07$ ).

A quadratic effect was observed for starch intake pre- and postweaning. There was no diet effect at wk 5 and 6, but calves that received the 10CS diet had a higher starch intake at wk 7 and 8 ( $P < 0.04$ ), even with reduced starch in the diet due to the higher inclusion of corn silage. Additionally, postweaning calves that re-

**Table 2.** Performance of calves in the preweaning (d 28–56) and postweaning periods (d 57–70), fed with 0, 10, and 20% of whole-plant flint corn silage in solid feed diets

Item	Diet <sup>1</sup>				P-value <sup>2</sup>			
	0CS	10CS	20CS	SEM	L	Q	A	A × D
Preweaning, d 28–56								
Feed intake, g DM/d								
Liquid diet	732.8	735.3	737.3	4.85	0.46	0.96	<0.01	0.99
Solid diet	631.7	847.4	667.3	87.5	0.77	0.07	<0.01	<0.01
Solid diet, % BW	0.99	1.28	1.08	0.098	0.48	0.04	<0.01	0.01
NDF	133.9	186.7	174.0	17.80	0.11	0.12	<0.01	<0.01
NDF, % BW	0.21	0.28	0.27	0.024	0.05	0.19	<0.01	0.03
peNDF <sup>3</sup> >4 mm	2.6	23.5	30.7	1.64	<0.01	0.01	<0.01	<0.01
Starch	290.9	349.6	270.5	31.72	0.81	0.05	<0.01	0.03
Growth, g/d								
ADG	710.0	771.9	695.2	38.32	0.59	0.18	0.01	0.05
EBWG <sup>4</sup>	690.0	719.5	641.9	35.22	0.34	0.21	0.01	0.06
Gain:feed ratio <sup>5</sup>	0.560	0.525	0.537	0.0204	0.44	0.35	<0.01	0.33
Postweaning, d 57–70								
Feed intake, g DM/d								
Solid diet	2,859.6	3,436.3	2,805.7	178.39	0.83	0.01	<0.01	0.03
Solid diet, % BW	3.60	4.20	3.62	0.178	0.93	0.01	<0.01	0.05
NDF	593.3	760.6	720.2	38.99	0.04	0.05	<0.01	0.26
NDF, % BW	0.75	0.91	0.90	0.036	0.01	0.07	<0.01	0.24
peNDF >4 mm	10.2	93.4	139.2	5.02	<0.01	0.01	<0.01	<0.01
Starch	1,269.3	1,417.4	1,114.7	75.26	0.16	0.02	<0.01	0.01
Growth, g/d								
ADG	1038.8	1,116.3	1,034.0	76.62	0.96	0.38	0.04	0.09
EBWG <sup>4</sup>	978.1	1,040.6	954.7	70.30	0.82	0.39	0.07	0.15
Gain:feed ratio <sup>5</sup>	0.384	0.344	0.376	0.0224	0.78	0.15	0.01	0.80
BW, kg								
Initial (d 28)	48.5	48.7	47.0	2.08	0.38	0.53	—	—
At weaning (d 56)	69.5	70.9	67.0	2.62	0.30	0.19	—	—
Final (d 70)	83.0	86.0	80.8	3.35	0.55	0.18	—	—

<sup>1</sup>0CS = 0% inclusion of corn silage on the diet; 10CS = 10% inclusion of corn silage on the diet; 20CS = 20% inclusion of corn silage on the diet; 15 calves per treatment.

<sup>2</sup>L = linear effect; Q = quadratic effect; A = age effect; A × D = interaction between diet and age.

<sup>3</sup>Physically effective neutral detergent fiber

<sup>4</sup>Empty BW gain (EBWG) was calculated according to equations given by Jahn and Chandler (1976).

<sup>5</sup>Considering liquid and solid DMI.

ceived the 0CS and 10CS diets had higher starch intake than the 20CS diet ( $P < 0.02$ ).

