Animal Nutrition 16 (2024) 326–337

Contents lists available at ScienceDirect

Animal Nutrition

journal homepage: http://www.keaipublishing.com/en/journals/aninu/

Original Research Article

How neonatal diet affects the long-term development of rumination behavior, rumen fermentation and feed digestion in dairy calves fed a high milk level?

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ARTICLE INFO

Article history:
Received 14 February 2023
Received in revised form
25 November 2023
Accepted 4 December 2023
Available online 30 December 2023

Keywords:
Neonatal calf
Early forage feeding
Rumination
Rumen fermentation
Nutrient digestibility

ABSTRACT

This study was to investigate growth performance, rumination development, rumen fermentation and feed digestion in young calves provided high volumes (about 20% of calf birth weight) of milk with or without forage inclusion and how these parameters correlate with each other. Immediately after birth, 160 newborn Holstein female calves (41.6 ± 4.2 kg of initial BW) were randomly divided into 2 treatments: 1) starter (CON, only starter) and 2) starter and hay (HAY, both starter and hay). The calves were fed their respective experimental diets from d 4 to 84, after which they were all introduced to similar diets until the end of the experiment on d 196. Treatment had no effect on growth and structural measurements throughout the experimental period. However, treatment had an effect on the other parameters, mainly during the post-weaning period. Forage supplementation tended to reduce starter dry matter intake ($P = 0.05$), while increasing the forage intake ($P < 0.01$) and the feed-to-gain ratio ($P < 0.01$). HAY calves had increased neutral detergent fiber (NDF) and physically effective NDF (peNDF) intakes ($P < 0.05$) and tended to lower ($P < 0.01$) starch intake compared to CON calves. The HAY calves had a higher rumination time ($P < 0.01$), ruminal pH ($P < 0.01$), and acetate-to-propionate ratio ($P = 0.05$) compared to the CON calves. Spearman correlation analysis showed that rumination time was positively related to the ruminal pH at d 84 ($P = 0.01$) and 196 ($P = 0.02$). The HAY calves had similar apparent total-tract digestibility of dry matter (DM), NDF and ether extract (EE), but lower digestibility of organic matter (OM, $P = 0.03$), crude protein (CP, $P < 0.01$) and starch ($P < 0.01$) compared to those of the CON calves at week 12. Furthermore, there were no positive relationships between rumination time and nutrient digestibility or between rumination time per kilogram DM and nutrient digestibility. In conclusion, feeding hay to calves fed a high milk level improved rumination during the post-weaning period only, without a concomitant effect on growth performance throughout the experimental period, suggesting no detrimental effect of feeding forage in calves fed high milk level.
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1. Introduction

Rumination behavior is a key marker that distinguishes ruminants from monogastric animals. Rumination is a physiological process that promotes the breakdown and decomposition of herbivorous feed and stimulates digestion (Heinrichs and Lesmeister, 2005). The development of the rumination behavior accompanies the development of rumen in calves. Although the rumen is not fully developed in calves, the onset of rumination begins at a very

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Peer review under responsibility of Chinese Association of Animal Science and Veterinary Medicine.

Production and Hosting by Elsevier on behalf of KeAi

https://doi.org/10.1016/j.aninu.2023.12.003
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young age of approximately 1 to 2 weeks (Babu et al., 2004; Swanson and Harris, 1958). Following the transition from pseudo-monogastric to full forestomach-based digestion (Huber, 1969), the rumination time increases rapidly and stabilizes to around 7 to 8 h/d in adulthood (Adin et al., 2009). In theory, greater rumination improves feed mastication, reducing particle size and ultimately enhancing feed digestibility. Moreover, rumination can stimulate saliva production and flow into the rumen, neutralizing the acids, which helps to optimize a healthy ruminal environment and maintain the appropriate ruminal fluid pH (Daneshvar et al., 2015; Hosseini et al., 2019; Kim et al., 2016; Xiao et al., 2018). Consequently, the risk of rumen acidosis is minimized, further improving calf health, growth performance (Khan et al., 2016; Xiao et al., 2020), and rumen development (Coverdale et al., 2004; Porter et al., 2007).

It is well known that rumination is highly dependent on the dietary composition. Forages, high in physically effective fiber, have an enormous ability to improve the extent of rumination in mature dairy cows (Beauchemin, 2018). Similarly, previous studies have reported that forage inclusion in the diet of calves enhances rumination activity (Castells et al., 2012; Krause and Oetzel, 2006). Most of the studies that have assessed the effects of forage on calf rumination to date mainly fed calves with conventional low levels of milk at approximately 4 to 5 L/d (equivalent to approximately 10% of calf birth weight) (Castells et al., 2012; Ebnali et al., 2016; Lin et al., 2018). While conventional milk feeding has been shown to encourage starter intake and promote early rumen development (Jasper and Weary, 2002), dietary inclusion of the less energy-dense forages contributed to poor weight gains in calves (Hill et al., 2008; Maktabi et al., 2016). On the contrary, Khan et al. (2011) reported that providing hay to calves fed with high milk level (8 L/d, equivalent to approximately 20% of calf birth weight) resulted in increased solid feed intake and rumen development. However, it is not fully clear how forage feeding affects rumination, rumen fermentation and feed digestion, as well as the association between rumination and feed digestion in calves fed high milk level. Moreover, although forage inclusion might have the ability to improve the rumination time of calves in the short term, continuous investigation on its inclusion from early life on subsequent rumination and rumen fermentation in the long term (beyond 84 d of life) has not been elucidated.

