University of São Paulo
“Luiz de Queiroz” College of Agriculture

Forage sources in total mixed rations for dairy calves: effects on performance, metabolism, behaviour, and development of the gastrointestinal tract

Ariany Faria de Toledo

Thesis presented to obtain the degree of Doctor in Science. Area: Animal Science and Pastures

Piracicaba
2024

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versão revisada de acordo com a Resolução CoPGr 6018 de 2011

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CONTENTS

RESUMO

Fontes de forragem na ração total para bezerros leiteiros: efeitos no desempenho, metabolismo, comportamento e desenvolvimento do trato gastrointestinal

Este estudo teve como objetivo investigar a ração total misturada (TMR) com níveis crescentes de silagem de milho de planta inteira na dieta de bezerros leiteiros e seus efeitos no desempenho, metabolismo e comportamento. Além disso, avaliar os efeitos da inclusão de fontes de forragem no desempenho, metabolismo, comportamento alimentar e desenvolvimento do trato gastrointestinal de bezerros leiteiros. Para este estudo foram realizados dois experimentos. Em ambos os experimentos, durante os primeiros 28 dias de vida, todos os bezerros receberam 3 L de leite integral duas vezes ao dia, ração comercial peletizada *ad libitum* e nenhuma forragem. Após isso, a dieta sólida foi alterada para os respectivos tratamentos. Os bezerros foram blocados e distribuídos aleatoriamente para os tratamentos de acordo com sexo e peso corporal (PC) aos 28 dias de vida. No primeiro estudo, quarenta e cinco bezerros Holandeses foram distribuídos em 1 de 3 tratamentos dietéticos. Três TMRs com teor crescente de silagem de milho de planta inteira [0, 10 ou 20% com base na matéria seca] foram comparados: 0SM, 10SM ou 20SM, respectivamente. Os bezerros foram desaleitados gradualmente dos 52 aos 56 dias de idade, e foram avaliados por mais 14 dias após o desaleitamento. O consumo de dieta sólida foi medido diariamente, enquanto o PC e os indicadores metabólicos do metabolismo intermediário foram avaliados semanalmente. Amostras ruminais foram coletadas para determinar o pH ruminal e as concentrações de ácidos graxos voláteis (AGV). A análise comportamental foi realizada nas semanas 7 (pré-desaleitamento) e 10 (pós-desaleitamento). No segundo estudo experimental, quarenta e oito bezerros Holandeses foram distribuídos em 1 de 4 tratamentos. Os tratamentos consistiram em concentrado moído grosseiramente sem forragem (CON); ou ração total misturada contendo 7,5% com base na MS de feno de Tifton-85 de média qualidade (FM) ou baixa qualidade (FB); ou 10% com base na MS de silagem de milho (SM). O consumo de dieta sólida, desempenho, PC, indicadores metabólicos do metabolismo intermediário, líquido ruminal e comportamento foram realizados conforme descrito no experimento 1. Duas semanas após o desaleitamento, foram abatidos 5 bezerros por tratamento. As partes anatômicas do trato gastrointestinal foram pesadas com e sem conteúdo e realizada análise histológica. A inclusão de 10% de silagem de milho na ração total de bezerros leiteiros maximizou o consumo de ração e antecipou os ciclos de ruminação pré e pós-desaleitamento. Além disso, os resultados sugerem que 7,5% de feno, independentemente da qualidade, e 10% de silagem de milho na ração total com alto teor de amido aumentaram o consumo de alimentos sólidos, beneficiam a saúde ruminal e promovem maior preenchimento intestinal sem efeitos negativos no peso corporal final. Conclui-se que todas as fontes de forragem incluídas na ração total apresentaram benefícios sobre o consumo de ração e comportamentais, reforçando a necessidade de fibra da forragem nas dietas pré e pós-desaleitamento.

Palavras-chave: Enchimento intestinal, Efetividade, Ruminação

ABSTRACT

Forage sources in total mixed rations for dairy calves: effects on performance, metabolism, behaviour, and development of the gastrointestinal tract

This study aimed to investigate total mixed ration with increasing levels of whole-plant flint corn silage (WPFCS) in the diet of dairy calves and its effects on performance, metabolism, and behavior. In addition, evaluate effects of forage inclusion and sources on performance, metabolism, feeding behavior, and development of the gastrointestinal tract of dairy calves. For this study, two experiments were carried out. In both experiments, during the first 28 days of life, all calves received 3 L of whole milk twice daily, a commercial pelleted starter, and no forage ad libitum. After that, the solid diet was changed to the respective dietary treatments. Calves were blocked and randomly assigned to dietary treatments according to sex and body weight (BW) at 28 days of life. In the first experimental study, forty-five Holstein calves were assigned to 1 of 3 dietary treatments. 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. Calves were gradually weaned from 52 to 56 days of age but were evaluated for an additional 14 days postweaning. Feed intake was measured daily, while body weight (BW) and metabolic indicators of intermediate metabolism were evaluated weekly. Rumen samples were taken to determine rumen pH and volatile fatty acid (VFA) concentrations. Behavioral analysis was carried out on weeks 7 (preweaning) and 10 (postweaning). In the second experimental study, forty-eight Holstein calves were assigned to 1 of 4 dietary treatments. Treatments consisted of a no-forage **coarsely ground** starter (CON); or total mixed ration containing 7.5% on DM basis of *Tifton* hay of either medium quality (MH) or low quality (LH); or 10% on DM basis of corn silage (CS). Feed intake, performance, BW, metabolic indicators of intermediate metabolism, ruminal fluid, and behavior were carried out as described in experiment 1. Two weeks after weaning, 5 per treatment, were harvested. The anatomical parts of the gastrointestinal tract were weighed with and without contents, and histological analysis was conducted. The inclusion of 10% corn silage in the TMR of dairy calves maximized feed intake and anticipated the rumination cycles pre- and post-weaning. In addition, the results suggest that 7.5% of hay, regardless of the quality, and 10% of corn silage in high-starch mixed diets increased solid feed intake, and benefit rumen health and promote greater gut fill without negative effects on final body weight. In conclusion, all forage sources included in the TMR showed feed intake and behavior benefits reinforcing the need for fiber from forage in pre- and post-weaning diets.

Keywords: Corn silage, Gut fill, Effectiveness, Tract filling, Rumination

1. INTRODUCTION

Replacement dairy calves represent the second highest production cost for dairy farms; and feed accounts represent 70% of these costs (Brown et al., 2005). In this sense, recent research is constantly looking for improvements in management and nutrition to reduce costs during the initial period, focusing attention on the acceptability of ingredients to promote early intake of a solid diet and high growth rates.

Feeding diets high in starch and reduced particle size can result in a high fermentation rate and VFA production (Laarman et al., 2012), which would benefit rumen development. However, as they age, the increasing intake reduces rumen pH and may depress the dry matter intake (Hill et al., 2008; Mojahedi et al., 2018; Toledo et al., 2020). In these situations, it is common to observe variations in the solid diet intake around weaning, which can delay the digestive tract development process and reduce weight (Kehoe et al., 2018).

Studies that evaluated the provision of forage during the preweaning period, mainly in the form of chopped hay, observed improvements in starter intake and, consequently, weight gain during the transition phase (Castells et al. 2012; Daneshvar et al. 2015; Toledo et al. 2020). This effect is observed especially when the concentrate used has reduced particle size, such as coarsely ground or pellet, and diets that use highly processed grains rich in carbohydrates (Beiranvand et al., 2013; Daneshvar et al., 2015; EbnAli et al., 2016). Furthermore, including forage can benefit individually housed calves, anticipating the rumination activity, increasing the time spent eating, and reducing non-nutritive oral behaviors (Poczynek et al., 2019; Horvath et al., 2020).

Although current data on the role of fiber in the solid diet of calves reinforces the positive effects on gastrointestinal development and potential increase in productivity in future life, the different sources of forage in total mixed diets and the benefits on performance need to be investigated. Elucidating the effects of different fiber sources in the calves' diet can help develop feeding programs for this transition period, allowing calves to express their productive potential.

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2. WHOLE-PLANT FLINT CORN SILAGE INCLUSION IN TOTAL MIXED RATIONS FOR PRE - AND POST -WEANED DAIRY CALVES

Abstract

Assuming that acetic acid plays a minor role in the development of ruminal epithelium of pre-weaned dairy calves, the supply of fiber for growing calves has been neglected. A greater research focus has been done in including starch and non-fibrous carbohydrates in solid feed for pre-weaned 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 an essential step for helping calves to 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 days 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 days of life, the calves were managed homogenously 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 days of age but were evaluated for an additional 14 days postweaning. Feed intake was measured daily, while body weight (BW) and metabolic indicators of intermediate metabolism were evaluated weekly. Ruminal fluid was collected at 6, 8, and 10 weeks of age. Behavioral analysis was carried out at weeks 7 (preweaning) and 10 (postweaning). There was a quadratic effect for dry matter intake (DMI) from week 7 until week 10, with higher intake for the 10CS diet as compared with the 0CS and 20CS diets. Consequently, the 10CS diet also promoted greater average daily gain (ADG) at weeks 8 and 9 compared with the 0CS and 20CS diets. However, the final BW was not affected by the different solid diets. Silage inclusion in calves' diet positively affected time spent ruminating and chewing pre- and post-weaning. The inclusion of fermented forages in early life as a fiber source did not impact the BW but tended to increase ruminal pH of pre- and post-weaned dairy calves. The inclusion of 10% corn silage in the TMR of dairy calves maximized feed intake. In addition, 10% of corn silage anticipated the rumination cycles pre- and post-weaning.

Keywords: Corn Silage, Effective Fiber, Forage, Rumination.*

2.1 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). While this benefits

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*A. F. Toledo, S. C. Dondé, A. P. Silva, A. M. Cezar, M. G. Coelho, C. R. Tomaluski, G. F. Virgínio Jr, J. H. C. Costa, C. M. M. Bittar. Whole-plant flint corn silage inclusion in total mixed rations for pre - and post -weaned dairy calves. Journal of Dairy Science, Volume 106: 6185–6197. March 2023. https://doi.org/10.3168/jds.2023-23494.

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 Junior 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 (Castells et al., 2012; Khan et al., 2011). 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 to calf starters with NDF from non-forage sources. 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.

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

The Flint-type corn (*Zea mays ssp*. dentura) 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 facilitate management for farmers who already have this forage available.

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.

2.2 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 (range 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 (Protocol no. 8560150621).

Animals, experimental design, and treatments

Forty-five newborn Holstein calves (36 males and 9 females; 36.89 ± 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 ± 0.21% Brix, suggesting no failure of passive immune transfer (Godden et al., 2019).

The calves were individually housed, initially in suspended pens (1.13×140 cm) until 14 days of age, and then in wood hutches (1.35 m height, 1 m width, and 1.45 m depth), when 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 6L/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% EE, 10.2% ash, 35.7% NFC, 22.4% Starch), and after 28 days 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 ± 1.212 kg) and distributed in three treatments with 15 calves each: 1) 0CS - Total mixed ration with 0% of WPFCS; 2) 10CS – Total mixed ration with 10% of WPFCS; 3) 20CS – Total mixed ration with 20% of WPFCS. The treatment diets were formulated to be isonitrogenous since 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 weeks 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.

Table 1. Ingredients and chemical composition of experimental solid feed diets

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[2]	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[3] > 4 mm, %	0.34	2.63	4.85	44.89
peNDF[3] > 1.18 mm, %	10.62	12.52	16.40	50.15

[1]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. NFC = non-fiber carbohydrates.

[2]Composition: Ca 20%; P 6.5%; F 650 ppm; K 1%; Mg 7%; S 0.7%; Co 25 ppm; Cu 800 ppm; Cr 20 ppm; I 40 ppm; Fe 1400 ppm; Mn 1500 ppm; Si 18 ppm; Zn 3200 ppm; Vit. At 140,000 IU / kg; Vit. D3 50,000 IU / kg; Vit. E 1500 IU / kg; Vit. B1 250, Vit B2 250,000 ppm; Vit B6 250 ppm; Vit. B12 250 ppm; Niacin 400 ppm; B.C. Pantothenic 500 ppm; B.C. Folic acid 20 ppm; Biotin 10 ppm; B.H.T 800 ppm, Sodium monensin 900 ppm.

[3]Physically effective neutral detergent fiber.

Weaning, measurements, and sampling

All calves received 6 L/d of whole milk divided into two meals (07:00 and 17:00 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 per day), 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 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 4th week of life, using three 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 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 1mm 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, USA; AOAC International, 2002; method 968.06), and CP was calculated by multiplying the total nitrogen by 6.25. Ether extract (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 Ankon 2000 fiber analyzer (Ankom Tech. Corp., Fairport, NY). Heat stable α-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: NFC (%) = 100% − (% NDF + % CP + % fat + % 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 three 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 hours 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 ± 0.13% solids, 3.96 ± 0.10% fat, 3.30 ± 0.05% protein, and 4.44 ± 0.04% lactose) by Fourier transform infrared spectroscopy (Lefier et al., 1996). The concentration of total milk solids was used to calculate total dry matter intake 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 weeks 6, 8, and 10. The sampling was performed oroesophageally 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°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): (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 ≥ 3, when the rehydration protocol based on oral fluid administration (dextrose, salt, and bicarbonate) was provided 6 hours 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 two hours after morning feeding at weeks 6, 8, and 10, and 4 g of fecal matter was added to 4 mL of deionized 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 variables 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 (07:00 h) for 10 hours at weeks 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 the SAS (2002). The P-value ≤ 0.05 was adopted as a significant effect and considering trend $0.05 > P < 0.10$. The following statistical model was used for the variables analyzed as repeated measures over time: $Yijk = \mu + Di + bj + eij + Ik + (Di)Ik + eijk$. Where μ = overall mean; Di = fixed effect of diet; bj = random effect of block; eij = residual error (A); Ik = fixed effect of age; (Di)Ik = fixed effect of diet × age interaction, and eijk = residual error (B). Covariance matrices were tested and defined according to the lowest value obtained for "Akaike's Information Criterion corrected" (AICC). Fixed variables were evaluated using the following statistical model: $Yij = \mu + Di + bj + eij$. Where μ = overall mean; Di = diet effect; bj = random effect of block; and eij = 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.

2.3 Results

Chemical composition of experimental diets

The WPFCS used in the experimental diets followed the average chemical composition commonly observed in Brazil, with 25% of starch and 50% of NDF (Table 1). The silage *in vitro* NDF digestibility indicates that diet fiber quality is above expectation compared to other roughages commonly used to feed calves. The KPS was 70%, considered an excellent level, leaving the starch more available for use (Ferreira and Mertens, 2005). Also, the percentage of long particles (8- and 19-mm sieves) was lower than 10%, reducing the probability of feed selection 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 1 - A; Table 2). There was no diet effect at weeks 5 and 6; however, a quadratic effect was observed for weeks 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 weeks 9 and 10. A similar response was observed for total solid feed DMI expressed as a percentage BW (DMI%BW) pre- and postweaning.