There was an interaction between diet and age for ADG in the preweaning period (Figure 2A). There was no diet effect at wk 5, but at wk 6, a decreasing linear effect occurred as the inclusion of silage increased in the diet ( $P = 0.03$ ). On the other hand, a quadratic effect was observed at wk 8, when calves fed the 10CS diet had the greatest ADG ( $P < 0.05$ ). The same effect was observed for empty body weight gain (EBWG) in the preweaning period (Figure 2B). The solid diets also affected the ADG in the postweaning period, and at wk 9, a quadratic effect was observed, with the highest ADG for calves fed the 10CS diet ( $P < 0.05$ ). In contrast, no diet effect was observed for EBWG in the postweaning period.

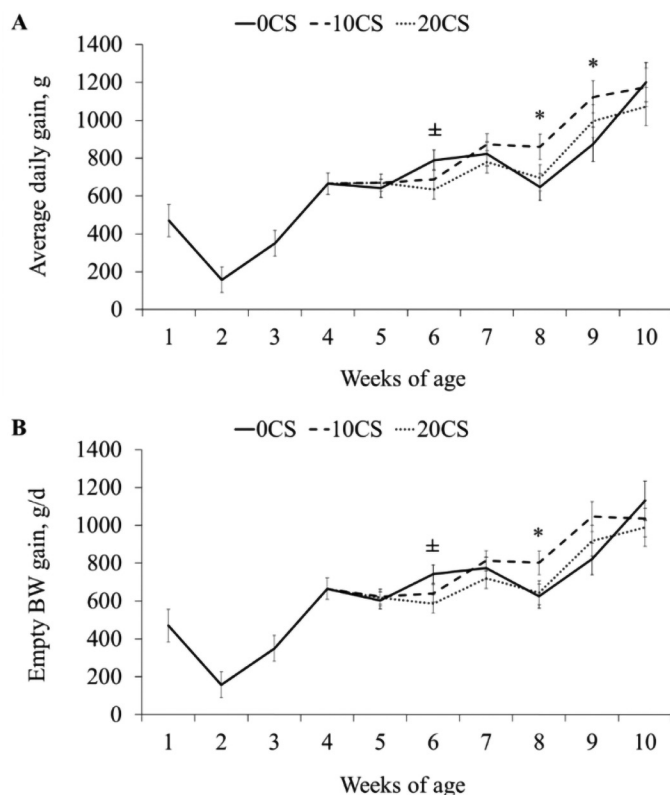
All calves had similar BW at the beginning of the study. However, despite the greater ADG in some of the weeks, it was not possible to observe a difference in

BW at weaning (d 56) and the end of the study (d 70). The feed efficiency of calves was also not affected by the experimental solid feed diets or the interaction of diet and age.

### Ruminal and Fecal Variables

There was no interaction between diet and age for ruminal and fecal variables at wk 6, 8, and 10. There was a trend for the total molar concentration of SCFA, with a linear decrease with the inclusion of corn silage in the diet (Table 3). The molar concentration of propionate, acetate, and butyrate was not affected by the diets. Despite this, the acetate:propionate ratio was affected by age, with an increase in concentrations from wk 6 to 10.

There was a decreasing linear effect on ruminal ammonia-nitrogen concentrations as silage levels increased in the diet. Also, there was a trend for a linear effect in



**Figure 2.** (A) Average daily gain (g/d) of calves during the whole period.  $\pm$  Denotes decreasing linear effect ( $P = 0.03$ ) for the average daily gain of the calves with the increase of inclusion of silage in the diet. \*Denotes quadratic effect. Calves that received the 10CS diet had a greater average daily gain than the 0CS and 20CS diets. ( $P < 0.05$ ). (B) Empty body weight gain (g/d) of calves during the whole period.  $\pm$  Denotes decreasing linear effect ( $P = 0.02$ ) for the empty BW gain (EBWG) of the calves with the increase of inclusion of silage in the diet. \*Denotes quadratic effect. Calves that received the 10CS diet had a greater EBWG than the 0CS and 20CS diets. ( $P = 0.02$ ). Calves ( $n = 15/\text{treatment}$ ) were fed with 0CS = 0% inclusion of corn silage on the diet; 10CS = inclusion of 10% corn silage on the diet; 20CS = inclusion of 20% corn silage on the diet. Error bars represent SEM.