Considering that early life is a critical stage in the development of a young calf and early forage feeding could encourage rumen development, we hypothesized that early forage feeding could improve the rumination behavior of calves fed high levels of milk during not only the dietary treatment stage but also in subsequent periods when all the experimental calves were introduced to the same diet. Thus, the aims of this study were 1) to detect, under high levels of milk feeding, the short- and long-term effects of neonatal diet (forage inclusion or not) on the development of rumination even after introducing all the calves to the same diet and 2) to investigate whether the changes in rumination would further affect the feed digestion and how they correlated with each other.

2. Materials and methods

2.1. Animal ethics statement

This animal experiment was conducted according to the Regulation of the Laboratory Animals (3rd edition, 2017) promulgated by Decree No. 676 of the State Council, China. Administration of the animal care protocol was approved by the Animal Care and Use Committee of China Agricultural University (Approval No. AW82211202-1-1, Beijing, China), and all efforts were made to minimize animal suffering.

2.2. Animals, feeding and housing

A priori statistical power analysis used primary response variables, dry matter intake (DMI), average daily gain (ADG) and rumination to determine an adequate number of animals per treatment. We calculated the statistical power using G*Power software (version 3.1) to obtain power of 0.80 as one minus type II error ($1 - \beta$), where β is the probability of making a type II error, and the probability (α) of making a type I error is 0.05. The effect sizes for DMI, ADG and rumination behavior were determined based on previous results (Castells et al., 2015; Ebnali et al., 2016) and were 0.23, 0.40 and 0.48, respectively. From our calculation, the minimum sample size was 154, 52 and 36 calves for DMI, ADG and rumination behavior, respectively. Thus, we projected that a sample size of 160 calves was more than sufficient to investigate our main objectives in this study.

One hundred and sixty neonatal female Holstein calves (41.6 ± 4.2 kg of initial BW) were enrolled in this study. To minimize the effects of maternal differences on newborn calves, the dams from the same farm (Modern Farming (Baoji) Co., Ltd) raised in the same barn and fed the same transition diet before calving were used. Newborn calves with birth weight ($\geq$30 kg) were separated from their dams immediately after birth and then transferred to individual polyethylene hutches with a fenced area (inside dimensions of the hutches were approximately 1.15 m $\times$ 1.8 m, and the outside fenced area dimensions were approximately 1.2 m $\times$ 1.6 m). The calves received 4 L of colostrum (>22% Brix) within 1 h of life. Serum total protein at 24 h after colostrum feeding was determined in all calves and only those that achieved successful passive immunity transfer ($\geq$5.5 g/dL) were enrolled in this study. During the pre-weaning period, calves were offered pasteurized whole milk twice daily at 08:00 and 15:00 from d 2 to 56 through pails, which included 8 L/d from d 2 to 21, 10 L/d from d 22 to 42, 8 L/d from d 43 to 49, and 6 L/d from d 50 to 52, 4 L from d 53 to 55, and 2 L on d 56. Afterward, calves were completely weaned off milk on d 57. Milk was pasteurized using a milk pasteurizer (BR0.7-BS-1J, Honneur Agriculture and Technology Ltd., Beijing, China), where milk temperature was elevated and held at 72 °C for 15 s and then cooled to 38 °C. Calves were kept in the individual hutches until d 84 of life. Thereafter, they were transferred to one pen in a barn, where they were housed together. The pen was approximately 13 m wide by 180 m long, consisting of a feeding lane (3 m wide) and a lying area (10 m wide). The pen was equipped with 15 automatic water troughs, feed bunks in front of the feeding lane and an automatic cable scraping system. The calves raised in the pen had free access to feed and water all the time. The hutches and barn were cleaned and sterilized before introducing calves, according to the standard operating procedures of the farm. Sawdust was used as bedding material and was renewed on a weekly basis in the hutches as well as in the pen.

2.3. Experimental design and diets

Immediately after birth, the calves were randomly divided into 2 treatments: 1) starter (CON, only starter; $n = 80$) and 2) starter and hay (HAY, both calf starter and oat hay; $n = 80$). From d 4, all calves were introduced to their respective diets and remained on the same diet until d 84. The starter (Modern Farming Co. Ltd., Anhui, China) and oat hay (<2.5 cm) were offered as free choices in separate buckets every morning for ad libitum intake. From d 85 to 133, on a daily basis, all calves were offered the same starter (90% of diet) and oat hay (10% of diet) as a top-dressed ration (TDR, starter spread over hay), which allowed calves free access to either fresh starter or hay in the feed bunks. Calves were then offered a novel total mixed ration (TMR) from d 134 to 196. The TMR was fed once daily at

10:00 immediately after orts disposal. The TMR composition included alfalfa hay (39.5%), corn silage (32.5%), soybean meal (13.5%), steam-flaked corn (11.7%) and premix compound (2.9%) on a DM basis. All calves had free access to solid feed and water. The nutrient composition and particle size distribution of the diets are reported in Table 1.