There was an interaction between diet and age for NDF intake during preweaning and for peNDF > 4.00 mm intake during the pre and postweaning period (Figure 1 - B). There was no solid feed diet effect at week 5 for NDF intake; however, at week 6, we observed a trend for a linear increasing effect ($P = 0.09$) with the inclusion of silage in the diet. From weeks 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 weeks 9 and 10 ($P = 0.04$).

At weeks 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 1 - B). From week 7 to 8 we observed a quadratic effect, and calves fed 20CS and 10CS diets had the highest peNDF > 4.00 mm intake (*P* = 0.05). The same effect was observed postweaning (*P* = 0.03).

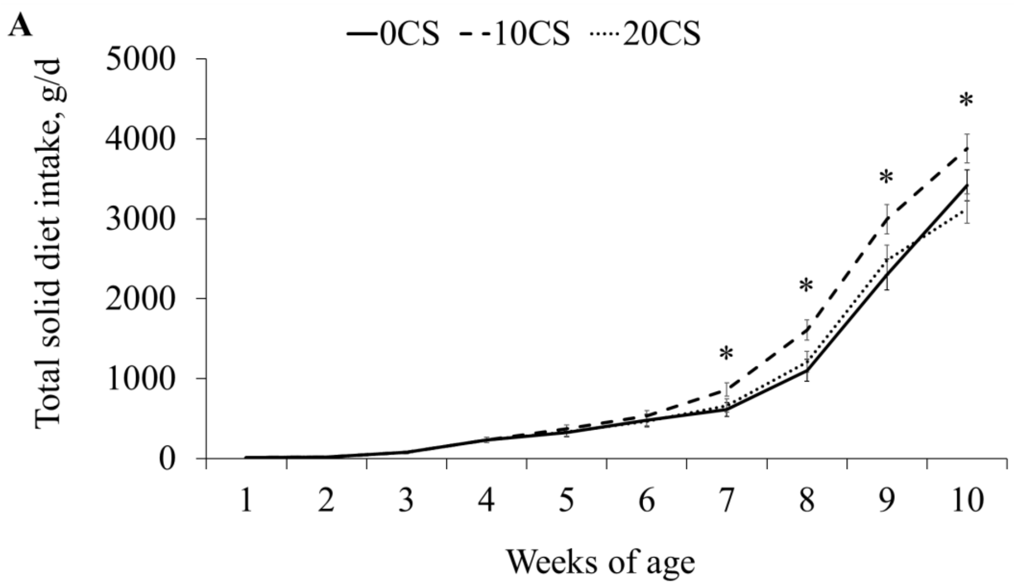
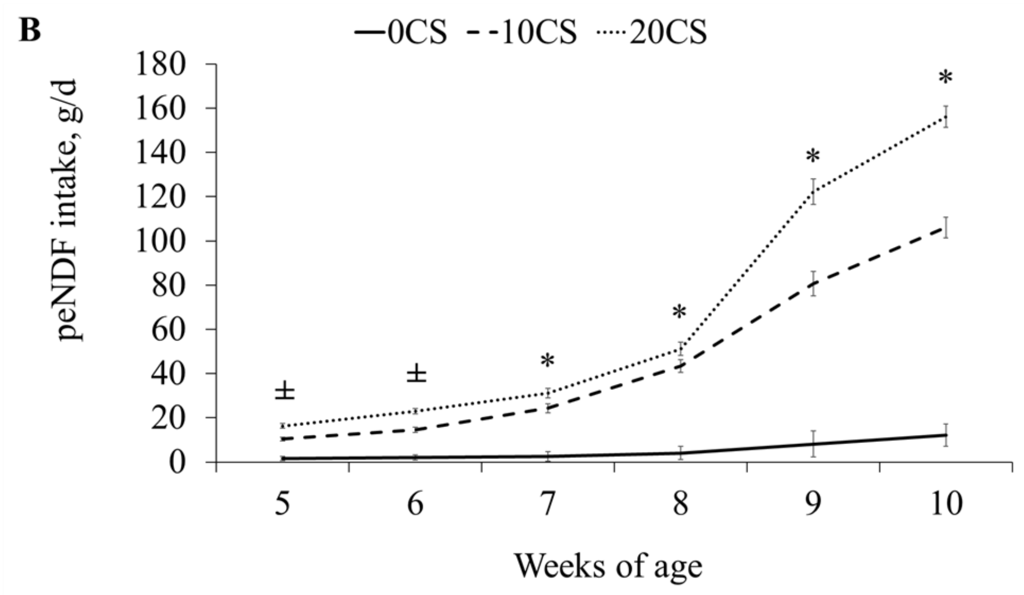


Figure 1. (A) Total solid diet intake (g/d) of calves during the whole period. *Denotes quadratic effect. Calves that received the 10CS diet had a higher total solid diet intake compared to the 0CS and 20CS diets ($P < 0.05$). Calves (n = 15/treatment) were fed with 0CS = 0% inclusion of corn silage on the diet; 10CS = inclusion of 10% corn silage on the diet; 20CS = inclusion of 20% corn silage on the diet.



(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/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.

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 weeks 5 and 6; however, we observed a quadratic trend effect at weeks 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 weeks 5 and 6, but calves that received the 10CS diet had a higher starch intake at weeks 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 received 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 2- A). There was no diet effect at week 5, but at week 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 week 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 2 – B). The solid diets also affected the ADG in the postweaning period; and at week 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.

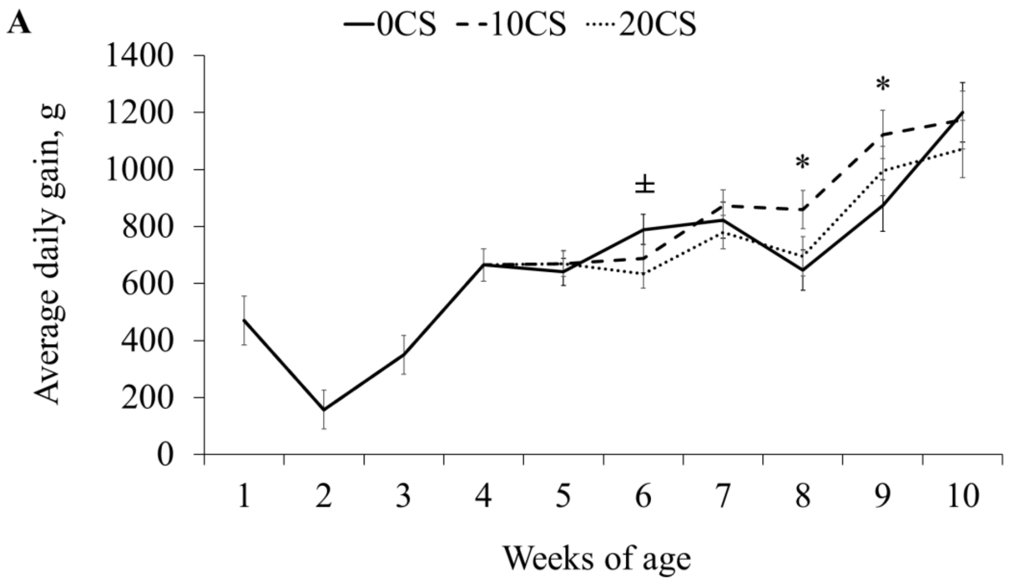
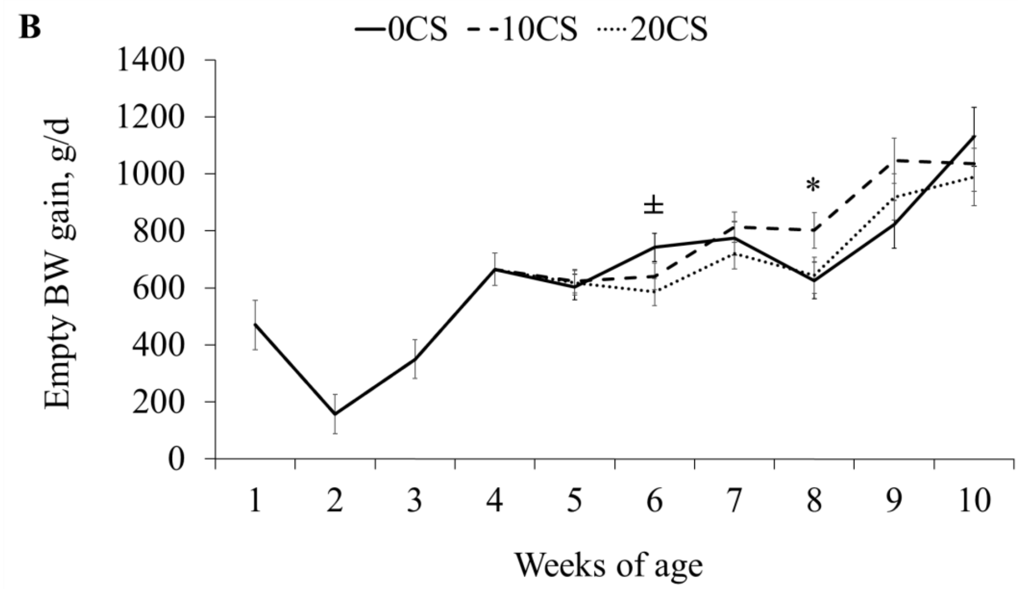


Figure 2. (A) Average daily gain (g/d) of calves during the whole period. ± Denotes decreasing linear effect ($P = 0.03$) for the average daily gain of the calves with the increase of inclusion of silage in the diet. *Denotes quadratic effect. Calves that received the 10CS diet had a greater average daily gain than the 0CS and 20CS diets. ($P < 0.05$).



(B) Empty body weight gain (g/d) of calves during the whole period. ± Denotes decreasing linear effect (P = 0.02) for the 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/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.

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 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.

Table 2. Performance of calves in the preweaning (d28-56) and postweaning periods (d57-70), fed with 0%, 10% and 20% of whole-plant flint corn silage in solid feed diets.

	Diet[1]			SEM	*P*-value[2]			
Item	0CS	10CS	20CS		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 > 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[4]	690.0	719.5	641.9	35.22	0.34	0.21	0.01	0.06
Gain:feed ratio[3]	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	3,436	2,805	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	1,417	1114,7	75.26	0.16	0.02	< 0.01	0.01
Growth (g/d)								
ADG	1,038.8	1,116.3	1,034.0	76.62	0.96	0.38	0.04	0.09
EBWG[4]	978.1	1,040.6	954.7	70.30	0.82	0.39	0.07	0.15
Gain:feed ratio[3]	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	-	-

[1]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.
[2]L = linear effect; Q = quadratic effect; A = age effect; D × A = interaction between diet and age.
[3]Considering liquid and solid DMI.
[4]Empty BW gain was calculated according to equations given by Jahn and Chandler (1976).

Ruminal and fecal variables

There was no interaction between diet and age for ruminal and fecal variables at weeks 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, acetate: propionate ratio was affected by age, with an increase in concentrations from weeks 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 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, and the interaction of these factors.

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

	Diet[1]			SEM	*P*-value[2]			
Item	0CS	10CS	20CS		L	Q	A	A×D
Total SCFA, mM	110.2	106.5	102.1	3.99	0.09	0.93	0.03	0.91
SCFA, mM/100 m*M*								
Acetate	58.8	58.8	59.9	0.70	0.28	0.51	< 0.01	0.45
Propionate	34.5	34.6	33.6	074	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[3]	1.76	1.76	1.83	0.065	0.44	0.65	< 0.01	0.86
NH3-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

[1]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.

[2]L = linear effect; Q = quadratic effect; A = age effect; D × A = interaction between diet and age.

[3]C2 = acetic acid; C3 = propionic acid.

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).

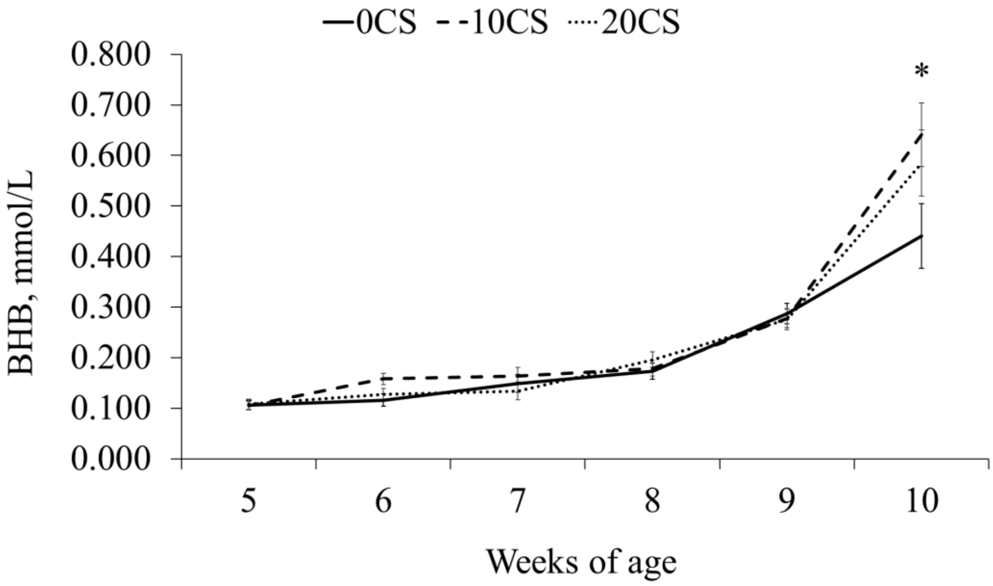


Figure 3. β-hydroxybutyrate concentration (BHB; mmol/L) of calves in the preweaning, d28-56, and postweaning, d57-70, feeding with different levels of corn silage in the diet. 0CS = 0% inclusion of corn silage on the diet; 10CS = inclusion of 10% with silage on the diet; 20CS = inclusion of 20% with silage on the diet; 15 calves per treatment. *Denotes trend of quadratic effect. The BHB increased at week 10 for calves fed the 10CS and 20CS diet compared to the 0CS ($P = 0.08$). Calves (n=15/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.

The BHB concentration increased at week 10 for calves fed the 10CS and 20CS diet compared to 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.

Table 4. Blood metabolites of calves in the preweaning (d28-56) and postweaning periods (d57-70), fed with 0%, 10% and 20% of whole-plant flint corn silage in solid feed diets.

	Diet[1]			SEM	*P*-value[2]			
Item	0CS	10CS	20CS		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

[1]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.

[2]L = linear effect; Q = quadratic effect; A = age effect; D × A = interaction between diet and age.

Behavior variables

At week 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 week 7.

At week 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 the 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.

Table 5. Ingestive behavior of calves in the preweaning (d28-56) and postweaning periods (d57-70), fed with 0%, 10% and 20% of whole-plant flint corn silage in solid feed diets. All values are min/10 h observation period.