ruminal pH (Table 3), with increasing values as silage was included. Fecal pH presented a quadratic effect, with higher values for calves fed the 10CS diet. The fecal score was not affected by diet, age, or the interaction of these factors.

### Blood Metabolites

There was no interaction between diet and age for blood metabolites, except for BHB. All blood parameters were affected by age at preweaning, with decreased glucose concentration and increased lactate, BHB, and urea concentrations as calves aged. During the preweaning period, the diets did not affect plasma glucose (Table 4). In contrast, a quadratic effect was observed postweaning, and the 10CS diet presented the highest

glucose concentration. A quadratic effect was observed for plasma lactate pre- and postweaning; the calves fed the 10CS diet presented the highest concentration.

Concentration of BHB was unaffected by diets in the preweaning period, but a significant interaction effect between diet and age was observed postweaning (Figure 3). The BHB concentration increased at wk 10 for calves fed the 10CS and 20CS diet compared with the 0CS ( $P = 0.04$ ). A linear trend effect was observed for decreased plasma urea concentration preweaning as the level of silage in the diet increased.

### Behavior Variables

At wk 7, a quadratic effect was observed for the behavior of eating the solid diet, with the greatest time spent by calves fed the 10CS diet. There was a decreasing linear effect for the time spent standing or lying idle with increasing levels of silage in the solid feed diets. There was an increasing linear effect for the time spent ruminating and a linear trend for chewing in preweaning with increasing levels of silage in the solid feed diets. Also, a quadratic trend for the number of meals ( $P = 0.07$ ) was observed. The experimental diets did not influence the other evaluated behavioral variables at wk 7.

At wk 10, there was a linear trend decrease in the time spent standing when silage was included in the solid diets ( $P = 0.06$ ; Table 5). Consequently, increases of silage in the diet tended to increase the time spent lying ( $P = 0.07$ ). A quadratic trend effect was observed for the time exploring the environment, with lower means for calves fed 10CS diet. There was a quadratic effect for the time spent ruminating, with higher times for the calves that received the 10CS and 20CS diets. In addition, there was an increasing linear effect for time spent chewing as the silage level in the solid feed diet increased. The other variables were not affected by the solid feed diets.

## DISCUSSION

### Intake and Growth Performance

In this study, including 10% of WPFCS in the calves' TMR diet increased the total solid DMI during the weaning process, providing more energy and protein for the calves. The moisture present in the silage may increase the palatability of the diet while supplying long particle fiber, increasing total solid diet for calves fed a moderate volume of liquid diet.

For young calves, fiber requirements still need to be better elucidated, with a recommendation of a minimum level of NDF from forage and precise information

**Table 3.** Ruminal fermentation parameters and fecal variables of calves at wk 6, 8, and 10 of age, fed with 0, 10, and 20% of whole-plant flint corn silage in solid feed diets

Item	Diet <sup>1</sup>				P-value <sup>2</sup>			
	0CS	10CS	20CS	SEM	L	Q	A	A × D
Total SCFA, <sup>3</sup> mM	110.2	106.5	102.1	3.99	0.09	0.93	0.03	0.91
SCFA, mM/100 mM								
Acetate	58.8	58.8	59.9	0.70	0.28	0.51	<0.01	0.45
Propionate	34.5	34.6	33.6	0.74	0.43	0.56	<0.01	0.77
Butyrate	6.4	6.4	6.4	0.27	0.92	0.97	0.01	0.12
C2:C3 ratio <sup>4</sup>	1.76	1.76	1.83	0.065	0.44	0.65	<0.01	0.86
NH <sub>3</sub> -N, mg/dL	16.6	13.6	12.2	1.32	0.02	0.61	<0.01	0.70
Ruminal pH	5.69	5.85	5.92	0.095	0.06	0.62	0.16	0.16
Fecal pH at 2 h	6.71	6.87	6.61	0.089	0.41	0.05	<0.01	0.18
Fecal score	1.6	1.6	1.6	0.09	0.97	0.77	0.27	0.81

