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# Forage sources in total mixed rations on rumen fermentation, gut fill, and development of the gastrointestinal tract of dairy calves

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**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, as well as impact gastrointestinal tract development. This study aimed to determine the effects of feeding forage sources with different qualities on rumen fermentation, gut fill, and development of the gastrointestinal tract of dairy calves. Forty-eight Holstein dairy calves were blocked according to sex and body weight (BW) at 28 days of life and randomly assigned to 1 of 4 dietary treatments. 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 crude 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, and 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 the length and width of papillae. The results suggest that 7.5% of *Tifton* 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.**

**Keywords** Dairy calves, Forage sources, Tract filling, Rumen fermentation, Histological

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[1]. The National Academies of Sciences, Engineering, and Medicine (NASEM)[2] presented examples of nutrient specifications in calf starters with varying nutrient content, but no maximum starch recommendations were presented. 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[3], stimulating epithelium growth and differentiation for improved absorption rates[4]. However, feeding NDF content from grounded concentrates and no forage may reduce ruminal pH around weaning[5,6].

Studies that reviewed the effects of forage provision on dairy calves have reported that highly fermentable diets require fiber from forage inclusion to increase starter intake as calves age[7,8]. However, while forage provision

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improves ruminal health, there is a concern about the long particles remaining in the rumen for an extended period compared to diets with no forage, increasing gut fill and gastrointestinal tract weight[9].

Moreover, the current tables with predicted nutrient concentrations needed to meet the Holstein dairy cattle requirements did not present an assessment of a minimum recommended level of fiber for calves weighing up to 120 kg[2], mainly due to insufficient information on the feedstuff's physical characteristics, such as particle size.

The evaluation of the physically effective neutral detergent fiber (peNDF) is a promising area of research in dairy calf nutrition. It merges information on the diet ingredients' chemical constituents (NDF) content and particle size[10,11]. Including peNDF in a TMR has been shown to promote great uniformity intake, resulting in lower feed sorting[12–14], and may promote positive effects on rumination and rumen buffering. This shows a step towards potential improvements in calf nutrition, encouraging further adjustments to assess the adequacy of dietary fiber.

In addition, feeding forage as part of TMR may ensure protein and energy intake that meets calf requirements and the effective fiber necessary to guarantee rumen health[12–14]. 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, warranting further investigation.

This study investigated the effects of feeding different *Tifton* hay qualities or corn 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.

## Materials and methods

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) and follow the relevant guidelines and regulations that apply. 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.[14]. 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.

### Animals and treatments

Forty-eight Holstein dairy 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 2 h after birth (10% of BW; > 22% Brix)[15]. To be enrolled in the study, calves needed to present a serum Brix % higher than 8.4% at 48 h of life. There were no differences ($P = 0.34$) among groups for passive immune transfer, with an average and standard deviation of $9.77 \pm 0.21$% Brix.

The calves were individually housed in suspended pens ($1.13 \times 1.40$ 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 10 L 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, totaling 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 ($47.95 \pm 1.905$ 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 stimulate 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 crude 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[7,16–19].

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

### Measurements and sample collection

Feed samples were collected monthly to determine the chemical composition of each offered ($n = 5$) and refused ($n = 5$) diets. The diets' metabolic energy (ME, Mcal/kg) was estimated using the NASEM program (2021).

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 (Table 1). The solid diet was offered every morning, just after milk feeding, and was available until the following morning. The orts were weighed for solid diet intake calculations (9094- Prix, Toledo Ltda., 4 g accuracy from 0 to 10 kg).

| Item | CON | MH | LH | CS | Medium quality hay | Low quality hay | Corn Silage |
|---|---|---|---|---|---|---|---|
| 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 | – | – | – |
| DM, % as fed | 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 |
| ME, Mcal/kg | 3.38 | 3.32 | 3.30 | 3.28 | – | – | – |
| 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[3] > 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 |

**Table 1**. Ingredient, chemical composition and particle size of experimental diets and forages (Toledo et al.)[15]. [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 described by (Toledo et al., 2024). [2]Composition: Ca 20%; P 6.5%; F 650 mg/kg; K 1%; Mg 7%; S 0.7%; Co 25 mg/kg; Cu 800 mg/kg; Cr 20 mg/kg; I 40 mg/kg; Fe 1400 mg/kg; Mn 1500 mg/kg; Se 18 mg/kg; Zn 3,200 mg/kg; Vitamin A 140,000 IU / kg; Vitamin D3 50,000 IU / kg; Vitamin E 1500 IU / kg; Vitamin B1 250 mg/kg, Vitamin B2 250,000 mg/kg; Vit amin B6 250 mg/kg; Vitamin B12 250 mg/kg; Niacin 400 mg/kg; Pantothenic acid 500 mg/kg; Folic acid 20 mg/kg; Biotin 10 mg/kg; Butylated hydroxytoluene 800 mg/kg; Sodium monensin 900 mg/kg. [3]peNDF: physically effective neutral detergent fiber > 4 mm.