2.4. Sample collection

2.4.1. Feed sampling and analysis

Daily milk and feed intakes were recorded based on the amount offered and refused by each calf from d 4 to 84. All types of feed were offered in sufficient amounts to ensure at least 10% orts. Forage intake to total solid intake ratio (FIR) was calculated based on daily solid feed DM intake (DMI). Representative samples of calf starter and oat hay were collected weekly and immediately frozen at −20 °C until further analysis for nutritional composition. Dry matter (DM), crude protein (CP), ether extract (EE), neutral detergent fiber (NDF), acid detergent fiber (ADF) and crude ash were analyzed following AOAC, 2000.

2.4.2. Body weight and structural measurements

All calves were weighed by a digital scale every two weeks for the first 12 weeks (d 1, 14, 28, 42, 56, 70 and 84) and then at week 28 (d 196). Withers height (vertical distance from the highest point of calf withers to the ground) and heart girth (the horizontal circumference of the calf body at the posterior edge of the scapula) were also recorded at these time points. The average daily gain (ADG) and feed efficiency (FE, a kilogram of DMI per kilogram of BW gain, until d 84) were calculated for every 2 time points between which measurements were taken for each calf before analysis.

Table 1
Nutrient composition and particle size distribution in the starter, hay, milk and TMR.[1]

Item	Starter[2]	Oat hay[3]	Milk	TMR[4]
Nutrient composition				
DM, %	88.7	88.0	13.0	50.7
CP, % of DM	22.8	6.4	27.4	17.4
Fat, % of DM	–	–	30.8	–
SNF, % of DM	–	–	69.2	–
Starch, % of DM	32.3	0.6	–	17.5
NDF, % of DM	17.0	47.7	–	34.6
ADF, % of DM	10.5	25.6	–	25.6
ASH, % of DM	7.4	4.6	–	–
EE, % of DM	1.7	1.4	–	3.0
ME[5], Mcal/kg of DM	2.9	2.2	5.4	2.5
Particles[6], %				
Long	0	39.0	–	8.4
Medium	17.4	25.1	–	42.0
Short	82.1	18.1	–	14.2
Fine	0.5	16.4	–	35.4

DM = dry matter; CP = crude protein; EE = ether extract; NDF = neutral detergent fiber; ADF = acid detergent fiber; SNF = solid non-fat; ME = metabolizable Energy; TMR = total mixed ration.

[1] This Table is adapted from Xiao et al. (2023).

[2] Calf starter was supplied by Modern Farming Co. Ltd, containing 33.6% corn, 18.8% fermented soybean meal, 14.1% soy hulls, 10.5% soybean meal, 6.3% wheat bran, 6.3% wheat flour, 5.4% whey powder, 5% premix compound (contained vitamin A 200,000 IU/kg, vitamin D 25,000 IU/kg, vitamin E 2,000 IU/kg, manganese 0.6 g/kg, iron 0.4 g/kg, copper 0.5 g/kg, zinc 2 g/kg, cobalt 4 mg/kg, iodine 16 mg/kg, and selenium 4 mg/kg) on a dry matter basis.

[3] Oat hay was cut to less than 2.5 cm using a stationary mixer (20 m^3, Trioliet Co., Ltd., Holland).

[4] TMR was composed of 39.4% alfalfa hay, 32.5% corn silage, 13.5% soybean meal, 11.7% steam flaked corn and 2.9% premix compound (contained vitamin A 300,000 IU/kg, vitamin D 80,000 IU/kg, vitamin E 2,000 IU/kg, Mn 2.0 g/kg, Cu 0.7 g/kg, Zn 2.5 g/kg, Co 20 mg/kg, iodine 15 mg/kg, and selenium 14 mg/kg) on a dry matter basis.

[5] ME for calf starter, hay, milk and TMR were calculated according to NRC (2001) equations.

[6] Samples for particle sizes were analyzed using a 3-sieve (19, 8, and 4 mm) Penn State Particle Separator (Nasco, Fort Atkinson, WI, USA).