<sup>1</sup>0CS = 0% inclusion of corn silage on the diet; 10CS = 10% inclusion of corn silage on the diet; 20CS = 20% inclusion of corn silage on the diet; 15 calves per treatment.

<sup>2</sup>L = linear effect; Q = quadratic effect; A = age effect; A × D = interaction between diet and age.

<sup>3</sup>SCFA = short-chain fatty acids.

<sup>4</sup>C2 = acetic acid; C3 = propionic acid.

about fiber sources that could be included in the diet. The wide range of fiber recommendations creates gaps for the inclusion of different levels of NFC in the diet, as examples of diets with high and low inclusion of starch are presented in NASEM (2021). The great variation in the diet composition for these calves may not allow maximum rumen development or may even result in metabolic disorders (Imani et al., 2017).

In this context, particle size and forage inclusion level must be adjusted to diets formulated for young calves. Panahiha et al. (2022) recently observed increased pre-weaning intake of corn silage versus alfalfa-based diets (581 vs. 376 g/d). Similar results were observed in this study, including 10% of WPFCS with almost 90% of particles ≤8mm in the TMR also resulted in higher intake when compared with calves that received no silage. However, particle size and silage quality can vary

drastically (Salvati et al., 2021), resulting in variable acceptability and allowing feed sorting by young calves.

Including fiber from forage sources in the diet of pre-weaned calves must be done carefully since they present an underdeveloped digestive tract with a low ingestion capacity (Khan et al., 2016). In the present study, including 10% of WPFCS proved to be the best alternative, stimulating solid feed intake and increasing the ingesting capacity of calves with advancing age due to the presence of effective fiber in the diet. On the other hand, we observed that the inclusion of 20% of WPFCS in the diet decreased the solid diet DMI, probably due to the rumen fill effect caused by the higher fiber inclusion with long particles, as previously reported with other fiber sources (Khan et al., 2016).

As pointed out by others, improved muscular development of the rumen may have contributed to the

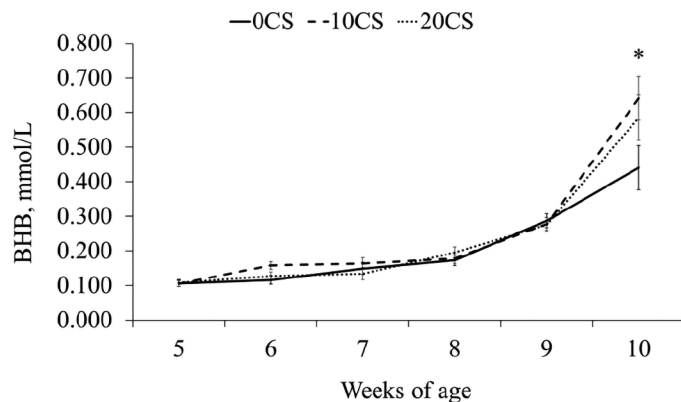
**Table 4.** Blood metabolites of calves in the preweaning (d 28–56) and postweaning periods (d 57–70), fed with 0, 10, and 20% of whole-plant flint corn silage in solid feed diets

