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Forage sources in total mixed rations early in life influence performance, metabolites, and behavior of dairy calves

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ABSTRACT

The objective of this study was to evaluate 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 BW at 28 d 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 (TMR) containing 7.5% on a dry matter (DM) basis of Tifton hay of either medium quality (MH) or low quality (LH); or 10% on a DM basis of corn silage (CS). During the first 28 d 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 d postweaning. 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 wk 7 (preweaning) and 10 (postweaning). Solid feed intake increased at wk 7 and 8 when MH, LH, and CS were included in TMR; the same results were observed postweaning. The diets did not affect the average daily gain and BW, 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 postweaning diets.

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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 percentage of NDF, NDF from forage, and physically effective NDF recommendations in the diet of calves with a BW up to 120 kg (NASEM, 2021). To assist scientific and practical recommendations, studies that characterize these particularities of each forage source area needed.

The cereal grains and the forage play different roles but complement each other as regard to the 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). In contrast, 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

The list of standard abbreviations for JDS is available at adsa.org/jds-abbreviations-24. Nonstandard abbreviations are available in the Notes.

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 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 ensures 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, 2013; Poczynek et al., 2020; 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., 2001).

In addition, hay and silage have different characteristics; hay consists almost exclusively of forage, and silage consists of forage and grains. Some corn silage (**CS**) varieties have higher grain content and stover digestibility than others (Ball et al., 2001), 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 with calves offered no forage.

MATERIALS AND METHODS

Animals and Treatments

All study procedures were approved by the "Luiz de Queiroz" College of Agriculture–University of São 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 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 hand-held 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–10-L 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 TMR 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 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 ~31.0% of grains and 69.0% of leaves and stems, resulting in 23.7% starch on a DM basis. Including 10% DM of CS 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[1]

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 mg/kg; I 4%; K 1%; Mg 7%; S 0.7%; Co 25 mg/kg; Cu 800 mg/kg; Cr 20 mg/kg; I 40 mg/kg; Fe 1,400 mg/kg; Mn 1,500 mg/kg; Se 18 mg/kg; Zn 3,200 mg/kg; vitamin A 140,000 IU/kg; vitamin D_3 50,000 IU/kg; vitamin E 1,500 IU/kg; vitamin B_1 250 ppm, vitamin B_2 250,000 mg/kg; vitamin B_6 250 mg/kg; vitamin B_{12} 250 mg/kg; niacin 400 mg/kg; pantothenic 500 mg/kg; folic acid 20 mg/kg; biotin 10 mg/kg; butylated hydroxytoluene 800 mg/kg, sodium monensin 900 mg/kg.

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 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 wk of life, 2 h after the morning feeding, using 3 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 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).

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 2 time points in wk 7 and 10, for a total observation time of 20 h/calf. Behavior was recorded by 4 trained observers who were blind to treatments.

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 1 mm Wiley mill (Marconi, Piracicaba, Brazil). The DM 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.

Table 2. Ethogram describing behaviors of calves during 10 h 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, or the hutch
Ruminating	Repeated chewing the solid feed

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

Joseph, MI; AOAC International, 2002; method 968.06), and CP was calculated by multiplying the total nitrogen by 6.25. Ether extract 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).

Samples of solid feed, chopped hay, CS, 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 (long: >19 mm; medium: between 19 and 8 mm; short: between 8 and 4 mm; and fine: <4mm), and physically effective NDF (**peNDF**) was calculated considering particles >4 mm, which were retained on the top 3 screens of the Penn State Particle Separator (Yang and Beauchemin, 2006). The in vitro true digestibility over 72 h was determined according to Goering and Van Soest (1970).