	Diet[1]			SEM	*P*-value[2]	
Item	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 / 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	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 / 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	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

[1]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.
[2]L = linear effect; Q = quadratic effect; A = age effect.

2.4 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 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 an increased preweaning intake of corn silage versus alfalfa-based diets (581 vs. 376 g/d). Similar results were observed in this study; the inclusion of 10% of WPFCS with almost 90% of particles ≤ 8mm in the TMR also resulted in higher intake when compared to calves that received no silage. However, the particle size and silage quality can vary drastically (Salvati et al., 2021), and may present variable acceptability and allow 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, the inclusion of 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).

Improved muscular development of the rumen may have contributed to the 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 (Wickramasinghe et al., 2022; Mitchell and Heinrichs, 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 (2022), who observed values of 0.7%.

Understanding the content of peNDF suitable for 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 when compared with the 10% WPFCS diet. As expected, these results suggest that higher silage inclusion rates at this age can impact 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 while the fiber will be fermented slowly in the reticulum-rumen. In addition, corn silage inclusion on a diet for pre-weaned calves may help increase motility, saliva production and entry in the rumen, and rumen colonization by microorganisms, while 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, as well as 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 to the exclusive starter supply (Khloe 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 ingredients from cereal grain provides more energy and protein that allows greater gain, as occurred at week 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 weeks 8 and 9.

The lower digestibility of the diet with WPFCS does not impact efficiency. Despite the greater ADG at week 8, it was not possible to observe differences in calves 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.

Since 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 to 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 et al., 2000).

Despite these limiting aspects, availability of silages with low aerobic stability has significantly decreased in recent years with advances in knowledge and technology in the conservation process (Salvati, 2021). In addition, the presence of acids and the concentration of soluble carbohydrates do not seem to decrease consumption or negatively impact rumen development in young calves (Suaréz et al., 2007).

Ruminal, 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 0CS 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 (Suarez et al., 2007; Terre et al., 2013).

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 0CS diet may have a higher passage rate of smaller particles through the rumen, resulting in greater amounts of 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). Cellulolytic microorganisms use ammonia-nitrogen as substrate to ferment fiber, reducing its concentration in the rumen as observed in the present study (Suarez et al., 2007; Castells et al., 2013). However, rumen samples were collected in just one moment - 2 hours 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 week 10. Thus, the increase in BHB can be related to the 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 rumen, which is absorbed and be converted to blood urea (Funaba et al., 1994). 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 et al., 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 0CS diet and may have occurred to prevent metabolic disorders such as acidosis with advancing age. Also, it was possible to observe that those calves spent more time and 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 both 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, 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 one due to the high fermentation rate resulting in low pH, and the second one 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.

2.5 Conclusion

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.

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3 FORAGE SOURCES IN TOTAL MIXED RATIONS EARLY IN LIFE INFLUENCES PERFORMANCE, METABOLITES, AND BEHAVIOUR OF DAIRY CALVES

Abstract

The objective of this study was to evaluate the effects of forage inclusion and sources on performance, metabolism, and feeding behavior of dairy calves. Forty-eight Holstein calves were blocked and randomly assigned to 1 of 4 dietary treatments according to sex, and body weight (BW) at 28 days of life to determine the effects of feeding forage sources (ensiled and dry), with different quality on performance, metabolites, and behavior. Treatments consisted of a no-forage coarsely ground starter (CON); or total mixed ration containing 7.5% on DM basis of *Tifton* hay of either medium quality (MH) or low quality (LH); or 10% on DM basis of corn silage (CS). During the first 28 days of life, all calves received 3 L of whole milk twice daily, a commercial pelleted starter and no forage, and water ad libitum. After that, the solid diet was changed to the respective dietary treatments. Calves were gradually weaned from 52 to 56 d of age and followed for 14 days post-weaning. Individual solid feed and milk intakes were recorded daily, and BW and metabolic indicators of intermediate metabolism were recorded weekly. Behavior was recorded, and the analysis was conducted on weeks 7 (preweaning) and 10 (post-weaning). Solid feed intake increased at weeks 7 and 8 when MH, LH, and CS were included in TMR; the same results were observed post-weaning. The diets did not affect the average daily gain and body weight, but the feed efficiency increased with the CON diet. The β-hydroxybutyrate concentration was greater in calves receiving TMR containing forage than CON diet. Furthermore, calves supplemented with forage had a greater rumination time. In conclusion, all forage sources included in the TMR showed feed intake and behavior benefits, reinforcing the need for fiber from forage in pre- and post-weaning diets.

Key words: TMR, particle size, fermented forage, effectiveness.

3.1 Introduction

During the preweaning period, dairy calves are commonly offered solid diets high in rapidly fermentable carbohydrates and processed grains to encourage early growth and rumen development. However, additional provision of forage may have a range of benefits, including stimulating intake, improving rumen health, and accommodating behavioral motivations to manipulate feed (Khan et al., 2011; Horvath and Miller-Cushon, 2019).

Previous research suggests that the effects of including forage in the diet of young calves are modulated by forage level, quality, source, method of presentation, and physical form of starter (Daneshvar et al., 2015; EbnAli et al., 2016). This variability in study outcomes has limited consensus on recommendations for the minimum % of NDF, NDF from forage, and physically effective NDF recommendations in the diet of calves with a body weight up to 120 kg (NASEM, 2021). To assist scientific and practical recommendations, studies that characterize these particularities of each forage source area are needed.

The cereal grains and the forage play different roles but complement each other as regards gastrointestinal tract maturation, especially around the weaning process (Kertz et al., 2017; Quigley et al., 2019). The cereal grains contained in the starter are mainly responsible for providing nutrients that meet the nutritional requirement of calves with advancing age and are strongly related to rumen epithelial development in young calves (Quigley et al., 2019; Aragona et al., 2020). On the other hand, the inclusion of forage can also stimulate intake and improve performance, reducing metabolic disorders caused by diets with high inclusion of starch and excessive grain processing (Gimeno et al., 2015; Kazemi-Bonchenari et al., 2017). In addition, calves can be motivated to consume forage, evidenced by sorting in favor of forage in a mixed diet, and provision of forage reduces non-nutritive oral behavior suggesting that it better accommodates a behavioral need to manipulate feed (Engelking et al., 2020).

Providing total mixed rations (TMR) to preweaning calves can result in a balanced diet, avoiding the grain intake replacement by forage observed when fed as a component diet. Feeding TMR might ensure adequate intake of protein and energy, but yet the fiber source, which guarantees a balanced rumen environment, thus increasing performance (Castells et al., 2012; Toledo et al., 2023). Despite that, the sorting behavior must be considered for better diet and fiber sources adjustments, such as particle size and digestibility. In some situations, sorting can lead to digestive disorders, and how sorting behavior develops in calves according to diet changes and early exposure to a TMR with different forage types need further investigation (DeVries et al., 2008; Miller-Cushon and DeVries, 2011).

Dairy calves voluntary forage intake, when offered as a component diet (starter and forage in separate buckets) in the preweaning period, tends to be between 4 and 10% of the total diet, depending on the source and quality (Castells et al., 2012 e 2013; Poczynek et al., 2019; Toledo et al., 2020). The different responses between sources may be related to the nutritional content, such as protein, NDF, and lignin, but also to moisture, digestibility, particle size linked to an increase in gut fill, and palatability that is influenced by texture, leafiness, or compounds that cause a forage to taste sweet, sour, or salty (Ball et al., 2021).

In addition, hay and silage have different characteristics; hay consists almost exclusively of forage, and silage consists of forage and grains. Some corn silage varieties have higher grain content and stover digestibility than others (Ball et al., 2021), and adjusting the forage inclusion in the diet may be necessary to achieve a desired animal response. This study aimed to investigate potential factors related to the performance, metabolites, and behavior that may be involved in the intake regulation of calves when ensiled or dry forage is offered by feeding a TMR to young calves. We hypothesize that calves fed a TMR with a low forage level, regardless of the source and quality, would have greater feed intake and growth and spend more time on nutritional behaviors compared to calves offered no forage.

3.2 Materials and Methods

Animals and treatments

All study procedures were approved by the "Luiz de Queiroz" College of Agriculture – University of Sao Paulo Institutional Animal Care and Use Committee (Protocol no. 8560150621). This study was conducted between July and November of 2022 at the calf facilities of the Animal Science Department of the "Luiz de Queiroz" College of Agriculture, Piracicaba, São Paulo, Brazil. During this period, the average temperature was 21°C (maximum of 30°C and minimum of 12°C), the mean relative humidity was 68%, and the average rainfall was 60.14 mm/ mo.

Forty-eight Holstein calves (36 males; 12 females), with initial BW of 35.60 ± 0.840 kg, from a commercial farm, were enrolled in this study. Calves were separated from their dam at birth, weighed, and fed with colostrum (10% of BW; > 60 mg/L of IgG, 2 h of birth; Godden et al., 2019). A blood sample was collected from the jugular vein 48 h after colostrum feeding, and the brix was analyzed using a handheld refractometer (Deelen et al., 2014). There were no differences (P = 0.34) among groups for passive immune transfer, with an average and standard deviation of 9.77 ± 0.21 % Brix.

The calves were individually housed in suspended pens (1.13×1.40 m) until 14 d of age, and after that in wood hutches (1.35 m height, 1.00 m width, and 1.45 m depth) with a bucket - 10L capacity for water and a rectangular trough (35.0 cm length, 24.0 cm width, 12.0 cm depth), and tethered by chain (2 m long), allowing an area for walking but no physical contact with other calves. A stationary brush was fixed at the side of each wood hutch. Calves received 3 L of whole milk twice daily (6 L/d; fed by a teat bucket) and water ad libitum. During the first 28 d of age, all calves received a commercial pelleted starter (87.4 % DM, 24.6 % CP, 17.7 % NDF, 38.5% NFC, Agroceres Multimix, Rio Claro, Brazil) and no forage. After that, calves were blocked according to their weight at 28 d and sex and distributed randomly into 1 of 4 dietary treatments (Table 1): No-forage coarsely ground starter (CON; n = 12); or a total mixed ration containing coarsely ground grains and varying sources of chopped forage: 7.5 % DM basis of medium quality *Tifton* hay (MH; n = 12), 7.5 % DM basis of low quality *Tifton* hay (LH; n = 12), or 10% DM basis of corn silage (CS; n = 12). The treatment diets were formulated to be isonitrogenous and the TMR to have a similar starch level because we aimed to evaluate and isolate the effects of forage source, composition, and effectiveness, included in the TMR, and determine the possible responses of calves. The corn silage included in the CS diet presented about 31.0% of grains and 69.0% of leaves and stems, resulting in 23.7% starch on a DM basis. Including 10% DM of corn silage in the diet resulted in 7.0% effective forage inclusion.

The weaning began at 52 d of age, with the decrease of 1 L/d, so that calves were completely weaned after 56 d. Calves were followed for 14 d following weaning.

Table 1. Ingredient of experimental diets.

Item	CON	MH	LH	CS
Ingredient, % DM				
Ground corn	55.10	49.00	48.20	45.40
Soybean meal	26.40	25.00	25.80	26.10
Wheat meal	15.00	15.00	15.00	15.00
Medium hay quality	-	7.50	-	-
Low hay quality	-	-	7.50	-
Corn Silage	-	-	-	10.00
Mineral and vitamin supplement[2]	3.50	3.50	3.50	3.50

[1]CON = 0% fiber from forage on the diet; MH = 7.5% medium quality hay on the diet; LH = 7.5% low quality hay on the diet; CS = 10% corn silage on the diet.
[2]Composition: Ca 20%; P 6.5%; F 650 ppm; K 1%; Mg 7%; S 0.7%; Co 25 ppm; Cu 800 ppm; Cr 20 ppm; I 40 ppm; Fe 1400 ppm; Mn 1500 ppm; Si 18 ppm; Zn 3200 ppm; Vit. At 140,000 IU / kg; Vit. D3 50,000 IU / kg; Vit. E 1500 IU / kg; Vit. B1 250, Vit B2 250,000 ppm; Vit B6 250 ppm; Vit. B12 250 ppm; Niacin 400 ppm; B.C. Pantothenic 500 ppm; B.C. Folic acid 20 ppm; Biotin 10 ppm; B.H.T 800 ppm, Sodium monensin 900 ppm.

Measurements and sample collection

The solid feed was offered every morning, just after milk feeding, and was available until the following morning, when orts were weighted for daily intake calculations (9094-Prix, Toledo Ltda., 4 g accuracy from 0 kg to 10 kg). Body weight was recorded weekly on a mechanical scale (ICS-300, Coimma Ltda., Dracena, SP, Brazil) before the morning liquid diet feeding.

Blood samples were collected weekly at 4, 5, 6, 7, 8, 9, and 10 weeks of life, 2 h after the morning feeding, using three 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 g for 20 min at 4 °C.

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

The behavior of each calf was characterized according to the ethogram described in Table 2, through live observation using instantaneous recording at 5 min intervals (as validated for calf feeding time by Miller-Cushon and DeVries, 2015). Behavior was recorded for 10 h beginning after morning feeding (0700 h) at two time points in weeks 7 and 10, for a total observation time of 20 h/calf. Behavior was recorded by 4 trained observers who were blind to treatments.

Table 2. Ethogram describing behaviors of calves for 10 hours observation.

Behavior[1]	Behavior description
Standing	Supported by its limbs
Lying	Lying down with the sternum facing the ground or with the body leaning to the side
Drinking milk	Sucking from the teat bucket during milk supply
Eating solid feed	With the head inside the feeder
Drinking water	With the head inside the water bucket
Sleeping	Lying down without moving and with closed eyes.
Urinating and defecating	Point event of defecating or urinating
Vocalizing	Point event of vocalization
Non-nutritive oral behaviors	Calf licking or sucking any surface or itself
Exploring	Calf interacting with the ground, the brush, and the hutch
Ruminating	Repeated chewing the solid feed

[1]Standing or lying down may be associated with a second activity.

Analytical procedures

Feed samples were collected monthly, to determine the chemical composition of the offered (n = 5), and refusals (n = 5) of each diet. Feed samples were oven-dried (MA035 - Marconi, Piracicaba, São Paulo, Brazil) at 55° C for 24 h, ground in a 1mm Wiley mill (Marconi, Piracicaba, Brazil). The dry matter and ash were determined according to AOAC International (2002, method 925.40; 942.05). Total nitrogen concentration was determined using the Leco TruMac® N apparatus (Leco Corporation, St. Joseph, MI, USA; AOAC

International, 2002; method 968.06), and CP was calculated by multiplying the total nitrogen by 6.25. Ether extract (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 Ankon 2000 fiber analyzer (Ankom Tech. Corp., Fairport, NY). Heat stable α-amylase and sodium sulfite were included in the NDF analysis. The non-fiber carbohydrates (NFC) content of the diets was estimated according to Mertens, (1997).