Item	Diet <sup>1</sup>				P-value <sup>2</sup>			
	0CS	10CS	20CS	SEM	L	Q	A	A × D
Preweaning, d 28–56								
Glucose, mg/dL	106.8	112.5	114.0	3.55	0.15	0.60	<0.01	0.64
Lactate, mg/dL	10.1	11.5	10.5	0.44	0.56	0.03	<0.01	0.91
BHB, mmol/L	0.136	0.152	0.142	0.008	0.63	0.19	<0.01	0.16
Urea, mg/dL	18.0	17.2	16.5	0.67	0.06	0.93	<0.01	0.52
Postweaning, d 57–70								
Glucose, mg/dL	75.3	81.2	74.8	2.29	0.87	0.03	0.21	0.58
Lactate, mg/dL	6.9	7.9	6.4	0.41	0.37	0.01	0.93	0.35
BHB, mmol/L	0.267	0.323	0.326	0.018	0.02	0.18	<0.01	0.04
Urea, mg/dL	29.6	31.6	29.8	1.19	0.90	0.18	0.82	0.60

<sup>1</sup>0CS = 0% inclusion of corn silage on the diet; 10CS = 10% inclusion of corn silage on the diet; 20CS = 20% inclusion of corn silage on the diet; 15 calves per treatment.

<sup>2</sup>L = linear effect; Q = quadratic effect; A = age effect; A × D = interaction between diet and age.





**Figure 3.**  $\beta$ -Hydroxybutyrate concentration (mmol/L) of calves preweaning, d 28–56, and postweaning, d 57–70, feeding with different levels of corn silage in the diet. 0CS = 0% inclusion of corn silage on the diet; 10CS = inclusion of 10% with silage on the diet; 20CS = inclusion of 20% with silage on the diet; 15 calves per treatment. \*Denotes trend of quadratic effect. Levels of BHB increased at wk 10 for calves fed the 10CS and 20CS diets compared with 0CS ( $P = 0.08$ ). Calves ( $n = 15/\text{treatment}$ ) were fed with 0CS = 0% inclusion of corn silage on the diet; 10CS = inclusion of 10% corn silage on the diet; 20CS = inclusion of 20% corn silage on the diet. Error bars represent SEM.

increased solid feed intake during the postweaning period (Coverdale et al., 2004; Khan et al., 2011; Castells et al., 2012). The high postweaning solid feed intake reported in this study is similar to the results reported with the inclusion of 10% of grass hay in the diet of weaned calves or those receiving a high volume of milk, but in an early step-down program (Mitchell and Heinrichs, 2020; Wickramasinghe et al., 2022). These results show that including fiber from silage in the diet may be an alternative to hay during the transition phase.

The calves that received diets with the inclusion of 10 and 20% of WPFCS presented an NDF%BW of 0.27% preweaning and 0.9% postweaning, which may indicate a level of physically effective NDF inclusion restrictive to feed intake around weaning. The NDF%BW intake of 0.9% is close to that of Mitchell and Heinrichs (2020), who observed values of 0.7%.

Understanding the content of peNDF suitable for the diets of young calves is crucial to adjust the level of inclusion of each ingredient in the diet. The 10CS diet allowed a high starch level content, while the peNDF (2.63% >4.00 mm) potentially provided mechanical stimulation that increased the reticulum-rumen physical capacity and muscle development. The silage particle size can help modulate rumen retention, reducing the escape of smaller particles, which will remain longer in the reticulum-rumen (Panahiha et al., 2022).

Although the NDF intake was similar for 10CS and 20CS diets in grams per day and % BW, the same did not occur for the intake of peNDF >4.00 mm. Calves

fed diets containing 20% WPFCS presented a higher intake of peNDF >4.00 mm due to the higher fiber inclusion than the 10% WPFCS diet. As expected, these results suggest that higher silage inclusion rates at this age can affect the ruminal fill, decreasing solid feed intake (Stobo et al., 1966).