The concentration of milk solids was used to calculate total DMI 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 DMI of the feed offered and the DM 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). The 48 calves were randomized into 12 complete blocks according to weight at 28 d of life and sex (9 males and 3 females). Feed intake, performance, and blood metabolites were analyzed as repeated measures over time: $Y_{ijk} = \mu + D_i + b_j + e_{ij} + I_k + (D_i)_{Ik} + e_{ijk}$, where μ = overall mean; D_i = fixed effect of diet; b_j = random effect of block; e_{ij} = residual error (A); I_k = fixed effect of age; $(D_i)_{Ik}$ = effect of diet × effect of age interaction; and e_{ijk} = residual error (B). Covariance matrices were tested and defined according to the lowest value obtained for Akaike's information criterion corrected. Ingestive behavior was evaluated as a nonrepeated measure using the following statistical model: $Y_{ij} = \mu + D_i + b_j + e_{ij}$, where μ = overall mean; D_i = diet effect; b_j = random effect of block; and e_{ij} = residual error. For all response variables, the means were obtained using the LSMEANS command. The model included the effects of 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 time period: preweaning (28–56 d) and postweaning (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* ≤ 0.10 was considered as a trend.

RESULTS

Diet and Forage Characteristics

The level of NDF, ADF, and lignin is higher in the LH, followed by medium hay and CS (Table 3). In contrast, the DM in vitro true digestibility over 72 h 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 2). 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. In contrast, 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-mm and 1.18-mm sieves. Therefore, peNDF >4 mm of the 2 diets containing hay were similar, with lower values for the CS diet. In contrast, the CS showed a higher percentage of particles distributed between the 19-mm 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.

Intake and Growth Performance

During the preweaning period, a significant interaction was observed between diet and age for solid feed intake (Figure 1A; Table 4). There was no diet effect at wk 5 and 6, however, the inclusion of forage increased the solid feed intake at wk 7 ($P = 0.02$) and tended to increase at wk 8 ($P = 0.07$). In addition, at wk 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 postweaning 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 postweaning.

There was an interaction between diet and age for NDF intake during the preweaning period (Figure 1B). There was no diet effect at wk 5 for NDF intake; however, at wk 6, TMR with forage increased NDF intake compared with CON ($P = 0.04$), with no difference among the evaluated forage sources. At wk 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 postweaning 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 with CS. A similar effect was observed for NDF intake as a percentage BW (NDF % BW), pre – and postweaning.

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

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 for 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 postweaning 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 with calves receiving a forage source. In contrast, no diet effect was observed for feed efficiency in the postweaning period.

Table 3. Chemical composition and particle size of experimental diets and forages[1]

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.