Samples of solid feed, chopped hay, corn silage, and each calf solid feed refusals were stored after the 10 h observation period for chemical composition and particle size analysis to evaluate particle sorting. The ingredients were separated using a Penn State 4-screen particle size separator (> 19 mm; > 8 mm: > 4 mm; > 1.18 mm, and bottle), and physically effective NDF (peNDF) was calculated considering particles > 4 mm, which were retained on the top three screens of the Penn State Particle Separator (Yang and Beauchemin, 2006). The in vitro true digestibility over 72 hours was determined according to Goering and Van Soest (1970).

The concentration of milk solids was used to calculate total dry matter intake and feed efficiency. Sorting behavior was quantified as the actual intake of each fraction (long: >19 mm, medium: < 19, > 8 mm, short: < 8, > 4 mm, and fine: < 4 mm), expressed as a percentage of the predicted intake of each fraction (Leonardi and Armentano, 2003). The predicted intake of each fraction was calculated as the product of the dry matter intake of the feed offered and the dry matter percentage of that fraction in the fed TMR. Values >100% indicate sorting for that particle size, and values < 100% indicate sorting against that particle size.

Statistical analysis

Data were screened for normality before analysis using the PROC UNIVARIATE, and then analyzed using the PROC MIXED procedure of SAS 9.4 (SAS Institute, Inc., Cary, NC, USA). The 48 calves were randomized into 12 complete blocks according to weight at 28 days of life and sex (9 males and 3 females). Feed intake, performance, and blood metabolites were analyzed as repeated measures over time: $Yijk = \mu + Di + bj + eij + Ik + (Di)Ik + eijk$. Where μ = overall mean; Di = fixed effect of diet; bj = random effect of block; eij = residual error (A); Ik = fixed effect of age; (Di)Ik = effect of diet × effect of age interaction, and eijk = residual error (B). Covariance matrices were tested and defined according to the lowest value obtained for "Akaike's Information Criterion corrected" (AICC). Ingestive behavior was evaluated as a non-repeated measure using the following statistical model: $Yij = \mu + Di + bj + eij$. Where μ = overall mean; Di = diet effect; bj = random effect of block; and eij = residual error. For all response variables, the means were obtained using the LSMEANS command. The model included the effects of diet, week (age of calves), and the interaction between diet and age 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. Data were analyzed separately by period: preweaning (28–56 d) and post-weaning (57–70 d) periods. Orthogonal contrasts were used to analyze 3 preplanned comparisons between treatment groups: 1) comparison between calves receiving no forage and calves receiving any forage source: control × forage (CON × F; where F = LH + MH + CS calves); 2) comparison between calves receiving corn silage and calves receiving hay: corn silage × hay (CS × H; where H = LH + MH); and 3) comparison between calves receiving hay of different quality: hay – medium quality × hay – low quality (MH × LH). The *P*-value ≤ 0.05 was adopted as a significant effect, and $0.05 > P \leq 0.10$ was considered as a trend.

3.3 Results

Diet and forage characteristics

The level of NDF, ADF, and lignin is higher in the low-quality hay, followed by medium-hay and corn silage (Table 3). On the other hand, the DM in vitro true digestibility over 72 hours is higher for the CON, followed by MH, CS, and LH diets. However, NDF digestibility is higher for MH, followed by CS, CON, and LH diets. The DM of the diets was similar, except for the lower value for CS (Table 3). As designed, diets contained similar levels of CP. The NDF of the diets was lower for the CON diet, followed by the CS diet, and higher values for MH and LH diets. The same effect was observed for ADF. On the other hand, the starch content of the CON diet was higher, followed by lower levels for the TMR with forage inclusion. In addition, the NFC remained higher for the CON diet, followed by the CS diet, and lower levels for MH and LH diets.

The hay particle distribution was similar, regardless of the hay quality, with a higher percentage between the 8- and 1.18-mm sieves. Therefore, peNDF > 4 mm of the two diets containing hay were similar, with lower values for the CS diet. On the other hand, the corn silage showed a higher percentage of particles distributed between the 19- and 8-mm sieves. The average particle size of corn silage was greater than that of medium or low-quality hay (9.80; 4.04, 3.91 mm, respectively), and the CS diet presented a greater average particle size, followed by the MH, LH, and CON diets.

Table 3. Chemical composition and particle size of experimental diets and forages

Item	CON	MH	LH	CS	Medium quality hay	Low quality hay	Corn Silage
DM, %	87.32	86.74	86.70	77.43	85.17	85.57	36.88
Chemical composition, % DM							
CP	22.48	21.82	21.54	22.53	17.00	13.23	8.20
NDF	17.54	21.66	21.38	19.35	71.30	77.30	43.78
ADF	6.66	10.22	11.80	9.25	35.43	38.87	24.28
Lignin	-	-	-	-	4.00	5.20	2.85
Ash	6.18	5.94	5.70	6.75	7.30	7.60	3.80
Ether extract	3.82	3.86	4.38	4.00	0.93	0.90	3.15
Starch	47.72	41.14	42.84	41.18	1.40	2.70	23.70
NFC	54.18	46.92	47.00	49.05	15.20	12.90	36.60
In vitro digestibility, %							
DM	83.00	79.80	77.50	79.50	60.00	55.70	60.50
NDF	52.10	54.20	51.20	53.20	57.00	50.10	53.80
Particle size distribution, %							
19 mm	0.00	0.00	0.00	1.61	0.00	0.00	14.88
8 mm	0.00	2.33	2.92	6.85	21.69	23.08	55.87
4 mm	3.84	19.19	14.82	13.91	26.51	29.23	16.19
1.18 mm	79.09	65.12	67.64	66.94	32.53	32.31	10.44
Bottom pan	17.07	13.37	14.61	10.69	19.28	15.38	2.61
peNDF[2] > 4 mm, %	0.75	3.70	3.12	2.81	34.82	37.45	37.76
Average Particle Size (mm)	2.10	2.55	2.46	2.74	3.91	4.04	9.80

[1]CON = 0% fiber from forage on the diet; MH = 7.5% medium quality hay on the diet; LH = 7.5% low quality hay on the diet; CS = 10% corn silage on the diet.

[2]peNDF: physically effective neutral detergent fiber > 4 mm.

Intake and growth performance

During the preweaning period, a significant interaction was observed between diet and age for solid feed intake (Figure 1 - A; Table 4). There was no diet effect at weeks 5 and 6, however, the inclusion of forage increased the solid feed intake at week 7 (*P* = 0.02) and tended to increase at week 8 (*P* = 0.07). In addition, at week 8, calves fed both TMR containing hay (MH and LH) had greater solid feed intake than animals fed with CS (*P* = 0.03), and calves fed with LH tended to have greater solid feed intake than MH (*P* = 0.07). Including forage in the TMR also increased the solid feed intake during the post-weaning period; however, there was no difference among the evaluated forage sources. A similar response was observed for solid feed intake expressed as a percentage of BW, pre- and post-weaning.

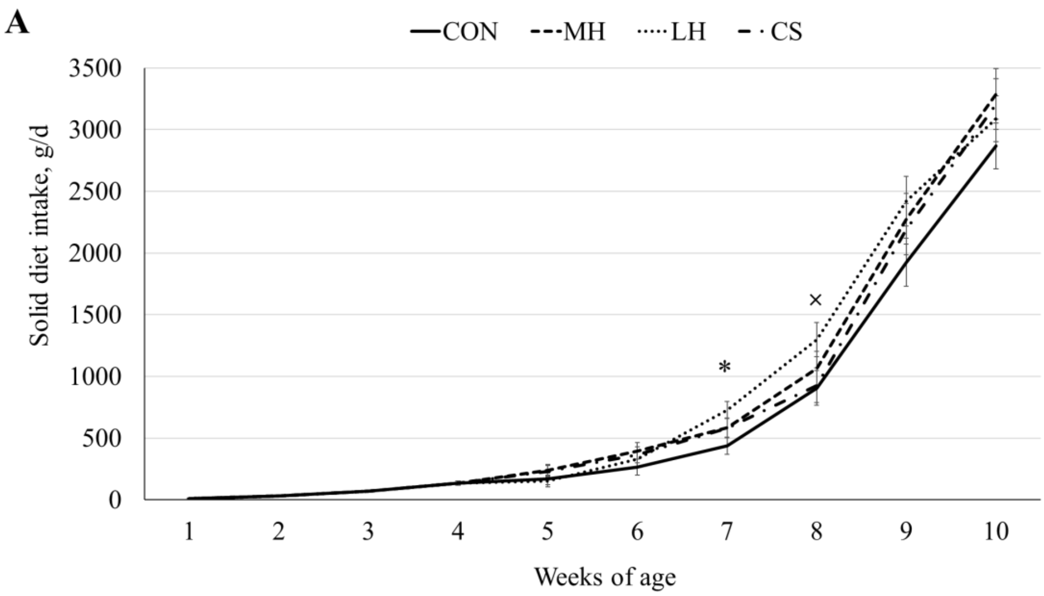

Figure 1. (A) Solid diet intake (g/d) of calves during the whole period. *Denotes forage effect. Calves that received the MH, LH, and CS diets had a higher solid diet intake compared to CON (*P* = 0.02); ×Denotes CS and Hay effects. Calves fed with hay, MH and LH, presented higher solid diet intake than animals fed with CS (*P* = 0.03), and calves fed with LH tended to present higher solid diet intake than MH (*P* = 0.07).

There was an interaction between diet and age for NDF intake during the preweaning period (Figure 1 - B). There was no diet effect at week 5 for NDF intake; however, at week 6, TMR with forage increased NDF intake compared to CON (*P* = 0.04), with no difference among the evaluated forage sources. At weeks 7 and 8, the inclusion of forage also increased NDF intake (*P* = 0.02), and calves fed MH or LH had greater NDF intake than animals fed CS

($P < 0.05$). In addition, between the calves provided different hay qualities, calves fed LH had greater NDF intake than MH ($P = 0.02$). During the post-weaning period, the inclusion of forage in the diet increased the NDF intake, and calves fed with hay (MH and LH) also had greater NDF intake compared to CS. A similar effect was observed for NDF intake as a percentage BW (NDF % BW), pre – and post-weaning.

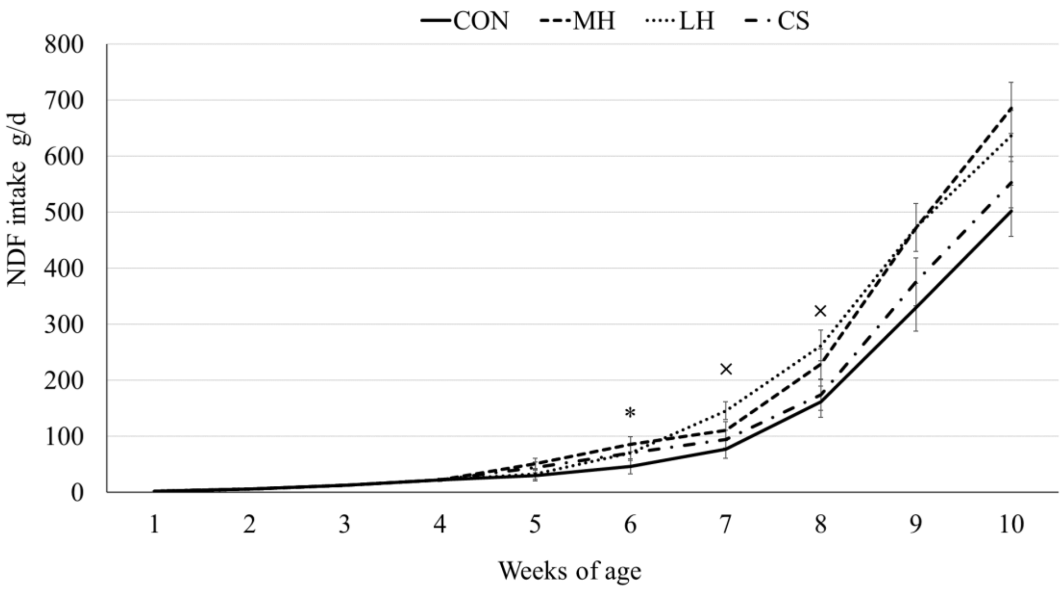

Figure 1. (B) Neutral detergent fiber intake (NDF; g/d) of calves during the whole period. *Denotes forage effect. Calves that received the MH, LH, and CS diets had a higher NDF intake compared to CON ($P = 0.04$); × Denotes forage effects between the sources. Calves fed with hay, MH and LH, presented higher NDF intake than animals fed with CS ($P < 0.05$), and calves fed with LH presented higher NDF intake than MH ($P = 0.02$).

There was an interaction between diet and age for peNDF > 4.00 mm intake during the pre – and post-weaning periods (Figure 1 – C). The inclusion of forage increased the peNDF intake during weeks 5 to 10 ($P < 0.01$). In addition, the TMR with hay, MH and LH, increased the peNDF intake compared to CS during weeks 7 to 9 ($P = 0.01$), and MH increased the peNDF intake compared to LH at week 10 ($P = 0.01$).

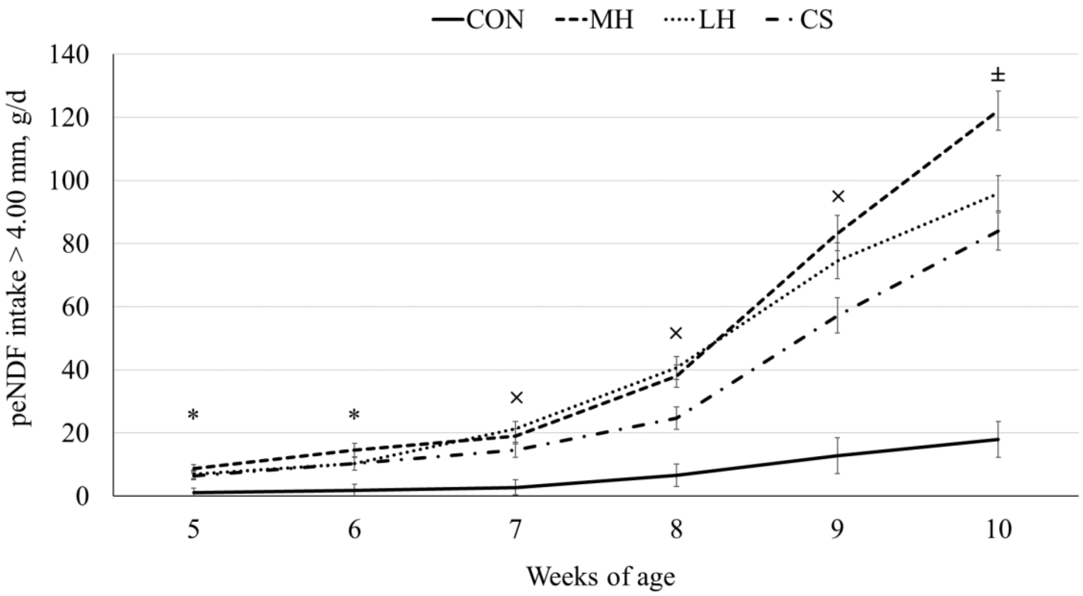

Figure 1. (C) Physically effective neutral detergent fiber intake (peNDF; g/d) of calves during the whole period. *Denotes forage effect. Calves that received the MH, LH, and CS diets had a higher peNDF intake compared to CON (P = 0.04); × Denotes forage effects between the sources. Calves fed with hay, MH and LH, presented higher peNDF intake than animals fed with CS ($P < 0.05$); ± Denotes forage effects between the sources. Calves fed with MH presented higher peNDF intake than LH (P = 0.01).