2.4.3. Rumen fermentation

Rumen fluid was collected on d 1, 35, 84 and 196 of age by a flexible esophageal tube (2 mm of wall thickness and 6 mm of internal diameter; Anscitech Co., Ltd, Wuhan, Hubei, China) from all calves 2 h after the morning feeding. The first 10 mL of rumen fluid was discarded to avoid saliva contamination. Rumen fluid was filtered through 4 layers of cheesecloth. Ruminal pH was measured immediately with a glass electrode pH meter (HORIBA Advanced Techno Co. Ltd., Osaka, Japan). The rumen fluid samples were processed for analysis of volatile fatty acids (VFA) concentration based on the method described by Erwin et al. (1961) with modifications using gas chromatography (6890N, Agilent Technologies, Wilmington DE, USA). Briefly, an initial centrifugation (3,000 × *g*) of the samples for 10 min was performed to remove particulate matter. Aliquots (1 mL) of the supernatant were mixed with 0.2 mL of 25% metaphosphoric acid solution containing the internal standard 2-ethyl butyric acid in a 1.5-mL centrifuge tube, placed in an ice water bath for more than 30 min, and centrifuged at 10,000 × *g* for 10 min. The supernatants were then placed in gas chromatography vials that were capped and refrigerated until the concentrations of VFA were determined. The column selection was adjusted and some chromatographic operating conditions were optimized based on Erwin et al. (1961). The chromatographic column was HP-INNOWax capillary column and set to a constant flow mode (flow: 2.0 mL/min, mean linear velocity: 38 cm/s). The gas chromatograph parameters were set as follows: carrier gas, N_2; injection volume, 0.4 μL; injection temperature, 220 °C; split ratio, 40:1, and 2-ethyl butyric acid was selected as the internal standard. Based on the established integral parameter and correction curve, the content of each component of the unknown sample was obtained by the internal standard calculation method.

2.4.4. Rumination behavior

Previous works have evaluated the feasibility of using acceleration sensors to monitor the rumination behavior in calves (Hill et al., 2017; Roland et al., 2018; Reynolds et al., 2019). Those studies reported that sensors were suitable for reliably identifying and detecting rumination. In this study, rumination behavior was recorded for all calves using an ear-attached 3-dimensional accelerometer sensor (Monitoring Ear Tag Flex, Allflex Livestock Intelligence eSense; SCR Engineers Ltd., Netanya, Israel). The electronic identification tag fitted with a sensor was placed in the calf's left ear. To minimize the risk of the sensor's position influencing the recordings, the sensor was centered between the 2 typical major veins within the proximal one-third of the ear. Data from the sensor were collected through a router and sent to the producer's computer, where the data are stored and processed by the Young Stock application and Heatime Pro$^+$ software (Allflex Livestock Intelligence; SCR Engineers Ltd., Netanya, Israel) to generate hourly and daily measurements recorded in minutes per each calf. Daily rumination time (min/d) was continuously recorded from d 1 to 196 and downloaded from the software for further analysis.

2.4.5. Apparent total-tract nutrient digestibility

After weaning, total-tract apparent digestibility was determined in 40 calves per treatment following the method used by Soltani et al. (2020). Twenty-one fecal grab samples (thrice daily at week 12 from d 78 to 84) per calf were gathered directly from the rectum (at 06:00,14:00 and 22:00, 8-h intervals) to estimate apparent total-tract nutrient digestibility by quantifying acid insoluble ash

(AIA) as an internal marker in the feed (corrected for refusals) and fecal samples as described by Van Kuelen and Young (1977). Briefly, all calves were rectally stimulated with sterile gloves to facilitate the collection of fecal samples. Individual daily calf fecal samples were composited (equal amounts on a wet weight basis) into one sample and frozen at −20 °C. The samples were then pooled by calf over the 7 d to obtain a single composite (combined on an equal wet weight basis) sample for further analysis. During analysis, the composite fecal samples were thawed at room temperature and placed in aluminum trays in an oven at 60 °C until completely dried (approximately 72 h). Dried fecal samples and representative feed samples (starter or oat hay) over the 7 days were ground through a 1-mm screen in a Wiley mill (Arthur H. Thomas, Philadelphia, PA) and analyzed for their chemical composition (CP, starch, fat, ash, NDF and ADF) as described previously. Individual calf AIA and nutrient percentage in feed consumed were corrected based on the starter and oat hay intake over the 7 days. The equation used to calculate digestibility was as follows:

Nutrient digestibility (%) = 100 − [100 × (nutrient in feces, %) × (AIA in DM consumed, %)]/[(AIA in feces, %) × (nutrient in consumed DM, %)].