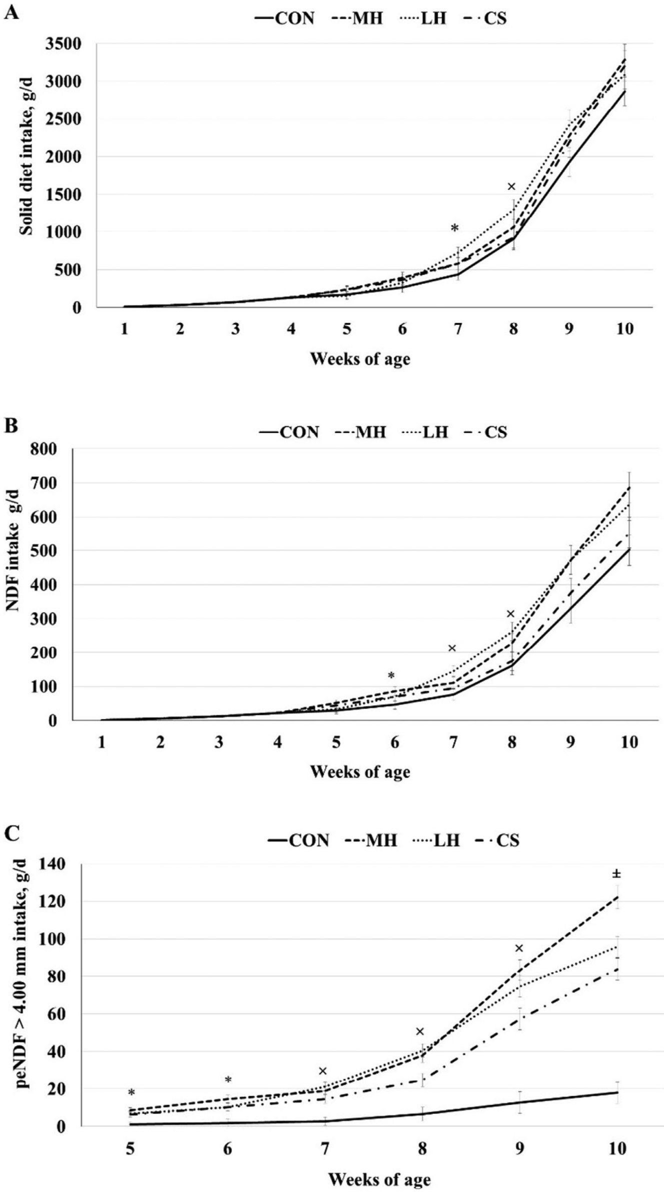

Figure 1. (A) Solid diet intake (g/d) of calves during the whole period. *Denotes forage effect. Calves that received the 7.5% medium-quality hay (MH), 7.5% low-quality hay (LH), and 10% corn silage (CS) diets had a higher solid diet intake compared with 0% fiber from forage (CON; $P = 0.02$); × denotes 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 ($P = 0.07$). (B) Neutral detergent fiber intake (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 with 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 ($P = 0.02$). (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 with 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 ($P = 0.01$). Error bars indicate SEM.

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.

Ingestive and Sorting Behavior

At wk 7, calves fed diets containing forage spent more time consuming their solid feed ($P < 0.02$) and ruminating ($P < 0.04$) compared with 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 wk 7.

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

Sorting of each particle size fractions is shown in Figure 2. Only the CS diet presented particles larger than 19 mm (Table 3). At wk 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 with MH ($P = 0.01$; Figure 2). In contrast, at wk 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 wk 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 wk 7 ($P = 0.01$) and wk 10 ($P = 0.03$).

Table 4. Preweaning and postweaning 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]				
	CON	MH	LH	CS		CON × F	CS × H	MH × LH	A	A × D
Preweaning (d 28–56)										
Liquid diet, g of DM/d	677.37	675.25	677.79	673.39	7.289	0.80	0.69	0.78	<0.01	1.00
Solid diet, g of DM/d	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, g of DM/d	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[3] >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[4]	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	—	—
Postweaning (d 57–70)										
Solid diet, g of DM/d	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, g of DM/d	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[4]	0.455	0.415	0.377	0.403	0.032	0.12	0.86	0.41	<0.01	0.99
Final BW (d 70), kg	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]Contrasts between groups (control × forage [CON × F]; corn silage × hay [CS × H]; medium-quality hay × low-quality hay [MH × LH]); A = age effect; A × D = interaction between age and diet.

[3]peNDF = physically effective neutral detergent fiber >4 mm.

[4]Considering liquid and solid diet intake.

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. In contrast, low levels of forage inclusion can benefit intake and performance during pre- and postweaning, 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 effect in

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

Item	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, 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, 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]Contrasts between groups (control × forage [CON × F]; corn silage × hay [CS × H]; medium-quality hay × low-quality hay [MH × LH]); A = age effect; A × D = interaction between age and diet.

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

	Diet[1]					P-value[2]		
Item	CON	MH	LH	CS	SEM	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 or 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
Postweaning, 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 or 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]Contrasts between groups (control × forage [CON × F]; corn silage × hay [CS × H]; medium-quality hay × low-quality hay [MH × LH]).

the intake and sorting of particles and, consequently, on performance (Khan et al., 2020; Zhang et al., 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 wk 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 postweaning, 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 wk 10. As expected, the capacity for effective fiber intake in postweaning 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% postweaning, 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 effect 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 in addition to providing nutrients. In contrast, the particle size and moisture might be the main factors that limit fiber intake when TMR with similar nutrients is fed to pre- and postweaning 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