Calves fed the 10CS diet showed a higher starch intake due to the higher solid feed intake. Replacing cereal starch with starch from silage grains in the diet may be beneficial because grains were affected by the action of enzymes from microorganisms during the fermentation and storage process. The starch may be released more quickly, whereas the fiber will be fermented slowly in the reticulum-rumen. In addition, corn silage inclusion on a diet for preweaning calves may help increase motility, saliva production and entry in the rumen, and rumen colonization by microorganisms, whereas starch and NFC provide substrate to sustain growth rates.

In the present study, the silage characteristics allowed the calves to perform well during weaning without affecting efficiency when 10% of WPFCS was included in the TMR. On the other hand, solid feed intake was reduced due to rumen fill with the inclusion of 20% WPFCS in the diet.

In this sense, adjustments must be made according to the ingredients used as the main sources of energy and protein, and the quality of the silage. Despite that, calves that received 20CS diet showed EBWG similar to calves receiving the other diets, indicating that higher inclusions of silage do not affect performance but may limit consumption in this phase.

Previous results that evaluated the inclusion of silage in the diet of calves had shown an increase in total intake, ADG, and BW, probably due to the feed palatability when 15% of corn silage was included (Mirzaei et al., 2016). On the other hand, higher levels of corn silage (30 or 60%; 75 or 100%) did not offer benefits compared with the exclusive starter supply (Kehoe et al., 2019).

The levels of NDF and peNDF intake observed with the inclusion of 10% of WPFCS indicate that, despite the higher level of substrates that ferment slowly, the forage characteristics may allow the maintenance of ruminal pH, resulting in increases in solid diet intake with advancing age. At the end of the weaning period, the calves fed the 10CS diet presented higher accumulated energy consumption, potentially allowing better gastrointestinal tract development and greater weight gain, as suggested by Quigley et al. (2019).

In the first weeks of life, due to the low solid diet intake, the starter with NDF levels between 15 and 25% may be satisfactory to meet the requirements of the calves, as recommended by Davis and Drackley (1998). It is expected that the higher consumption of

**Table 5.** Ingestive behavior of calves in the preweaning (d 28–56) and postweaning periods (d 57–70), fed with 0, 10, and 20% of whole-plant flint corn silage in solid feed diets; all values are min/10-h observation period

Item	Diet <sup>1</sup>			SEM	P-value <sup>2</sup>	
	0CS	10CS	20CS		L	Q
Preweaning, 7 wk						
Standing	236.0	251.6	249.7	13.38	0.43	0.53
Lying	364.0	348.4	350.3	12.89	0.43	0.53
Sleeping	115.0	122.8	127.8	11.56	0.45	0.88
Suckling liquid diet	14.6	12.8	13.2	1.98	0.65	0.62
Eating solid diet	24.1	38.1	28.7	4.66	0.50	0.04
Drinking water	6.1	10.9	8.2	2.06	0.51	0.16
Urinating or defecating	2.2	0.9	0.8	0.61	0.13	0.54
Vocalizing	1.7	2.3	3.4	0.90	0.22	0.87
Non-nutritive oral behavior	61.3	60.0	61.3	7.25	0.99	0.87
Standing or lying idle	292.0	255.6	220.7	13.36	0.01	0.96
Exploring environment	30.7	32.0	39.8	6.34	0.21	0.94
Ruminating	52.4	64.5	95.0	7.45	0.03	0.92
Chewing	77.2	103.4	102.0	8.57	0.06	0.20
Number of meals	3.7	5.1	4.1	0.55	0.53	0.07
Time eating meal	6.7	7.3	7.2	0.53	0.54	0.58
Postweaning, 10 wk						
Standing	284.3	245.9	249.0	12.69	0.06	0.18
Lying	315.7	354.1	351.0	13.13	0.07	0.20
Eating solid diet	115.0	106.6	99.9	11.92	0.39	0.95
Drinking water	13.9	14.7	12.6	3.24	0.78	0.72
Sleeping	80.4	71.3	61.8	8.35	0.13	0.98
Urinating or defecating	1.4	1.0	1.3	0.71	0.92	0.66
Vocalizing	0.4	0.7	0.3	0.38	0.96	0.49
Non-nutritive oral behavior	30.4	39.3	33.3	5.35	0.69	0.25
Standing or lying idle	273.7	238.2	245.2	18.63	0.50	0.25
Exploring environment	34.6	18.7	27.5	6.68	0.41	0.09
Ruminating	49.6	106.6	118.2	9.32	<0.01	0.05
Chewing	167.0	211.3	226.2	13.12	0.01	0.31
Number of meals	9.7	9.0	9.5	0.62	0.85	0.44
Time eating meal	12.4	11.4	11.1	0.99	0.30	0.75