2.5. Statistical analyses

Before analysis, data were checked for normality using the D'Agostino–Pearson omnibus normality test of GraphPad Prism version 8 (GraphPad Software Inc.). Calf was the experimental unit. Data for milk and solid feed DMI and nutrient DMI were summarized for each calf by week and analyzed separately by stage of life (Pre-weaning, d 1 to 56; Post-weaning, d 57 to 84; Total period, d 1 to 84). Data for BW, ADG, FE (until d 84) and structural growth at d 1, 28, 56, 70, 84 and 196 were collected for each calf and were also analyzed separately by stages of life. Data for ruminal pH and VFA were analyzed by sample collecting time at d 1, 35, 84, and 196. As data for daily rumination time was continuously recorded until the end of the experiment (d 196), it was summarized for each calf by week and then analyzed separately by the stage of dietary change (Pre-weaning, d 1 to 56; Post-weaning, d 57 to 84; Top-dressing, d 85 to 133; TMR, d 134 to 196). All data were analyzed as a randomized complete block design using the MIXED procedure of SAS (version 9.4, SAS Institute Inc., Cary, NC) with time (week or day) as repeated measures for the dependent variables. The model included the fixed effects of treatment, time and their interactions (treatment × time), and the random effect of calf within the treatment. Various covariance structures, including compound symmetry, simple, unstructured, first-order autoregressive and first-order antedependence, were examined and the best-fitted structure for the model was chosen based on the lowest Akaike information criterion. Spearman correlation coefficients were calculated to determine relationships between rumination time and rumen fermentation and feed digestibility indicators in SAS. The resulting correlation matrix was visualized in the Cor Heatmap Plot generated by the Hiplot applications (version 0.1.2). For each correlation, the correlation coefficient and *P*-value were obtained. The correlation coefficient values ranged from −1 to +1, with larger absolute values indicating a stronger relationship while positive and negative values indicated the direction of the association. Significant differences were declared at $P \leq 0.05$ and trends at $0.05 < P \leq 0.10$ for all the analyses.

3. Result and discussion

3.1. Feed intake and growth performance

The least-square means for feed intake-related and growth performance parameters are shown in Tables 2–4. No treatment differences were found in milk, starter, total solid and total DMI before weaning. After weaning, calves fed solely starter (CON, 2405.0 g/d) had greater starter DMI than those exposed to both starter and forage (HAY, 2264.8 g/d, $P = 0.05$). However, there were no differences in total DMI, mainly due to the significantly higher oat hay intake in HAY compared to the CON calves (248.1 g/d vs. 0.0 g/d, $P < 0.01$). We also found out that the starter and oat hay DMI increased over time ($P < 0.01$), while FIR dropped dramatically over time ($P < 0.01$) from 21.4% before weaning to 10.0% after milk withdrawal in HAY group. Similarly, our previous results showed that the FIR decreased over time, with calves preferring to consume more starter after weaning (Xiao et al., 2018). Given that dairy cows have a strong preference for sweet flavors that reflect higher energy levels (Ginane et al., 2011), it is easy to understand why calves in the HAY group could experience a gradual increase in the proportion of starter intake over time. Concomitant with the increase in age, the withdrawal of the high-nutrient milk diet most likely encourages the calves to turn to starter while trying to meet their taste preferences and energy requirements. Since there is a paucity of research on the effect of milk feeding on feed preferences, further studies are required to understand whether the milk feeding level affects the forage intake ratio in calves.

Regarding nutrient intake, similar CP, fat, starch, and ME intakes were found between treatments before weaning. After weaning, consistent with the increase of oat hay intake, the HAY calves had higher NDF, physically effective NDF (peNDF) and ADF intakes than CON calves. Including forage limited the starter intake, leading to a trend towards lower starch intake in the HAY than in CON calves (Table 3).

Consistent with similar feed and nutrient intakes, the CON calves did not differ in BW, ADG and structural growth measurements compared with the HAY calves in all periods (Table 4). However, a higher feed-to-gain ratio was found in HAY (2.68) than in CON calves (2.51, $P < 0.01$) during the post-weaning period, attributable to numerically higher total DMI in HAY but similar ADG between the two treatments.

Controversy remains on whether forage supplementation affects feed intake and growth performance in calves. Similar to some of the previous studies, this study found that dietary forage inclusion did not affect total solid DMI and growth performance (body weight, body size and daily gain) of calves (Khan et al., 2011; Mirzaei et al., 2015; Xiao et al., 2018). However, forage inclusion increases the feed-to-gain ratio (Beiranvand et al., 2014; Hibbs et al., 1956; Hill et al., 2008; Suárez et al., 2006), implying that HAY calves had a lower feed utilization efficiency. These observations could be related to the differences in nutrient composition and digestibility of starter and oat hay. Compared with starter, oat hay contains more fiber, which is more difficult to digest and might increase the accumulation of feed in the digestive tract, resulting in reduced feed utilization efficiency (Conrad and Hibbs, 1956; Hibbs et al., 1956). Reducing the consumption of highly digestible (e.g., starch), while elevating the hard-to-digest nutrients (e.g., NDF and ADF), could have increased the feed-to-gain ratio in the HAY calves.

3.2. Rumination behavior development and rumen fermentation

Table 5 presents the age at which calves started to ruminate, whereby the HAY calves tended to begin ruminating earlier than CON calves (13.1 vs. 14.4 d of age, $P = 0.09$), implying that offering forage to calves could stimulate rumination in calves. Similarly, a recent study reported that the age at first rumination was positively correlated with the age at which calves began to eat bedding straw (Wang et al., 2021), suggesting that consuming physically effective fiber earlier in life is key to initiating the rumination behavior.

Table 2
Effect of early forage inclusion on DMI and FIR before and after weaning.