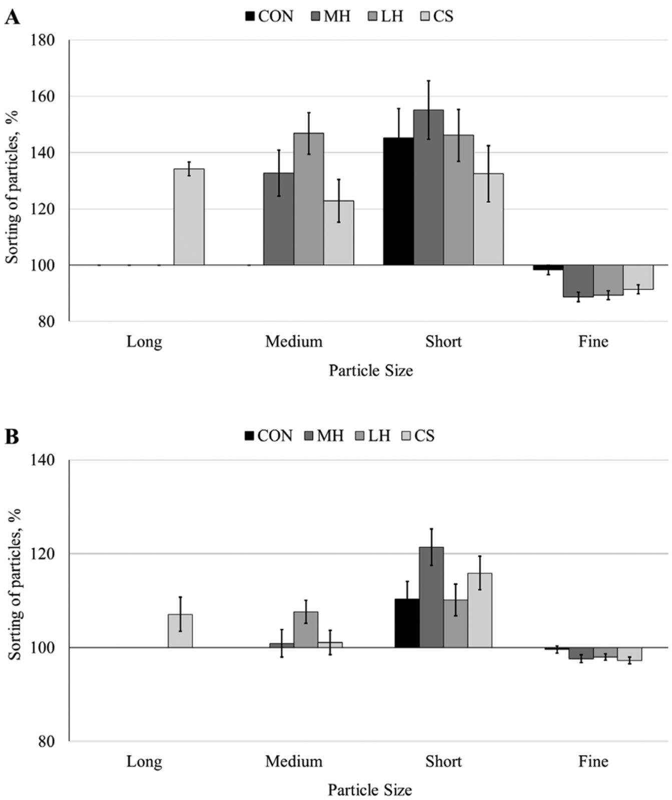

Figure 2. (A) Mean ± SEM intake of the particle fractions of a TMR (expressed as a % of predicted intake) when calves were offered 0% fiber from forage on the diet (CON), 7.5% medium-quality hay on the diet (MH), 7.5% low-quality hay on the diet (LH), and 10% corn silage on the diet (CS) at 49 d and (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).

Quigley et al. (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 CS: 19.4% NDF, 2.8% peNDF) increased the fiber content and the effectiveness provided in the TMR compared with the CON diet (17.5% NDF; 0.8% peNDF). The increase in the NDF level associated with peNDF >4 mm and an average particle size of 4 mm for hay and 9.8 mm for CS promoted early intake of solid feed without affecting the performance of dairy calves. Poczynek et al. (2020), 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 CS 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 DMI (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 DM, 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 CS 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). In contrast, 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, affecting 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. (2014) 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). As a consequence of rumen development, metabolism of end products are also more efficient. Therefore, differences in blood metabolites concentrations are expected when pre and postweaning periods are compared. In the present study, we have observed a forage effect decreasing lactate and increasing BHB concentrations preweaning, but that was not observed postweaning.

Ingestive and Sorting Behavior

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 postweaning. In addition, postweaning, the CS diet resulted in longer feeding when compared with 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 CS 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 with calves providing no forage and the provision of CS having an intermediate effect. The main differences between medium and LH 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 CS 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 with 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. In contrast, 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 preweaning 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 also 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 nonfiber 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 postingestive 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 postweaning (Miller-Cushon et al., 2013). However, this needs to be consistently reported (Xiao et al., 2018). Postweaning 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 wk. 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 affects the ruminal environment, intake, and performance of calves.

CONCLUSIONS

Regardless of quality, increasing the NDF level with fiber from forage is essential for preweaning diets with high starch inclusion. A TMR with CS, containing 19.4% NDF and 2.8% peNDF on a DM basis, does not negatively affect 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 postweaning. Hay is the preferable forage source in the diet during the transition phase between preweaning and postweaning. However, CS is a potential alternative source of fiber to be included in TMR for calves. In any case, adjusting particle size and the nutrients for feeding as a TMR must be considered.

NOTES

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Abbreviations used: CON = no-forage coarsely ground starter; CS = corn silage; LH = low-quality Tifton hay; MH = medium-quality Tifton hay; peNDF = physically effective neutral detergent fiber.

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