The experimental diets did not affect the age at which the calves achieved 15 kg of cumulative NFC intake (64.5, 64.3, 63.9, 65.3 ± 1.48 – CON, MH, LH, CS, respectively), although the forage diets had lower starch content (P = 0.92).

The diet did not affect the ADG in the pre- or post-weaning periods (Table 4). All calves had similar BW at the beginning, at weaning (d 56), and at the end of the study (d 70). Consequently, diet affected feed efficiency of calves during the preweaning period, with greater feed efficiency in calves fed with CON compared to calves receiving a forage source. In contrast, no diet effect was observed for feed efficiency in the post-weaning period.

Table 4. Preweaning and post-weaning feed intake and performance of calves fed with different sources and qualities of fiber in total mixed diets.

Item	Diet[1]				SEM	*P*-value[2]				
	COM	MH	LH	CS		CON×F	CS×H	MH×LH	A	A×D
Feed intake, g of DM/d										
Preweaning (d 28-56)										
Liquid diet	677.37	675.25	677.79	673.39	7.289	0.80	0.69	0.78	< 0.01	1.00
Solid diet	425.21	552.74	625.92	508.81	73.281	0.05	0.26	0.37	< 0.01	0.04
Solid diet, % BW	0.67	0.88	0.98	0.79	0.106	0.05	0.22	0.48	< 0.01	0.02
NDF	74.36	115.80	131.83	96.23	14.362	0.01	0.10	0.40	< 0.01	0.02
NDF, % BW	0.12	0.19	0.21	0.15	0.022	0.01	0.06	0.43	< 0.01	0.01
peNDF > 4 mm	2.68	19.72	19.03	13.63	2.051	< 0.01	0.02	0.79	< 0.01	< 0.01
ADG, g/d	637.80	619.86	649.56	687.63	31.499	0.66	0.18	0.47	< 0.01	0.48
Feed efficiency[3]	0.599	0.535	0.515	0.554	0.0256	0.02	0.34	0.57	0.02	0.75
BW, kg										
Initial (d 28)	48.33	47.23	48.13	48.09	1.905	0.51	0.62	0.34	-	-
At weaning (d 56)	64.85	64.21	65.78	64.51	2.577	0.99	0.75	0.39	-	-
Post-weaning (d 57-70)										
Solid diet	2,371	2,801	2,698	2,667	187.121	0.05	0.67	0.64	< 0.01	0.39
Solid diet, % BW	3.13	3.67	3.53	3.47	0.196	0.04	0.57	0.58	< 0.01	0.58
NDF	416.07	579.08	554.49	464.28	42.477	0.01	0.03	0.63	< 0.01	0.39
NDF, % BW	0.547	0.755	0.735	0.610	0.0427	0.01	0.01	0.74	< 0.01	0.92
peNDF > 4 mm	14.87	102.81	83.45	70.09	5.732	< 0.01	0.01	0.03	< 0.01	< 0.01
ADG, g/d	1,055	1,069	1,015	986.88	90.564	0.74	0.59	0.65	0.05	0.46
Feed efficiency[3]	0.455	0.415	0.377	0.403	0.032	0.12	0.86	0.41	< 0.01	0.99
Final BW (d 70)	79.02	79.04	78.65	76.85	3.755	0.74	0.45	0.90	-	-

[1]CON = 0% fiber from forage on the diet; MH = 7.5% medium quality hay on the diet; LH = 7.5% low quality hay on the diet; CS = 10% corn silage on the diet; 12 calves per treatment. [2]Contrastst between groups (Control × Forage; Corn silage × Hay; Medium quality hay × Low quality hay); A = age effect; D × A = interaction between diet and age.

[3]Considering liquid and solid diet intake.

Blood metabolites

There was no interaction between diet and age for blood metabolites (Table 5). The lactate concentration was higher in calves receiving CON than MH, LH, and CS diets during the preweaning period. In contrast, the BHB concentration was higher in calves receiving forage (MH, LH, and CS) than CON diets. The glucose, lactate and BHB concentrations were not affected by diets during the postweaning period. All blood metabolites were affected by age, except for lactate during the postweaning period, with decreased glucose concentration and increased BHB as calves aged.

Table 5. Preweaning and post-weaning blood metabolites of calves fed with different sources and qualities of fiber in total mixed diets.

	Diet[1]				SEM	*P*-value[2]				
	CON	MH	LH	CS		CON×F	CS×H	MH×LH	A	A×D
Preweaning, d 28–56										
Glucose, mg /dl	113.29	115.60	108.39	108.90	3.561	0.57	0.47	0.15	< 0.01	0.83
Lactate, mg /dl	12.18	10.83	10.92	10.92	0.558	0.03	0.95	0.91	< 0.01	0.71
BHB[3], mmol / l	0.113	0.128	0.126	0.126	0.0068	0.10	0.90	0.85	< 0.01	0.27
Postweaning, d 57–70										
Glucose, mg / dl	83.79	86.65	87.22	87.99	2.886	0.28	0.76	0.89	0.01	0.27
Lactate, mg / dl	6.31	6.83	7.18	8.10	0.716	0.15	0.17	0.68	0.59	0.33
BHB[3], mmol / l	0.302	0.299	0.327	0.360	0.0215	0.31	0.11	0.35	< 0.01	0.30

[1]CON = 0% fiber from forage on the diet; MH = 7.5% medium quality hay on the diet; LH = 7.5% low quality hay on the diet; CS = 10% corn silage on the diet; 12 calves per treatment.

[2]Contrastst between groups (Control × Forage; Corn silage × Hay; Medium hay × Low hay); A = age effect; D × A = interaction between diet and age.

Ingestive and sorting behavior

At week 7, calves fed diets containing forage spent more time consuming their solid feed (*P* < 0.02) and ruminating (*P* < 0.04) compared to those fed the CON diet (Table 6). In addition, a trend was observed for greater ruminating time for calves fed hay, either MH and LH, than CS (*P* < 0.10). The experimental diets did not influence the other evaluated behavioral variables at week 7.

At week 10, there was a trend for increased time spent standing and less time spent lying when calves were fed CS as compared to either MH or LH (*P* < 0.08). Calves fed diets containing forage tended to spend more time eating compared to CON (*P* < 0.10). Calves fed the CS diet spent more time eating than calves fed TMR containing hay (MH and LH; *P* < 0.10). There was also a trend for greater rumination time for forage-fed calves as compared to CON diet (*P* < 0.07), with no difference among forage sources. Other behavior variables were not affected by the diets.

The sorting of each particle size fraction is shown in Figure 2. Only the CS diet presented particles larger than 19 mm. At weeks 7 and 10 of age, calves fed with CS preferentially sorted the TMR for long particles (*P* < 0.01; 134.2 ± 2.60%, and 107.1 ± 3.50%, respectively). Calves that received diets with forage preferentially sorted the TMR for medium particles, with greater sorting in favor of medium particles by calves provided LH compared to MH (*P* = 0.01; Figure 2). On the other hand, at week 10 there was no evidence of sorting the medium particle fraction, excepting a tendency for calves on the LH diet to sort in favor of medium particles (*P* = 0.08).

The intake of short particles exceeded the predicted value at weeks 7 and 10, but no difference among the diets was observed, suggesting that calves similarly sorted in favor of the short particles. Calves fed with a forage diet sorted more against fine particles than the CON group at week 7 (*P* = 0.01) and week 10 (*P* = 0.03).

Table 6. Ingestive behavior at weeks 7 and 10 of age of calves fed with different sources and qualities of fiber in total mixed diets.

	Diet[1]				SEM	*P*-value[2]		
	COM	MH	LH	CS		CON×F	CS×H	MH×LH
Min/10 h observation								
Preweaning, 7 wk								
Standing	227.08	216.15	221.15	218.75	14.795	0.64	0.99	0.81
Lying	375.83	381.15	374.62	369.17	14.429	0.96	0.63	0.75
Drinking milk	10.00	8.84	8.84	9.58	0.480	0.12	0.23	1.00
Eating solid feed	24.52	38.03	35.66	37.85	4.615	0.02	0.85	0.70
Drinking water	5.30	6.28	6.63	7.38	1.859	0.49	0.68	0.89
Sleeping	96.02	106.36	85.18	115.19	12.883	0.66	0.20	0.21
Urinating / defecating	3.75	2.30	1.15	2.91	1.140	0.22	0.39	0.46
Vocalizing	6.24	3.82	6.18	4.57	2.632	0.65	0.89	0.52
Non-nutritive oral behavior	56.20	46.25	52.11	49.18	8.439	0.46	0.99	0.61
Exploring environment	33.84	34.39	31.52	34.25	8.777	0.96	0.89	0.80
Ruminating	50.42	78.81	88.53	62.08	10.458	0.04	0.10	0.51
Post-weaning, 10 wk								
Standing	242.92	256.54	229.62	275.83	14.045	0.52	0.08	0.19
Lying	357.08	343.46	370.38	324.17	14.456	0.52	0.08	0.19
Eating solid diet	91.60	105.16	91.52	126.18	8.760	0.10	0.01	0.26
Drinking water	8.33	3.84	6.92	7.92	2.013	0.39	0.32	0.29
Sleeping	75.83	67.69	80.00	59.58	10.373	0.59	0.28	0.41
Urinating / defecating	3.32	3.47	1.92	2.07	1.159	0.53	0.66	0.34
Vocalizing	2.13	2.25	1.46	2.97	1.304	0.95	0.49	0.66
Non-nutritive oral behavior	22.18	24.30	19.98	30.11	5.016	0.64	0.22	0.52
Exploring environment	23.99	18.09	14.21	20.66	7.305	0.38	0.56	0.65
Ruminating	67.03	108.42	120.90	110.37	12.114	0.01	0.77	0.46

[1]CON = 0% fiber from forage on the diet; MH = 7.5% medium quality hay on the diet; LH = 7.5% low quality hay on the diet; CS = 10% corn silage on the diet; 12 calves per treatment.

[2]Contrastst between groups (Control × Forage; Corn silage × Hay; Medium hay × Low hay); A = age effect; D × A = interaction between diet and age.

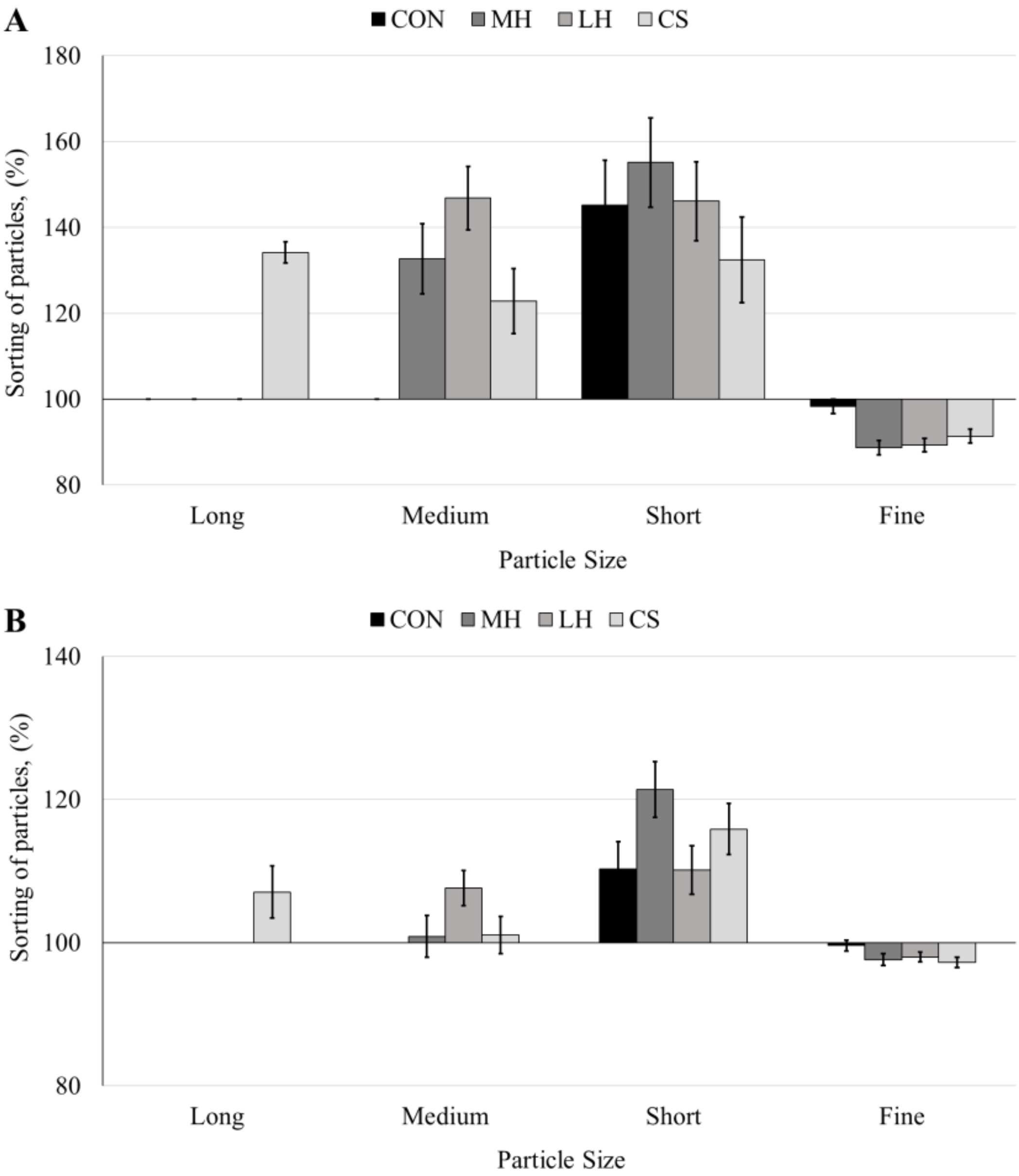

Figure 2. (A) Mean ± SEM intake of the particle fractions of a TMR (expressed as a % of predicted intake) when calves were offered CON = 0% fiber from forage on the diet; MH = 7.5% medium quality hay on the diet; LH = 7.5% low quality hay on the diet; CS = 10% corn silage on the diet at 49 d, and (B) at 70 d. Results are from individually housed calves (n = 12). Analyses were based upon the predicted intake of each particle fraction measured as disappearance after 10 h of feeding. Values >100% indicated sorting for that particle size, and values <100% indicated sorting against that particle size. Particles were separated into 4 fractions: long (>19 mm), medium, (< 19, > 8 mm), short (< 8, > 4 mm), and fine (< 4 mm).