Item	Trt[1]		SEM	*P*-value		
	CON	HAY		Trt	Time[2]	Trt × Time
Starter DMI, g/d						
Pre-weaning (d 4 to 56)	178.6	152.5	21.56	0.39	<0.01	0.71
Post-weaning (d 57 to 84)	2405.0[a]	2264.8[b]	50.62	0.05	<0.01	0.41
Total period (d 4 to 84)	920.7	856.6	27.92	0.11	<0.01	0.16
Hay DMI, g/d						
Pre-weaning (d 4 to 56)	0.0[b]	27.7[a]	4.12	<0.01	<0.01	<0.01
Post-weaning (d 57 to 84)	0.0[b]	248.1[a]	14.63	<0.01	<0.01	<0.01
Total period (d 4 to 84)	0.0[b]	101.2[a]	8.07	<0.01	<0.01	<0.01
Total solid DMI[3], g/d						
Pre-weaning (d 4 to 56)	178.6	180.3	21.85	0.96	<0.01	0.09
Post-weaning (d 57 to 84)	2405.0	2514.0	51.99	0.14	<0.01	0.58
Total period (d 4 to 84)	920.7	958.2	28.74	0.36	<0.01	0.48
FIR, %						
Pre-weaning (d 4 to 56)	0.0[b]	21.4[a]	1.12	<0.01	<0.01	<0.01
Post-weaning (d 57 to 84)	0.0[b]	10.0[a]	0.66	<0.01	<0.01	<0.01
Total period (d 4 to 84)	0.0[b]	17.6[a]	0.82	<0.01	<0.01	<0.01
Milk DMI, g/d						
Pre-weaning (d 4 to 56)	1145.9	1144.0	5.43	0.81	<0.01	0.02
Total DMI[3], g/d						
Pre-weaning (d 4 to 56)	1324.2	1324.4	17.91	0.99	<0.01	0.02
Post-weaning (d 57 to 84)	2405.0	2514.0	51.99	0.14	<0.01	0.58
Total period (d 4 to 84)	1684.4	1721.0	28.05	0.36	<0.01	0.15

DMI = dry matter intake; FIR = forage to total solid feed intake ratio; Trt = treatment; SEM = the standard error of the mean.
[a, b]Different letters indicate a significant difference ($P \leq 0.05$).
[1] CON = calves fed starter without forage from d 1 to 84, HAY = starter and forage were fed to calves as free choices from d 1 to 84. From d 85 to 196, calves were fed the same diet.
[2] For all variables, data were summarized by week. Period: Data were analyzed for the pre-weaning, post-weaning and total period.
[3] Total solid DMI = Starter DMI + Hay DMI, and Total DMI = Milk DMI + Total solid DMI.

Table 3
Effect of early forage inclusion on nutrient intake before and after weaning.

Item	Trt[1]		SEM	*P*-value		
	CON	HAY		Trt	Time[2]	Trt × Time
CP intake, g/d						
Pre-weaning (d 4 to 56)	354.6	350.0	3.88	0.40	<0.01	0.05
Post-weaning (d 57 to 84)	548.4	532.3	11.52	0.33	<0.01	0.57
Total period (d 4 to 84)	418.2	410.8	6.13	0.33	<0.01	0.15
EE intake, g/d						
Pre-weaning (d 4 to 56)	356.0	355.3	1.70	0.80	<0.01	0.01
Post-weaning (d 57 to 84)	40.9	42.0	0.87	0.38	<0.01	0.67
Total period (d 4 to 84)	250.9	250.9	1.28	0.98	<0.01	0.01
Starch intake, g/d						
Pre-weaning (d 4 to 56)	57.7	49.4	6.95	0.40	<0.01	0.73
Post-weaning (d 57 to 84)	776.8	733.0	16.35	0.06	<0.01	0.43
Total period (d 4 to 84)	297.4	277.3	9.02	0.12	<0.01	0.18
NDF intake, g/d						
Pre-weaning (d 4 to 56)	30.4	39.1	4.12	0.14	<0.01	<0.01
Post-weaning (d 57 to 84)	408.9[b]	503.4[a]	10.92	<0.01	<0.01	0.03
Total period (d 4 to 84)	156.5[b]	193.9[a]	5.98	<0.01	<0.01	<0.01
peNDF intake, g/d						
Pre-weaning (d 4 to 56)	0.0[b]	5.15[a]	0.75	<0.01	<0.01	<0.01
Post-weaning (d 57 to 84)	0.0[b]	46.2[a]	2.72	<0.01	<0.01	<0.01
Total period (d 4 to 84)	0.0[b]	18.8[a]	1.50	<0.01	<0.01	<0.01
ADF intake, g/d						
Pre-weaning (d 4 to 56)	18.7	23.1	2.47	0.22	<0.01	<0.01
Post-weaning (d 57 to 84)	252.5[b]	301.3[a]	6.40	<0.01	<0.01	0.07
Total period (d 4 to 84)	96.7[b]	115.8[a]	3.51	<0.01	<0.01	<0.01
ME intake, Mcal/d						
Pre-weaning (d 4 to 56)	6.71	6.68	0.050	0.74	<0.01	0.02
Post-weaning (d 57 to 84)	6.97	7.11	0.150	0.51	<0.01	0.68
Total period (d 4 to 84)	6.79	6.83	0.080	0.78	<0.01	0.10