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 h after feeding, and the initial portion was discarded to avoid saliva contamination[20]. 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[21] and ammonia-nitrogen (NH3-N)[22] analysis.

The fecal score was evaluated daily for its consistency[23], 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[24].

## Slaughter data and morphometrics parameters

Twenty calves, 5 per treatment, were harvested 2 weeks after weaning, and anatomical parts of the anterior (reticulo-rumen, omasum, abomasum) and posterior (small and large intestine) portions of the gastrointestinal tract (GIT) 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 and empty compartments (reticulo-rumen, omasum, abomasum, small and large intestine). Therefore, true empty body weight (EBW) was calculated as BW and gastrointestinal tract filling subtraction. The empty BW was also calculated using the equation described in a previous study[25].

The reticulo-rumen volume was measured[6]. 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[26]. Histological sections were stained with hematoxylin and eosin, embedded in paraffin wax, and sectioned[27].

## 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.[14].

## 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 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:

$$\mathrm{Yijk} = \mu + \mathrm{Di} + \mathrm{bj} + \mathrm{eij} + \mathrm{Ik} + (\mathrm{Di})\,\mathrm{Ik} + \mathrm{eijk}.$$

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:

$$\mathrm{Yij} = \mu + \mathrm{Di} + \mathrm{bj} + \mathrm{eij}$$

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* ≤ 0.10 was considered a trend.

# Results

## Ruminal and fecal parameters

There was no interaction between diet and age for ruminal and fecal variables at weeks 6, 8, and 10 (Tables 2 and 12 calves per treatment). The CON diet increased the total VFA concentration ($P < 0.01$). The molar proportion of acetate increased, while the propionate decreased for calves fed with forage diets, with no difference among the evaluated forage sources ($P = 0.01$). Consequently, the acetate: propionate ratio was lower for calves fed with the CON diet ($P = 0.02$). 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 ($P = 0.03$; $P < 0.01$, respectively).

Feeding forage increased ruminal and fecal pH compared to the CON diet, with no difference between sources ($P < 0.01$; $P = 0.01$, respectively). Ruminal pH increased, and fecal pH decreased as calves aged. However, the fecal score was not affected by diet or age.

## Slaughter and morphometrics parameters

The experimental diets did not affect the slaughter BW (Table 3 and 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 ($P = 0.09$). The empty GIT, the reticulo-rumen, omasum, abomasum, and intestines weights were not affected by experimental diets. Adding *Tifton* hay to the diets increased the reticulo-rumen volume compared to CS, with no difference among hay qualities ($P = 0.01$).

Forage diets, MH, LH, and CS, tended to increase the gastrointestinal tract fill weight compared to the CON diet, with no difference between sources ($P = 0.07$). However, the experimental diets did not affect the true and empty body weights calculated with equation[13]. 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 (Tables 4 and 5 calves per treatment; ($P = 0.08$); the same results were observed for papillae from the caudal portion of the caudal central blind sac ($P = 0.04$). Nevertheless, the

| | Diet[1] | | | | | P-value[2] | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Item | COM | MH | LH | CS | SEM | 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 mM | | | | | | | | | | |
| 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 |
| Acetate: propionate ratio | 1.54 | 1.83 | 1.77 | 1.76 | 0.084 | 0.02 | 0.70 | 0.65 | < 0.01 | 0.80 |
| NH3-N, mg/dl | 17.91 | 13.47 | 14.01 | 15.64 | 1.428 | 0.03 | 0.26 | 0.78 | < 0.01 | 0.25 |
| Ruminal pH[3] | 5.67 | 6.02 | 6.10 | 5.92 | 0.084 | < 0.01 | 0.19 | 0.49 | < 0.01 | 0.47 |
| Fecal pH[3] | 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 |

**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. [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]Measured 2 h after morning feeding.

| | Diet[1] | | | | | P-value[2] | | |
|---|---|---|---|---|---|---|---|---|
| | CON | MH | LH | CS | SEM | 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 | | | | | | | | |
| Full gastrointestinal tract | 15.42 | 17.12 | 17.53 | 16.44 | 1.157 | 0.09 | 0.16 | 0.73 |
| Empty gastrointestinal tract | 6.49 | 7.02 | 6.74 | 6.39 | 0.457 | 0.62 | 0.32 | 0.64 |
| Gastrointestinal tract fill | 8.93 | 10.14 | 10.79 | 9.53 | 0.812 | 0.07 | 0.18 | 0.43 |
| Reticulo-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 |
| Reticulo-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 | | | | | | | | |
| Full gastrointestinal tract | 19.37 | 20.79 | 20.82 | 20.22 | 0.962 | 0.22 | 0.98 | 0.96 |
| Empty gastrointestinal tract | 8.09 | 8.49 | 8.04 | 8.10 | 0.301 | 0.71 | 0.60 | 0.25 |
| Gastrointestinal tract 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 |
| Reticulo-rumen volume, L as % BW | 20.45 | 22.11 | 21.37 | 18.87 | 1.227 | 0.79 | 0.04 | 0.62 |

**Table 3**. Slaughter parameters of calves fed with different sources and qualities of fiber in total mixed diets. [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. [3]Empty BW calculated by adjusting for gut fill, as Jahn et al. (1976) described.

| | Diet[1] | | | | SEM | P-value[2] | | |
|---|---|---|---|---|---|---|---|---|
| | CON | MH | LH | CS | | CON×F | CS×H | MH×LH |
| Papillae number per 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 | 0.323 | 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 |

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

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 ($P = 0.03$).