3.4 Discussion

Intake and growth performance

Dairy calf feeding programs often encourage the use of highly fermentable carbohydrates and processed grains in the diet to maximize growth potential in early life

(Omidi-Mirzaei et al., 2018; Makizadeh et al., 2020). However, those programs may increase the risk of acidosis, which may affect performance because of the higher oscillation in solid feed intake and animal welfare. On the other hand, low levels of forage inclusion can benefit intake and performance during pre- and post-weaning, and the quality and source may promote a different desired calf response.

Corn silage is the most used forage for cows on dairy farms (Ferraretto et al., 2018). Feeding a forage already used on the farm to feed dairy cows might facilitate the management of the farm with no need to produce or buy another source of fiber for calves. However, the variation in particle size and quality between different silos is a factor that must be considered because of its impact in the intake and sorting of particles and, consequently, on performance (Khan et al., 2020; Jun Zhang et a., 2023). In addition, TMR with silage inclusion to feed young calves must be prepared daily to avoid undesirable fermentation. In contrast, chopped hay diets can be stored longer, and it is more manageable to manipulate the particle size that meets the requirements of growing animals.

Including forage increased solid feed intake at weeks 7 and 8, and hay increased the intakes of solid feed, NDF%BW, and peNDF at the weaning week, with high values for the LH diet. These results suggest that the greater fiber effectiveness of hay and the lower particle size are important for high starch diets, providing a greater intake of solid feed and fiber for milk-fed calves. The CS diet may be efficient in promoting a greater intake of a solid diet as the calves age, but the longer particle size of corn silage may result in the accumulation of undigested material due to the low physical development of the reticulorumen (Khan et al., 2011), suggested by the low fiber intake.

During post-weaning, including forage in the TMR also increased the solid feed intake; however, there was no difference among the evaluated forage sources, and the peNDF intake was higher for calves fed with the MH diet at week 10. As expected, the capacity for effective fiber intake in post-weaning may be greater for a better-quality fiber with advancing age (Mitchell and Heinrichs, 2020).

The inclusion of hay, regardless of quality, showed similar values of NDF%BW intake of 0.20 % in pre- and 0.7% post-weaning, with lower values for the CS diet, 0.15%, and 0.6 %, these results suggest that dry forage with similar particle size in the diet may result in a similar calf response due to the low inclusion level. There was no effect of hay nutritional contents in the intake of solid diet when final TMR nutrients are adjusted, especially the accumulated intake of NFC, which has a greater impact on the development of the rumen epithelium and weight gain of calves. The fiber from forage offered as a TMR will stimulate motility and maintain rumen health besides providing nutrients. On the other hand, the particle size and moisture might be the main factors that limit fiber intake when TMR with similar nutrients is fed to pre- and post-weaning calves.

Forage quality can be an issue when fed to calves as a diet component, reducing the total nutrient intake that affects weight gain, such as energy and protein (Castells et al., 2012). When a TMR is fed for dairy calves, one of the benefits is the adjustment of nutrients, minimizing adverse effects of fiber quality.

Despite the inclusion of forage in the TMR, the NFC content of the diets remained high (54.2% CON, 46.9% MH, 47.0% LH, 49.1% CS), and this may have promoted a compensation in solid diet intake to reach the accumulated 15kg intake of NFC at the same age. According to Quigley (2019), this accumulated intake of NFC suggests digestive tract maturation. The diet energy density improves dairy calves feed efficiency (Aragona et al., 2020), as observed in the present study with calves receiving the CON diet. However, the lower feed efficiency of the animals fed with TMR-containing forage was due to the increase in the voluntary intake of a solid diet, but higher NDF, lower NFC, and *in vitro* DM digestibility.

Regardless of the inclusion of forage reducing the starch content, the concentrations in all diets are above 41%, classified as high starch diets according to NASEM (2021), which may have contributed to maintaining the ADG and the BW of the calves during the pre-and post-weaning. The CON, MH, and LH diets present greater inclusion of dry ground corn, which has less ruminal digestion than starch from the CS diet. It was expected that the greater digestibility of corn in the CS diet could increase weight gain, but it was not possible to observe these effects in the present study, perhaps due to the level of inclusion in association with the underdeveloped rumen.

The low inclusion levels of forage (7.5 % of chopped hay – 21.7% NDF; 3.7% peNDF and 10% of corn silage – 19.4% NDF; 2.8% peNDF) increased the fiber content and the effectiveness provided in the TMR compared to the CON diet (17.5% NDF; 0.8% peNDF). The increase in the NDF level associated with peNDF > 4mm and an average particle size of 4 mm for hay and 9.8 mm for corn silage promoted early intake of solid feed without affecting the performance of dairy calves. Poczynek et al. (2019), evaluating increased NDF levels in the ground starter (22% and 31%) by using fiber from co-product, did not observe differences in intake and performance, but providing free access to coast-cross hay resulted in behavior benefits. As observed in the present study, increasing NDF level by feeding forage and providing peNDF may have benefits over providing fiber from no forage ingredients, but the TMR nutrients must be adjusted to help regulate intake without reducing performance.

The forage nutritional quality must be considered to adjust dietary nutrients to maintain high growth rates in calves. Furthermore, the level of fiber inclusion when corn silage is used must be based on the level of grain and forage present in the silage, not to exceed voluntary fiber intake that promotes gut filling and low weight gain.

Including low levels of forage in the TMR of calves may help regulate the intake, increasing intake capacity and total dry matter intake (Imani et al., 2017). However, forages greatly vary in nutritional value (Khan et al., 2020) and may interact with other factors in the diet, such as dry matter, protein content, and particle size, as regards the effects on the performance and behavior of calves. It is possible to observe different responses when calves are fed with different sources of fiber (Castells et al., 2013), corroborating our study.

Therefore, the inclusion of silage in the diet of dairy calves should be based on the levels of starch and NDF to balance with other ingredients. Levels between 10 and 15% of corn silage in the diet have been reported in the literature, demonstrating benefits without negatively affecting the intake and performance of calves (Mirzaei et al., 2016; Toledo et al., 2023). On the other hand, inclusions between 20 and 50% may result in a ruminal filling effect, decreasing intake and delaying the development of the tract and growth (Kehoe et al., 2019).

Blood metabolites

Butyrate is a product of solid-feed fermentation in the rumen and is crucial for the development of the ruminal epithelium, papillae growth, and, respectively, development of the digestive system, impacting the metabolism of the calf (Suarez-Mena et al., 2017). As calves age, the hepatic metabolic activity shifts from glycolytic to gluconeogenic, and the relationship between starter intake and the blood metabolites indicates rumen development (Baldwin et al., 2004).

Deelen et al. (2016) suggest that blood BHB is positively correlated with starter intake by the calf, and the study by Khan et al. (2020) found higher BHB concentrations at weaning in calves fed forage compared with those fed only concentrate. In addition, there is also a negative relationship between blood glucose with age and starter intake (Quigley et al., 1994). In the present study, the concentration of BHB was higher for calves fed diets containing forage than CON, corroborating with the higher consumption of solid feed during the preweaning period. We also observed that glucose concentrations reduced with advancing age regardless of the diet, suggesting adequate digestive tract development. In contrast, the intake of the CON diet provided higher total lactate in the blood, suggesting calves are at more risk of acidosis.

When calves are close to weaning and presenting high solid diet intake, rumen is more developed and fermentation is more efficient, with higher rumination time and a more efficient buffering from saliva entering the rumen (Baldwin et al., 2004). Because of rumen development, metabolism of end-products is also more efficient. Therefore, differences in blood metabolites concentrations are expected when pre and post weaning periods are compared. In the present study, we have observed a forage effect decreasing lactate and increasing BHB concentrations pre-weaning, but that was not observed post-weaning.

Ingestive and sorting behaviour

The composition of the diet, especially the physical form, influences the time needed for calves to achieve high amounts of solid intake (Khan et al., 2016). Including forage in the TMR increased the time spent eating solid feed in pre- and post-weaning. In addition, post-weaning, the CS diet resulted in longer feeding when compared to diets containing hay, either MH or LH diets. Similarly, increased feeding time has been observed previously when forage is provided (Horvath and Miller-Cushon, 2019). Our observed increase in time spent eating solid feed, associated with more time standing and less time lying down, may indicate a slower rate of feed intake, which may reduce the intake of large amounts of starter in a small period, preventing excessive fermentation and acid accumulation in the rumen. Thus, including corn silage in the diet may be a strategy to increase meal frequency and duration, resulting in longer eating periods (Kargar and Kanani, 2019).

We found that forage sources influenced rumination time, with the provision of hay increasing rumination time compared to calves providing no forage and the provision of corn silage having an intermediate effect. The main differences between medium and low-quality hay are the protein, NDF, and lignin content level. As the diets were balanced to present similar levels of nutrients, the results suggested that particle size's effectiveness may not differ when using different hay qualities.

The corn silage used in the CS diet presented 36.9% of grains and 63.1% of leaves and stems, resulting in 23.7% starch on a DM basis. Therefore, including 10% DM of corn silage in the diet resulted in 7.0% effective forage inclusion, with high moisture and lower peNDF content than hay-containing diets. That may have promoted less effectiveness in stimulating motility and rumination than dry forage sources but allowed intermediate stimulation compared to the CON diet.

We found that calves could extensively sort their mixed ratio, consistent with previous findings (Miller-Cushon and DeVries, 2011; Costa et al., 2016). Specifically, we found that calves did not sort against the long, medium, and short particles consisting primarily of forage, especially for long particles of the CS diet, as expected. On the other hand, calves that received TMR with forage, regardless of the source, selected against fine particles. Our findings suggest that, during the preweaning, calves were motivated to consume forage and were selecting in favor of fractions containing primarily forage. Similar to the present findings, Miller-Cushon et al. (2013) described sorting in favor of hay in preweaned calves but no sorting for or against different ration components after weaning.

Calves select feeds with nutrient contents that meet the specific requirements as they age and according to the diet offered (Bach et al., 2012; Miller-Cushon and DeVries, 2015). During preweaning, the liquid diet meets most of the calves' energy requirements for maintenance and growth, and sorting for long and against fine particles may be more common at this stage to prevent excessive fermentation in the underdeveloped rumen, especially in diets with high non-fiber carbohydrates inclusion (Miller-Cushon et al., 2013; Costa et al., 2016).

Our observed lack of sorting in favor of forage after weaning may reflect the shift from a greater reliance on the solid feed diet to meeting energy requirements following milk removal. Engelking et al. (2020) suggest that calf preference for long particles in the diet may decrease with declining milk provision, so the greater energy demand for growth may reduce the sorting against the fine particles and the preference for long particles. These results support the idea that sorting may occur because ruminants can make dietary choices based on nutritional demands and post-ingestive feedback (Forbes and Kyriazakis, 1995), and this behavior can be manifested in preweaning calves fed TMR, as previously described (Miller-Cushon and DeVries, 2011; Engelking et al., 2020).

Longer-term effects of early feed sorting behavior still need to be better understood. Some evidence suggests that early exposure to a TMR may influence feed sorting following dietary transitions post-weaning (Miller-Cushon et al., 2013). However, this needs to be consistently reported (Xiao et al., 2018). Post-weaning feed sorting may also depend on early dietary exposure. Miller-Cushon and DeVries (2011) found that calves fed hay before weaning initially demonstrated a preference for forage particles when switched to a mixed ration containing (DM basis) 40% hay and 60% concentrate but developed a preference for grain particles after 4 weeks. Feed sorting additionally depends on particle sizes (Miller-Cushon et al., 2013), such that further studies with different particle size are needed to understand the sorting behavior in favor of or against the long particles and how this impacts the ruminal environment, intake, and performance of calves.

3.5 Conclusions

Regardless of quality, increasing the NDF level with fiber from forage is essential for preweaning diets with high starch inclusion. A total mixed ration with corn silage, containing 19.4% NDF and 2.8% peNDF on a dry matter basis, does not negatively impact performance but increases feed intake and benefits behavior. A mixed ration with chopped hay containing 21% NDF and 3.0% peNDF also benefits behavior and maximizes the feed intake around weaning and post-weaning. Hay is the preferable forage source in the diet during the transition phase between preweaning and post-weaning. However, corn silage is a potential alternative source of fiber to be included in total mixed rations for calves. In any case, adjusting particle size and the nutrients for feeding as a total mixed ration must be considered.

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4 FORAGE SOURCES IN TOTAL MIXED RATIONS ON RUMEN FERMENTATION, GUT FILL, AND DEVELOPMENT OF THE GASTROINTESTINAL TRACT OF DAIRY CALVES

Abstract
The inclusion of forage sources in calf diets is often discussed, and the main point debated is whether the inclusion level, particle size, source, and how forage is offered may impact gut fill and reduce body weight gain. This study aimed to determine the effects of feeding forage sources with different quality on rumen fermentation, gut fill, and development of the gastrointestinal tract of dairy calves. Forty-eight Holstein calves were blocked and randomly assigned to 1 of 4 dietary treatments according to sex and body weight (BW) at 28 days of life. Treatments consisted of a no-forage coarsely ground starter (CON); or total mixed rations containing 7.5% on DM basis of *Tifton* hay of either medium quality (MH) or low quality (LH); or 10% on DM basis of corn silage (CS). The nutritional content, including protein, NDF, lignin, and *in vitro* digestibility, was used as forage quality criteria. During the first 28 days of life, all calves received 3 L of whole milk twice daily, a commercial pelleted starter ad libitum, but no forage. After that, the solid diet was changed to the respective dietary treatments. Rumen samples were taken to determine rumen pH and volatile fatty acid (VFA) proportions. Calves were gradually weaned from 52 to 56 d of age, and 20 calves, 5 per treatment, were harvested two weeks after weaning. The anatomical parts of the gastrointestinal tract were weighed with and without contents, and histological analysis of rumen epithelium was conducted. The CON diet increased total VFA concentration compared to forage diets. The forage diets increased rumen pH, fecal pH, and gut fill. However, regardless of the source, the forage provision did not affect empty body weight. In addition, the forage provision increased the number of papillae in the rumen, but diets did not influence length and width of papillae. The results suggest that 7.5% of hay, regardless of the quality, and 10% of corn silage in high-starch mixed diets benefit rumen health and promote greater gut fill without negative effects on final body weight.