DM = dry matter; CP = crude protein; EE = ether extract; NDF = neutral detergent fiber; peNDF = physically effective NDF; ADF = acid detergent fiber; ME = metabolizable energy; Trt = treatment; SEM = standard error of the mean.
[a, b]Different letters indicate a significant difference ($P \leq 0.05$).
[1] CON = calves fed starter without forage from d 1 to 84, HAY = starter and forage were fed to calves as free choices from d 1 to 84. From d 85 to 196, calves were fed the same diet.
[2] For all variables, data were summarized by week. Period: Data were analyzed for the pre-weaning, post-weaning and total period.

Table 4
Effect of early forage inclusion on BW, ADG, feed to gain ratio and structural growth before and after weaning.

Item	Trt[1]		SEM	*P*-value		
	CON	HAY		Trt	Time	Trt × Time
BW, kg						
Initial (d 1)	41.6	41.5	0.47	0.84	–	–
Final (d 196)	224.5	220.8	3.01	0.38	–	–
Pre-weaning (d 1, 14, 28, 42 and 56)	60.3	59.9	0.66	0.68	<0.01	0.49
Post-weaning (d 70 and 84)	98.3	98.2	1.10	0.95	<0.01	0.67
Total period (d 1 to 84)	71.1	70.7	0.75	0.81	<0.01	0.64
Overall (d 1, 14, 28, 42, 56, 70, 84 and 196)	85.2	84.7	1.17	0.74	<0.01	0.58
ADG, kg/d						
Pre-weaning (d 1 to 56)	0.65	0.63	0.010	0.50	<0.01	0.13
Post-weaning (d 57 to 84)	1.00	0.97	0.020	0.32	0.88	0.30
Total period (d 1 to 84)	0.76	0.75	0.010	0.30	<0.01	0.24
Overall (d 1 to 196)	0.81	0.80	0.010	0.31	<0.01	0.24
Feed-to-gain ratio[2]						
Pre-weaning (d 1 to 56)	2.91	3.06	0.237	0.65	<0.01	0.74
Post-weaning (d 57 to 84)	2.51[b]	2.68[a]	0.040	<0.01	<0.01	0.99
Total period (d 1 to 84)	2.77	2.93	0.159	0.48	<0.01	0.86
Withers height, cm						
Initial (d 1)	76.6	76.2	0.32	0.45	–	–
Final (d 196)	111.7	111.5	0.49	0.72	–	–
Pre-weaning (d 1, 14, 28, 42 and 56)	82.2	81.7	0.25	0.16	<0.01	0.93
Post-weaning (d 70 and 84)	92.5	92.0	0.35	0.36	<0.01	0.81
Total period (d 1 to 84)	85.1	84.6	0.26	0.19	<0.01	0.99
Overall (d 1, 14, 28, 42, 56, 70, 84 and 196)	88.5	88.0	0.26	0.23	<0.01	0.99
Heart girth (cm)						
Initial (d 1)	79.0	78.5	0.33	0.28	–	–
Final (d 196)	140.4	140.9	0.80	0.71	–	–
Pre-weaning (d 1, 14, 28, 42 and 56)	88.6	88.3	0.30	0.47	<0.01	0.48
Post-weaning (d 70 and 84)	105.5	105.0	0.41	0.46	<0.01	0.48
Total period (d 1 to 84)	93.6	92.9	0.35	0.14	<0.01	0.60
Overall (d 1, 14, 28, 42, 56, 70, 84 and 196)	99.3	99.0	0.34	0.62	<0.01	0.49

BW = body weight; ADG = average daily gain; Trt = treatment; SEM = standard error of the mean.
[a, b]Different letters indicate a significant difference ($P \leq 0.05$).
[1] CON = calves fed starter without forage from d 1 to 84, HAY = starter and forage were fed to calves as free choices from d 1 to 84. From d 85 to 196, calves were fed the same diet.
[2] Feed-to-gain ratio = Total DMI/ADG.