<sup>1</sup>0CS = 0% inclusion of corn silage on the diet; 10CS = inclusion of 10% corn silage on the diet; 20CS = inclusion of 20% corn silage on the diet; 15 calves per treatment.

<sup>2</sup>L = linear effect; Q = quadratic effect.

ingredients from cereal grain provides more energy and protein that allows greater gain, as occurred at wk 6. However, as the fiber requirement increases according to BW and rumen development, calves that do not receive fiber from forage in the diet may present variable solid feed intake due to the high fermentation rate and acidosis risk (Baldwin et al., 2004). On the other hand, as expected, the higher intake by calves fed with 10CS diet also provided more substrates for greater ADG at wk 8 and 9.

The lower digestibility of the diet with WPFCS does not affect efficiency. Despite the greater ADG at wk 8, it was not possible to observe differences in calf BW because of rumen fill. Even so, other benefits, such as higher ruminal and fecal pH, increased ruminating time, and possible better total-tract development attributed to higher accumulated nutrient intake can be achieved when calves receive a 10CS diet.

Because WPFCS is a fermented feed, it is not a feedstuff commonly recommended by nutritionists for calves and still raises some concerns in the industry.

Three hypotheses are associated with the low intake of silage compared with hay. The high content of acids may affect acceptability; the low concentration of soluble carbohydrates and energy availability may delay rumen development (Kehoe et al., 2019); and finally, its lower aerobic stability and the high prevalence of toxic substances produced during the deteriorating process (Ranjiti and Kung, 2000). Despite these limiting aspects, the availability of silages with low aerobic stability has significantly decreased in recent years with advances in knowledge and technology in the conservation process (Salvati et al., 2021). In addition, the presence of acids and the concentration of soluble carbohydrates do not seem to decrease consumption or negatively affect rumen development in young calves (Suárez et al., 2007).

#### **Ruminal and Fecal Variables, and Blood Metabolites**

The trend of a linear effect on total SCFA concentration in the rumen as WPFCS is included in the diet

agrees with previously observed results with other forage sources for calves (Castells et al., 2013; Terré et al., 2013b). The higher concentration of NFC in the OCS diet allows intensive microbial fermentation in the rumen, giving rise to high production of SCFA and a drop in ruminal pH (Mirzaei et al., 2016). The WPFCS may provide the required physical factor to improve ruminal pH and stimulate musculature and physical development of the reticulum-rumen, as previously reported in the literature (Khan et al., 2011).

Despite that, a decrease in butyrate concentrations or acetate:propionate ratio was not observed. These results indicate that even with the inclusion of an ingredient with lower digestibility in the diet, it is possible to maintain the concentrations of metabolites that stimulate epithelium development (Suárez et al., 2007; Terré et al., 2013b).

The higher level of larger particles in the diet should decrease the passage rate of smaller particles, reducing fermentation in the large intestine, which has previously been correlated with fecal pH (Castells et al., 2013). The OCS diet may have a higher passage rate of smaller particles through the rumen, resulting in greater substrate for subsequent fermentation in the cecum. However, despite the quadratic effect observed for fecal pH, observed values indicate a slight drop in pH that may suggest an increase in cecum fermentation.