Key words: gut fill, effectiveness, tract filling, histological.*

4.1 Introduction

Dairy calves need to access a solid diet alongside adequate volumes of liquid diet early in life to support high growth rates and rumen development, thus accelerating the weaning transition process (Montoro et al., 2013). The NASEM (2021) provides examples of nutrient specifications in calf starters, with varying protein and starch content. A solid feed with high starch content, around 36.9% may increase the total volatile fatty acids (VFA) in the rumen, particularly butyric and propionic acids (Laarman and Oba, 2011), stimulating epithelium growth and differentiation for improved absorption rates (Baldwin et al., 2004). However, a

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solid feed with low NDF content, around 18.9%, and no effective fiber may reduce ruminal pH and starter intake around weaning (Porter et al., 2007; Toledo et al., 2020).

Studies that reviewed the effects of forage provision on dairy calves have reported improvements in starter intake and rumen pH (Castells et al. 2012; Daneshvar et al. 2015). However, they noted that while forage provision improves ruminal health, the long particles may remain in the rumen for an extended period compared to diets with no forage, increasing gut fill and gastrointestinal tract weight (Imani et al., 2017).

The forage provision during preweaning has raised concerns regarding the impact of fiber on reduced intake due to gut fill, although this has not been properly measured (Hill et al., 2008; Khan et al., 2011; Montoro et al., 2013; Toledo et al., 2023). The most used equation for calculating empty body weight (EBW) in dairy calves is from Jahn et al. (1976); however, this equation was developed for calves between 8 and 20 weeks of age with straw-level inclusion and may not be suitable for assessing gut fill during preweaning. On the other hand, additional studies that evaluated forage provision and its effects on gut fill did not report differences between forage and no-forage diets (Khan et al., 2011; Mirzaei et al., 2014), indicating the need for further investigation.

Feeding forage as part of TMR may ensure protein and energy intake that meet calf requirements, along with the effective fiber necessary to guarantee rumen health (Castells et al., 2012; Toledo et al., 2024). However, hay and silage have distinct characteristics, such as chemical composition and particle size, and their inclusion in a TMR may promote different responses in calves. Moreover, dairy calves may sort total mixed rations, affecting individual nutrient intake (Miller-Cushon and DeVries, 2017), reducing the effectiveness of fiber and the nutritive value of the diet for rumen health and development, warranting further investigation.

This study aimed to investigate the effects of feeding different qualities of hay or silage as part of a total mixed ration on rumen fermentation, gut fill, and development in young calves. We hypothesize that calves fed a TMR with a low forage level, regardless of source and quality, would exhibit better ruminal health and greater gastrointestinal tract development than calves offered no forage.

4.2 Materials and Methods

Animals and treatments

All study procedures were approved by the Luiz de Queiroz College of Agriculture – University of Sao Paulo, Institutional Animal Care and Use Committee (Protocol no. 8560150621). This study was conducted between July and November of 2022 at calf facilities of the Animal Science Department of the "Luiz de Queiroz" College of Agriculture, Piracicaba, Sao Paulo, Brazil, and performance and behavior data are presented in Toledo et al. (2024). During this period, the average temperature was 21°C, with a maximum of 30°C and a minimum of 12°C. The mean relative humidity during the study period was 68%, and the average rainfall was 60.14 mm/ mo.

Forty-eight Holstein calves (36 males and 12 females) from a commercial farm were enrolled in this study. Calves were separated from their dam at birth and weighed. The colostrum quality was measured using a refractometer and fed to calves within 2h after birth (10% of BW; > 22% Brix; Godden et al., 2019). Calves needed to present a serum Brix % higher than 8.4% at 48h of life to be enrolled in the study. There were no differences (P = 0.34) among groups for passive immune transfer, with an average and standard deviation of 9.77 ± 0.21 % Brix.

The calves were individually housed in suspended pens (1.13×1.40 m, slatted floor 0.8 m height) inside the barn until 14 d of age. After that, calves were housed in wood hutches (1.35 m height, 1.00 m width, and 1.45 m depth) tethered by chain (2 m long), allowing an area for walking inside and outside the shelter, but no physical contact with other calves. Wood hutches were distributed in a Paspalum notatum grass field frequently trimmed to ground level, so no grass was available for grazing. Calves had access to a bucket of 10L capacity for ad libitum drinking water and a rectangular trough (35.0 cm length, 24.0 cm width, 12.0 cm depth) for the solid diet. Calves received 3 L of whole milk (12.6% solids, 3.94% fat, 3.40% protein, and 4.42% lactose) twice daily, 6 L/d, fed by a teat bucket. During the first 28 days of age, all calves received a commercial pelleted starter (87.4 % DM, 24.6 % CP, 17.7 % NDF, 38.5% NFC; Agroceres Multimix, Rio Claro, Brazil) and no forage.

After that, calves were blocked according to their weight at 28 d (47.95 ± 1.905 kg) and sex and distributed randomly into 1 of 4 dietary treatments (Table 1): No-forage **coarsely ground** starter (CON; n = 12); or a total mixed rations containing coarsely ground grains and varying sources of chopped forage: 7.5 % DM basis of medium quality *Tifton* (*Cynodon dactylon*) hay (MH; n = 12), 7.5 % DM basis of low quality *Tifton* hay (LH; n = 12), or 10% DM basis of corn silage (CS; n = 12).

The treatment diets were formulated to be isonitrogenous, and the TMR had a similar starch level because we aimed to evaluate and isolate the effects of forage source, composition, and effectiveness to stimulating chewing, salivation, and ruminal health. The corn silage included in the CS diet presents about 31.0% grains and 69.0% leaves and stems, resulting in 23.7% starch on a DM basis. Including 10% DM of corn silage in the diet resulted in 7.0% effective forage inclusion. The nutritional content, including protein, NDF, lignin, and *in vitro* digestibility, were used as forage quality criteria. The TMR with forage inclusion started to be offered at 28 days due to the low voluntary intake of forage by calves in the first few weeks of life, as already reported in the literature (Coverdale et al., 2004; Khan et al., 2011; Castells et al., 2012 and 2013; Poczynek et al., 2020).

The weaning began at 52 d of age, with a decrease of 1 L/d, so calves were completely weaned after 56 d. Calves were followed for 14 days following weaning.

Measurements and sample collection

Feed samples were collected monthly, to determine the chemical composition of the offered (n = 5), and refusals (n = 5) of each diet. Both hays used in the diet were previously chopped and blended with the other ingredients using a horizontal mixer (Lucato, Limeira, Brazil). Similarly, the silage was harvested, chopped, ensiled, and mixed daily before feeding (Table1). The solid diet was offered every morning, just after milk feeding, and was available until the following morning. The orts were weighted for solid diet intake calculations (9094-Prix, Toledo Ltda., 4 g accuracy from 0 kg to 10 kg).

Body weight was recorded weekly on a mechanical scale (ICS-300, Coimma Ltda., Dracena, SP, Brazil), and performance results are present in Toledo et al. (2024).

A ruminal fluid sample was taken using an oro-esophageal tube (150 cm long, 1.3 cm internal diameter, and 0.2 cm wall thickness) connected to the vacuum pump (model TE-0581, Tecnal Ltda., Piracicaba, SP, Brazil) at weeks 6, 8, and 10 of age. The samples were collected 2 hours after feeding, and the initial portion was discarded to avoid saliva contamination (Terre et al., 2013). The sample was filtered through 2 layers of cheesecloth, and the pH was immediately measured with a digital potentiometer (Model tec-5, Tecnal Ltda, Piracicaba-SP, Brazil). A 10-mL subsample was immediately frozen (-10°C) for subsequent VFA analyses, according to Ferreira et al. (2016), and ammoniacal nitrogen (NH3-N), according to de Paula et al. (2017).

The fecal score was evaluated daily for its consistency according to Larson et al. (1977) as (1) normal and firm, (2) consistent and mushy, (3) mushy and slightly liquid, and (4) watery, and an average was obtained by week. Fecal samples were collected by rectal palpation two hours after morning feeding at weeks 6, 8, and 10. Moreover, 4 g of fecal matter was added to 4 mL of deionized water, and the pH was immediately measured according to Channon et al. (2004).

Slaughter data and morphometrics parameters

Twenty calves, 5 per treatment, were harvested two weeks after weaning, and anatomical parts of the gastrointestinal tract (GIT) anterior portion (reticulo-rumen, omasum, abomasum) and posterior portion (small and large intestine) were separated and weighed with and without contents (full and empty). Afterward, the compartments were separated into reticulo-rumen, omasum, abomasum, and intestines, washed with tap water, and weighed. Digestive tract filling was calculated by subtracting the weight of full GIT and empty compartments (reticulo-rumen, omasum, abomasum, small intestine, and large intestine). Therefore, true EBW was calculated as BW and gastrointestinal tract filling subtraction. The empty BW was also calculated using the equation described by Jahn et al. (1976).

The reticulo-rumen volume was determined according to Toledo et al. (2020). Subsequently, rumen tissue samples were collected from the cranial ventral sac, ventral portion of the caudal ventral blind sac, and the caudal portion of the caudal ventral blind sac; the samples were stored for 2 days in 4% buffered paraformaldehyde and then in 70% alcohol to the histological analyses. The number of papillae/cm^2 of the ruminal wall and histological measurements, including papillae length and width, were determined according to Lesmeister et al. (2004). Histological sections were stained with hematoxylin and eosin, embedded in paraffin wax, and sectioned (Odongo et al., 2006).

Analytical procedures

Feed samples were collected, and the chemical composition of the offered and refusals, particle size analysis, and the in vitro true digestibility were done according to the methods described in Toledo et al. (2024).

Statistical analysis

Blinding to treatment was not possible because the starter and TMR were weighed and fed immediately to the calves by the research team. Data were screened for normality before analysis using the PROC UNIVARIATE and then analyzed using the PROC MIXED procedure of SAS 9.4 (SAS Institute, Inc., Cary, NC, USA). The 48 calves were randomized into 12 complete blocks according to weight and sex at 28 days of life (9 males and 3 females). Each block consisted of 4 dietary treatments, and the blocks were established based on births occurring over 3 months. Ruminal parameters and fecal variables were analyzed as repeated measures over time: $Yijk = \mu + Di + bj + eij + Ik + (Di)Ik + eijk$. Where μ = overall mean; Di = fixed effect of diet; bj = random effect of block; eij = residual error A; Ik = fixed effect of age; (Di)Ik = effect of diet × effect of age interaction, and eijk = residual error B. Covariance matrices were tested and defined according to the lowest value obtained for "Akaike's Information Criterion corrected" (AICC). The model included the effects of diet, week (age of calves), and the interaction between diet and age 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 20 calves were randomly chosen from 5 complete blocks according to sex, only males, at 69 days of age. Slaughter and morphometrics parameters were evaluated as a non-repeated measure using the following statistical model: $Yij = \mu + Di + bj + eij$. Where μ = overall mean; Di = diet effect; bj = random effect of block; and eij = residual error. For all response variables, the means were obtained using the LSMEANS command, and the orthogonal contrasts were used to analyze 3 preplanned comparisons between treatment groups: 1) comparison between calves receiving no forage and calves receiving any forage source: control × forages (CON × F; where F = LH + MH + CS); 2) comparison between calves receiving corn silage and calves receiving hay: corn silage × hay (CS × H; where H = LH + MH); and 3) comparison between calves receiving hay of different quality: hay – medium quality × hay – low quality (MH × LH). The *P*-value ≤ 0.05 was adopted as a significant effect, and $0.05 > P \leq 0.10$ was considered a trend.

4.3 Results

Ruminal and fecal parameters

There was no interaction between diet and age for ruminal and fecal variables at weeks 6, 8, and 10 (Table 2; 12 calves per treatment). The CON diet increased the total VFA concentration. The molar proportion of acetate increased, while the propionate decreased for calves fed with forage diets, with no difference among the evaluated forage sources. Consequently, the acetate: propionate ratio was lower for calves fed with the CON diet. The butyrate molar concentration was not affected by experimental diets. The total VFA concentrations and individual VFA proportion increased as calves aged. The ruminal ammonia-nitrogen concentration increased with the CON diet more than forage diets and decreased as calves aged.

Feeding forage increased ruminal and fecal pH compared to the CON diet, with no difference between sources. Ruminal pH increased, and fecal pH decreased as calves aged. However, the fecal score was not affected by diet or age.

Table 2. Ruminal and fecal parameters at weeks 6, 8 and 10 of age of calves fed with different sources and qualities of fiber in total mixed diets.

	Diet[1]				SEM	*P*-value[2]				
Item	COM	MH	LH	CS		CON×F	CS×H	MH×LH	A	A×D
Total VFA, mM	83.89	68.51	65.73	71.55	3.266	< 0.01	0.28	0.56	< 0.01	0.40
VFA, mM/100 m*M*										
Acetate	54.47	57.94	58.24	56.98	0.898	0.01	0.31	0.81	< 0.01	0.82
Propionate	36.81	33.80	33.35	34.29	0.802	0.01	0.47	0.70	< 0.01	0.70
Butyrate	8.50	7.99	8.41	8.84	0.374	0.85	0.18	0.44	0.28	0.54
C2:C3 ratio[3]	1.54	1.83	1.77	1.76	0.084	0.02	0.70	0.65	< 0.01	0.80
N-NH3, mg/dl	17.91	13.47	14.01	15.64	1.428	0.03	0.26	0.78	< 0.01	0.25
Ruminal pH[4]	5.67	6.02	6.10	5.92	0.084	< 0.01	0.19	0.49	< 0.01	0.47
Fecal pH[4]	6.67	6.92	6.85	6.91	0.075	0.01	0.74	0.47	< 0.01	0.25
Fecal score	1.87	1.73	1.90	1.76	0.081	0.47	0.61	0.15	0.26	0.79

[1]CON = 0% fiber from forage on the diet; MH = 7.5% medium quality hay on the diet; LH = 7.5% low quality hay on the diet; CS = 10% corn silage on the diet.

[2]Contrasts between groups - CON × F: contrast between control diet and diets containing forages (MH, LH and CS); CS × Hay: contrast between diet containing 10% CS and diets containing 7.5% of hay (MH and LH); MH × LH: contrast between diet containing 7.5% of MH and 7.5% of LH; A = age effect; D × A = interaction effect between diet and age. 12 animals per treatment.

[3]C2:C3 ratio: = acetate: propionate ratio.

[4] Measured 2h after morning feeding.

Slaughter and morphometrics parameters

The experimental diets did not affect the slaughter BW (Table 3; 5 calves per treatment). On the other hand, the full GIT weight tended to be greater for calves that received the forage-containing diets, with no differences between sources. The empty GIT, the reticulo-rumen, omasum, abomasum, and intestines weights were not affected by experimental diets. Adding hay to the diets increased the reticulo-rumen volume compared to CS, with no difference among hay qualities.