## Discussion

### Ruminal and fecal parameters

It is crucial to understand that forage inclusion in diets with high starch content or reduced particle size can significantly enhance solid diet intake due to the rumen buffering effect[9,28]. This knowledge is particularly important near weaning, as solid diet intake gradually increases, and the volume of liquid diet remains fixed throughout the pre-weaning period.

Notably, physically effective dietary fiber has shown significant potential in maintaining rumen health for preweaning dairy calves[29]. Finding the ideal balance between peNDF and NFC in the diet of a young dairy calf is difficult due to low rumen capacity volume. However, it is essential to maintain adequate ruminal metabolism and improve dairy calves' performance.

Feeding TMR may favor fiber degradation kinetics in the rumen compared to a component diet (starter and forage)[30,31]; the interaction between the ingredients may favor the uniformity of the diet and promote great intake. However, these factors underscore the need to evaluate different scenarios and make necessary adjustments.

The performance results of this study suggest that including forage in coarsely ground starter, at 7.5% of both *Tifton* 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[14]. 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 starch bacteria, increasing propionate in the rumen but reducing rumen pH[18,32,33]. 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[4]. 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[14].

Including long particles from forage promoted a small change in the average particle size of the TMR. 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[12]. 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[19]. This reinforces the need for fiber from forage with a minimum percentage of peNDF greater than 4 mm 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[34,35]. 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[36], providing a healthy ruminal environment.

Forage diets usually favor cellulolytic fermenting bacterial growth and increase ruminal pH[37]. 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.

Previous research indicates that including 10% and 15% of corn silage (on a dry matter basis) in calves' diets can maintain rumen pH and improve the performance of preweaning dairy calves[12,38]. This is particularly beneficial for farms due to the availability of corn silage and logistical or cost constraints. However, it is important to note that providing a long particle size and a high-moisture diet for calves requires careful management. This includes daily evaluation of orts to ensure consistency and avoid variations in early intake management.

Moreover, Cynodon dactylon is a perennial tropical forage, and ruminants well accept cultivars such as Tifton-85, coast-cross, and Florakirk due to their thin stems and multiple leaves[39]. 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[6,19]. These findings were considered to establish forage-level inclusions in the present study.

## Slaughter and morphometrics parameters

Proper rumen development in the pre-weaning period is central to success during post-weaning. Providing forages to young calves may promote rumen health needed for optimal feed intake and nutrient absorption[40]. Nevertheless, the level and particle size of the forage supplied should be combined with the grain source provided in the starter.

Besides the rumen health benefits for young calves, 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[12,17,30]. The most used equation for calculating EBW in dairy calves was developed for calves between 8 and 20 weeks of age fed with straw-level inclusion and may not be suitable for assessing gut fill during preweaning[25]. 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[17,29], indicating the need for further investigation.

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 VFA and develop rumen papillae[29]. Consequently, calves may have compensated for the energy intake, and slaughter weight was not affected when comparing forage and no-forage diets.

Mirzaei et al.[41] evaluated 8% and 16% alfalfa inclusion in the calf's diet with medium (2.92 mm) and long (5.04 mm) particle 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[42,43]. 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[18,29].

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 fiber's lower digestibility 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[16,29].

The NASEM[2] 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.[25] 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 starter 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[14,17,30,41].

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 a 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 digestive tract's development process due to improved ruminal health and its effects on solid diet intake.

Lesmeister et al.[26] 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, 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[44,45].

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.

Previous research has limited the evaluation of the caudal ventral blind sac, and it appears this area is less developed in young calves[26]. 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. This promotes a variation in papillae width and height with no major differences among diets.

Feeding dairy calves *Tifton* hay or corn silage, combined with high starch and moderate peNDF content in a total mixed ration, is a strategy to increase rumen health and develop the rumen epithelium of weaned dairy calves. Forage supply, when part of a balanced diet, ensures the health and development of the calves without negatively affecting their growth.

## Data availability

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

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## Author contributions
C.M.M.B. conceived the study. A.F.T. made substantial contributions to the design of the work. C.M.M.B. provided experimental and laboratory resources. A.P.S., F. V. L. B, R. D. F. B, I. C. R. O, E. D. M collected data and samples. A.F.T, D. M. P, and C.M.M.B. analyzed the statistical data. All authors were involved in the interpretation of the data. A.F.T. drafted the manuscript, which was critically revised by C.M.M.B. All authors reviewed and approved the manuscript.

## Competing interests
The authors declare no competing interests.

## Ethics approval
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). We confirm that this study was carried out in compliance with the Animal Research: Reporting of In Vivo Experiments (ARRIVE) guidelines.

## Additional information
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