In addition, we observed a decrease in ammonia-nitrogen concentration in the rumen that may be related to a greater ruminal pH through an improved buffer capacity (Laarman et al., 2012; Castells et al., 2012, 2013). The cellulolytic microorganisms use ammonia-nitrogen as a substrate to ferment fiber, reducing its concentration in the rumen, as observed in the present study (Suárez et al., 2007; Castells et al., 2013). However, rumen samples were collected in just one moment, 2 h after the calves were fed, and may not represent variations throughout the day, being a limitation of this study.

The preweaning plasma glucose concentrations were unaffected by diets and were typical of calves. On the other hand, the greater intake of nutrients by calves that received the 10CS diet reflected the higher postweaning concentration of plasma glucose. As the rumen develops, calves depend on gluconeogenesis to provide most of the glucose needed. With advancing age, glucose concentrations decline, as is typical in ruminants (Hostettler-Allen et al., 1994).

The higher plasma lactate concentration may be linked to the higher consumption of starch by calves that received the 10CS diet. The greater availability of this substrate may have resulted in higher lactic fermentation (Laarman et al., 2012).

The BHB plasma concentrations were not affected by diets during the preweaning period. In ruminants, butyrate derived from carbohydrate breakdown is converted to BHB in the rumen wall. Although the concentrations were kept similar during preweaning, a rise in plasma BHB concentration linked to higher intake was observed at wk 10. Thus, the increase in BHB can be related to rumen development (Quigley and Bernard, 1992).

The blood urea concentrations tended to reduce as silage was included in the diet during the preweaning period and may reflect ammonia-nitrogen concentrations in the rumen, which is absorbed and converted to blood urea (Funaba et al., 1994). The cellulolytic microorganisms may incorporate ammonia-nitrogen in the cell, reducing the metabolite in the rumen and, consequently, the concentrations of urea in plasma (Castells et al., 2013).

### Behavior Variables

Providing fiber from forage, even at low levels such as 10% of WPFCS, increases motility and salivation, resulting in better use of nutrients without major changes in diet formulation (Nocek, 1997). The feeding behavior of calves can affect how the feed is digested and how nutritionists can adjust the diets to improve performance without negative effects on rumen health and welfare, as observed for cows (Johnston and DeVries, 2018). The higher preweaning number of meals by calves fed the 10CS diet in this study may have resulted in a more constant flow of nutrients to the rumen, avoiding large within-day depressions of pH.

Feed intake was lower for calves fed the OCS diet and may have occurred to prevent metabolic disorders such as acidosis with advancing age. Also, those calves spent more time standing or lying idle preweaning.

A linear effect was also observed on time spent chewing and ruminating as the inclusion of WPFCS in the diet increased pre- and postweaning. To achieve higher levels of nutrient consumption, rumen health, and animal welfare must be targeted. That could be attained through changes in ingestive behavior rather than energy and protein increases in the diets (Costa et al., 2016; Poczynek et al., 2020). It is common sense that chewing allows the particle size reduction, increasing the contact surface so that the fermentation and passage rates increase and the animal eventually returns to feeding (Khan et al., 2011). In addition, rumination is fundamental for keeping the rumen healthy and functioning. Thus, nutrient consumption will be optimized when calves ruminate at healthy levels, increasing reticulum-rumen pH buffering, as observed for

calves receiving the 10CS diet. On the other hand, both 0CS and 20CS diets proved inefficient: the first due to the high fermentation rate resulting in low pH and the second due to the rumen filling effect resulting in low consumption of total nutrients.

The present study did not evaluate the physical characteristics of the solid feed offered and the refusals, limiting the understanding of the feed sorting when calves were fed the 10CS and 20CS diets.

## CONCLUSIONS

Including 10% of WPFCS in the diets of young dairy calves is a strategy to increase total solid intake and decrease acidosis risk by increasing pH and ruminating activity around weaning.

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