Forage diets (MH, LH, and CS) tended to increase the digestive tract fill weight compared to the CON diet, with no difference between sources. However, the experimental diets did not affect the true and empty body weights calculated with the equation of Jahn et al. (1976). The full and empty GIT, digestive tract fill, reticulo-rumen, omasum, abomasum, and intestines evaluated as the % of BW were also unaffected by diets.

The number of papillae corresponding to the cranial ventral sac tended to be greater for calves receiving forage diets (MH, LH, and CS) than the CON diet (Table 4; 5 calves per treatment); the same results were observed for papillae from the caudal portion of the caudal central blind sac. Nevertheless, the diets did not affect the number of papillae in the ventral portion of the caudal ventral blind sac; however, calves fed MH present wider papillae than those fed LH.

Table 3. Slaughter parameters of calves fed with different sources and qualities of fiber in total mixed diets.

	Diet[1]				SEM	*P*-value[2]		
	CON	MH	LH	CS		CON×F	CS×H	MH×LH
Slaughter BW, kg	80.70	82.20	84.20	79.50	6.387	0.77	0.45	0.73
Weight, kg								
GIT full	15.42	17.12	17.53	16.44	1.157	0.09	0.16	0.73
GIT empty	6.49	7.02	6.74	6.39	0.457	0.62	0.32	0.64
GIT fill	8.93	10.14	10.79	9.53	0.812	0.07	0.18	0.43
Reticulum-rumen	1.67	1.75	1.78	1.55	0.202	0.89	0.32	0.90
Omasum	0.44	0.51	0.46	0.43	0.053	0.62	0.41	0.55
Abomasum	0.48	0.52	0.57	0.49	0.043	0.38	0.25	0.41
Intestines	3.91	4.23	3.92	3.93	0.245	0.61	0.54	0.31
Reticulum-rumen volume, L	16.43	18.02	18.10	15.41	1.256	0.34	0.01	0.93
True empty body weight, kg	71.77	72.13	73.41	69.97	5.740	0.98	0.56	0.83
Empty body weight, kg – Equation[3]	77.18	77.03	78.44	75.20	6.065	0.95	0.56	0.80
Weight, % BW								
GIT full	19.37	20.79	20.82	20.22	0.962	0.22	0.98	0.96
GIT empty	8.09	8.49	8.04	8.10	0.301	0.71	0.60	0.25
GIT fill	11.28	12.29	12.79	12.12	0.844	0.24	0.67	0.68
Reticulum-rumen	2.04	2.11	2.13	1.92	0.146	0.95	0.25	0.93
Omasum	0.55	0.60	0.55	0.53	0.043	0.80	0.44	0.45
Abomasum	0.61	0.62	0.68	0.61	0.042	0.45	0.36	0.38
Intestines	4.90	5.15	4.68	5.03	0.288	0.83	0.69	0.19
Reticulum-rumen volume, L as % BW	20.45	22.11	21.37	18.87	1.227	0.79	0.04	0.62

[1]CON = 0% fiber from forage on the diet; MH = 7.5% medium quality hay on the diet; LH = 7.5% low quality hay on the diet; CS = 10% corn silage on the diet.
[2]Contrasts between groups - CON × F: contrast between control diet and diets containing forages (MH, LH and CS); CS × Hay: contrast between diet containing 10% CS and diets containing 7.5% of hay (MH and LH); MH × LH: contrast between diet containing 7.5% of MH and 7.5% of LH; 12 calves per treatment.
[3]Empty BW calculated by adjusting for gut fill, as Jahn et al. (1976) described.

Table 4. Rumen morphometrics parameters of calves fed with different sources and qualities of fiber in total mixed diets.

	Diet[1]				SEM	*P*-value[2]		
	CON	MH	LH	CS		CON×F	CS×H	MH×LH
Papillae count, cm^2								
Cranial ventral sac	96.8	131.3	164.0	136.2	21.53	0.08	0.67	0.33
Ventral portion of caudal ventral blind sac	120.8	119.5	150.7	110.8	15.91	0.74	0.23	0.19
Caudal portion of caudal ventral blind sac	91.0	149.5	132.2	143.8	13.30	0.04	0.86	0.38
Papillae length, mm								
Cranial ventral sac	2.25	1.57	2.19	2.00	0.305	0.65	0.84	0.22
Ventral portion of caudal ventral blind sac	1.71	1.23	1.13	1.37	0.325	0.24	0.54	0.86
Caudal portion of caudal ventral blind sac	1.85	1.11	1.83	1.60	0.308	0.82	0.49	0.12
Papillae width, mm								
Cranial ventral sac	0.34	0.34	0.32	0.34	0.043	0.91	0.82	0.70
Ventral portion of caudal ventral blind sac	0.35	0.39	0.32	0.33	0.022	0.98	0.47	0.03
Caudal portion of caudal ventral blind sac	0.38	0.34	0.30	0.32	0.038	0.20	0.98	0.54

[1]CON = 0% fiber from forage on the diet; MH = 7.5% medium quality hay on the diet; LH = 7.5% low quality hay on the diet; CS = 10% corn silage on the diet.

[2]Contrasts between groups - CON × F: contrast between control diet and diets containing forages (MH, LH and CS); CS × Hay: contrast between diet containing 10% CS and diets containing 7.5% of hay (MH and LH); MH × LH: contrast between diet containing 7.5% of MH and 7.5% of LH; 5 calves per treatment.

4.4 Discussion

Ruminal and fecal parameters

It is well established that forage inclusion in diets with high starch content or reduced particle size can enhance solid diet intake due to the rumen buffering effect (Imani et al., 2017; Xiao et al., 2020). This is particularly important near weaning, as solid diet intake gradually increases, especially when the volume of liquid diet remains fixed throughout the weaning period.

The physical form of the starter also plays a role in the effects of fiber inclusion (Imani et al., 2017), as it correlates to degradation rate. Pelletized or textured starters tend to break down upon contact with saliva, resulting in reduced particle size when they reach the rumen, which facilitates bacterial adherence increasing degradation rate (Kim et al., 2016). Nevertheless, the decision to include forage appears to be primarily determined by the concentration of non-fibrous carbohydrates and starch in the diet.

The performance results of this study suggest that including forage in coarsely ground starter, at 7.5% of both hay qualities or 10% corn silage, led to increased solid feed intake around weaning and decreased feed efficiency, with no impact on final body weight (Toledo et al., 2024). However, there is concern regarding providing fiber with a longer retention time, as it may reduce the total nutrient intake available for fermentation, delay the development of the ruminal epithelium, and consequently decrease the growth rate immediately post-weaning.

The provision of a no-forage starter, as the CON in the present study, may favor the starch bacteria, increasing propionate in the rumen but reducing rumen pH (Suárez et al., 2007; Castells et al., 2013; Zhang et al., 2023). Beyond the lower rumen and fecal pH, these results suggest that the greater NFC and DM in vitro digestibility present in the CON diet favored the conditions for epithelial and metabolic development of the rumen (Baldwin et al., 2004). However, the inclusion of forage did not change the molar concentration of butyrate, probably because of the increased solid diet intake, suggesting that the intake of a balanced TMR may promote the same development of the ruminal epithelium as the no-forage diet (Toledo et al., 2024).

Including long particles from forage promoted a small change in the average particle size of the TMR. However, the concentration of peNDF increased from 0.75% in the CON diet to 2.8%, 3.1%, and 3.7% in the CS, LH, and MH diets, respectively. This greater effective fiber percentage is responsible for maintaining ruminal pH through changes in the fermentative parameters of the rumen, allowing an intake increase.

A recent study demonstrated that increasing peNDF from 0.34% to 2.63% and 4.85% by adding 10 and 20% of corn silage, respectively, linearly increased the ruminal pH of calves fed high-starch TMR (Toledo et al., 2023). On the other hand, increasing the NDF content in diets using co-products was not effective in raising ruminal pH due to the small average particle size (Poczynek et al., 2020), reinforcing the need for fiber from forage with a minimum percentage of peNDF greater than 4mm to maintain a healthy rumen environment for calves with a developing rumen.

Combining forage and coarsely ground grains can potentially increase ruminal health by increasing chewing time, eating time, and ruminating activity, and, therefore, increasing rumen pH through buffer content in the saliva flow (Nemati et al., 2016; Kim et al., 2016). In addition, the abrasion effect on the rumen wall, which removes excess small starch granules between the papillae and reduces keratinization, is mostly caused by the long fiber (Greenwood et al., 1997), providing a healthy ruminal environment.

Forage diets usually favor cellulolytic fermenting bacterial growth and increase ruminal pH (Žitnan et al., 1998). The differences in ammonia-nitrogen concentrations in the rumen may be explained by the greater utilization of cellulolytic bacteria and absorption rate in diets with higher pH, such as forage diets.

The inclusion of 10% and 15% of corn silage (on a dry matter basis) in calves' diets has been shown to maintain rumen pH and improve the performance of preweaning dairy calves (Mirzaei et al., 2017; Toledo et al., 2023). Silage provision benefits by improving the ruminal environment and serves as an alternative forage for preweaning calves' diets on farms that including hay in the TMR may not be practicable due to logistics, availability, or cost constraints. However, providing a high-moisture diet for calves emphasizes the importance of carefully mixing, feeding, and changing the diet daily to encourage consistent intake and avoid variations in early intake management.

Moreover, Cynodon dactylon is a perennial tropical forage, and cultivars such as Tifton-85, coast-cross, and Florakirk are well accepted by ruminants due to their thin stems and multiple leaves (Ribeiro et al., 2001). Previous studies provided coast-cross, and Tifton-85 hay ad libitum for milk-fed dairy calves and reported forage-to-concentrate ratio intake of 4:96 and 7:93 (Poczynek et al., 2020; Toledo et al., 2020). These findings were considered to establish forage-level inclusions in the present study.

Slaughter and morphometrics parameters

The greater solid diet intake observed toward the end of the study with forage provision can be attributed to the establishment of ruminal fermentation, which enhances the ability to absorb volatile fatty acids (VFA) and develop rumen papillae (Mirzaei et al., 2014). Consequently, calves may have compensated for their energy intake, and slaughter weight was not affected when comparing forage and no-forage diets.

Mirzaei et al. (2014) evaluated 8% and 16% alfalfa inclusion in the calf's diet with medium (2.92 mm) and long particle (5.04 mm) sizes and suggested that the effect of alfalfa particle size on rumen development of dairy calves depended on the level of supplementation. In the present study, the weight of the reticulo-rumen did not differ, probably because the inclusion level was lower than 10%, even with a larger particle size of corn silage (9.8 mm) compared to hay (4.0 mm).

The greater reticulo-rumen volume was observed in calves that received hay diets (MH and LH) than in the CS diet. The reduced particle size of chopped hay may have optimized the solid diet intake at the end of the experimental period, promoting greater stimulation for increased rumen musculature development (Tamate et al., 1962; Hamada et al., 1976). On the other hand, the long particle size from the fiber in hay and silage diets increased gut fill weight but did not reduce the true EBW, as mentioned in early research (Castells et al., 2013; Hill et al., 2008).

There are differences in the particle size, peNDF, and dry matter in vitro digestibility among the diets with different forage sources and no-forage diets, which may have affected ruminal kinetics and influenced the development of the gastrointestinal tract structures, especially in calves that express greater solid diet intake early in life.

The retention time and gut fill of calves fed forage may be influenced by the lower digestibility of fiber than starch and sugar. Many studies claimed that roughage increased gut fill due to its low ruminal fermentation rate, thereby curbing the starter feed intake, which has a higher energy density. However, these results are mostly observed when the inclusion level is greater than 10% or when forage is fed ad libitum (Hill et al., 2008; Coverdale et al., 2004).

The NASEM (2021) presented a ratio of EBW to BW of calves fed only liquid diet, liquid and solid diet, or solid diet exclusively, varying between 6 and 15%. The ratio calculated using the Jahn et al. (1962) equation was 6%, but the true ratio found in the present study was greater, around 12%. Additionally, the inclusion of hay with an average particle size of 4 mm and silage with 9.8 mm showed no change in the gut fill when adjusted for the percentage of BW. These results suggest that calves fed concentrate with no forage, 7.5% hay, or 10% corn silage with 0.8 to 3.7% peNDF present similar gut fill two weeks after weaning.

Previous studies that provided low levels of forage for calves, up to 10% inclusion, with several particle sizes (ranging from 3 to 12 mm), reported improved total solid diet intake, greater physical development of the reticulo-rumen, and increased time spent ruminating and chewing (Khan et al., 2011; Hill et al., 2008; Mirzaei et al., 2014; Toledo et al., 2024).

The low forage in the TMR and reduced particle size may ensure adequate fiber intake without negative effects on gut fill and performance. Even though the forage inclusion increased the gut fill weight, this was not true when this value was reported as percentage of BW, with no diet effect. These results suggest that providing a TMR with energy, protein, and low inclusion of long particles from forage, regardless of the source and quality, reduces the chances of a confounding effect of gut fill and calf body weight, and it may encourage farmers to incorporate forage into dairy calf diets earlier.

Despite requiring additional management, feeding low levels of forage in the TMR can accelerate the development process of the digestive tract due to improved ruminal health and its effects on solid diet intake.

Lesmeister et al. (2004) suggested a rumen tissue sampling technique for young calves. These study results indicated that samples taken from certain areas of the rumen represent rumen development as effectively as those taken from all areas. In the present study, the inclusion of forage tended to increase papillae number per square centimeter, in the cranial ventral sac and the caudal portion of the caudal ventral blind sac. The mechanisms related to stimulating the development of papillae include the possibility that butyrate and propionate metabolism by the ruminal epithelium induces an increase in blood flow and the possibility of a direct effect of butyrate or propionate on gene expression within the rumen (Sander et al., 1959; Glauber et al., 1991).

As expected, total VFA concentrations and the proportion of propionate in the rumen were greater for calves receiving the CON diet than forage diets. However, the proportion of butyrate remained the same regardless of diet, and that may be responsible for the greater number of papillae.

The evaluation of the caudal ventral blind sac is limited in previous research, and it appears this area is less developed in the young ruminant calves (Lesmeister et al., 2004). It is possible to observe that even with no effect among the diets, the papillae length was lower in this area but with greater papillae number per square centimeter than in the other areas, which may indicate that papillae differentiation is still in progress.

Nonetheless, the rumen is not completely developed during a transition phase, and the papillae may have irregular size and form, promoting a variation in papillae width and height with no major differences among the diets.

4.5 Conclusions

The inclusion of 7.5% hay or 10% corn silage with an average particle size of 4 mm and 9.8 mm, respectively, increasing the peNDF from 0.8 to 3.7% in the TMR for young calves, improved ruminal pH and did not affect empty body weight and the development of the rumen epithelium of weaned dairy calves. Forage provision combined with **c**oarsely ground starter in the total mixed ration for young calves should be stimulated since it improved ruminal health and did not affect the digestive tract fill as % of BW, suggesting no confounding effect on body weight.

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