The comparison between treatments on the duration of rumination time is shown in Fig. 1. There were no significant differences between groups during the pre-weaning period (d 1 to 56, 140.9 vs. 142.5 min/d). On the contrary, post-weaning (d 57 to 84), calves solely consuming starter had significantly lower daily rumination time than those consuming both starter and oat hay (230.3 vs. 275.5 min/d, $P = 0.01$). Inconsistent with previous results (Hosseini et al., 2019; Maktabi et al., 2016; Mirzaei et al., 2017), dietary forage inclusion did not increase the rumination time during the milk feeding stage. We speculate that the high amount of milk fed to calves in this study could have reduced solid feed intake, hindering drastic changes in rumination behavior, as calves in the HAY group only consumed a small amount of oat hay (27.7 g/d) and peNDF (5.15 g/d) before weaning. Forage intake has been positively associated with ruminal pH and negatively correlated with the severity of SARA at a breakpoint of 80 g/d (Laarman and Oba, 2011). Most likely, consuming a certain amount of forage is required to reduce the time ruminal pH falls below 5.8 and mitigate rumen acidosis in calves through augmenting rumination activity. We further did Spearman correlation to better understand the relationship between rumination and feed intake and ruminal pH after weaning. The results showed that the FIR, forage DM intake and long particle size NDF intake were positively ($r = 0.31, P < 0.01$; $r = 0.28, P < 0.01$; $r = 0.28$, $P < 0.01$, Fig. 2) correlated with the duration of daily rumination time in week 12. These results indicate that forage intake, especially its physically effective fiber, can strongly improve rumination behavior in calves.

Table 5
Effect of early forage inclusion on the age at first rumination and starter and forage consumption.

Item[1]	Trt[2]		SEM	*P*-value
	CON	HAY		
The age at first rumination (days of age)	14.4	13.1	0.54	0.09
The age at first starter consumption (days of age)	6.4	6.1	0.27	0.42
The age at first forage consumption (days of age)	–	7.3	0.33	–

Trt = treatment; SEM = standard error of the mean.
[1] The age at first rumination and starter and forage consumption represents the age at the onset of rumination and starter and forage consumption, respectively.
[2] CON = calves fed starter without forage from d 1 to 84, HAY = starter and forage were fed to calves as free choices from d 1 to 84. From d 85 to 196, calves were fed the same diet.

Upon transfer of all calves from individual hutches to group feeding pen from d 85 to 133 and feeding a top-dressed ration, unexpectedly, rumination time in the CON group began to increase, being significantly higher than that of the HAY group calves (308.8 vs. 285.1 min/d, $P = 0.02$, Fig. 1). As this is the first study to monitor the long term development and changes in rumination behavior of calves that experienced different feeding regimes in early life, no relevant results were available in the literature for cross reference. We conjectured that the more rumination time in CON calves, upon transition to a top-dress diet, might have been motivated by the introduction of forage in this period for the first time and hence, more time was needed to digest the available diet. However, from d 134 to 196 of age, when all calves were transferred to a TMR, the effect of treatment on rumination behavior faded away (CON vs.

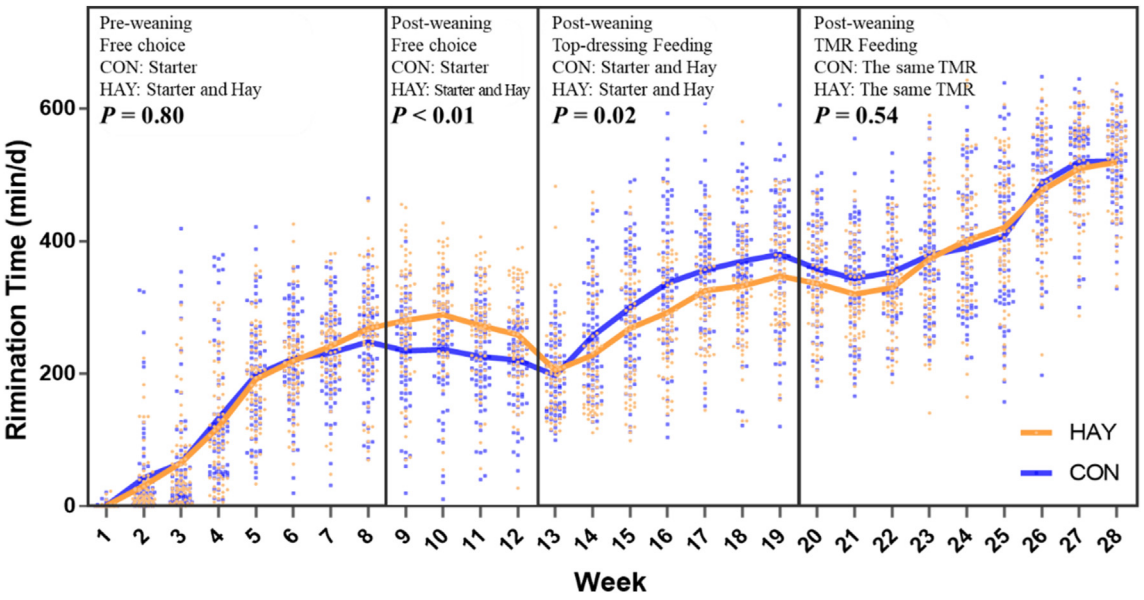

Fig. 1. Effect of early forage inclusion on the daily rumination time. CON = calves fed starter without forage from d 1 to 84, HAY = starter and forage were fed to calves as free choices from d 1 to 84. From d 85 to 196, calves were fed the same diet.

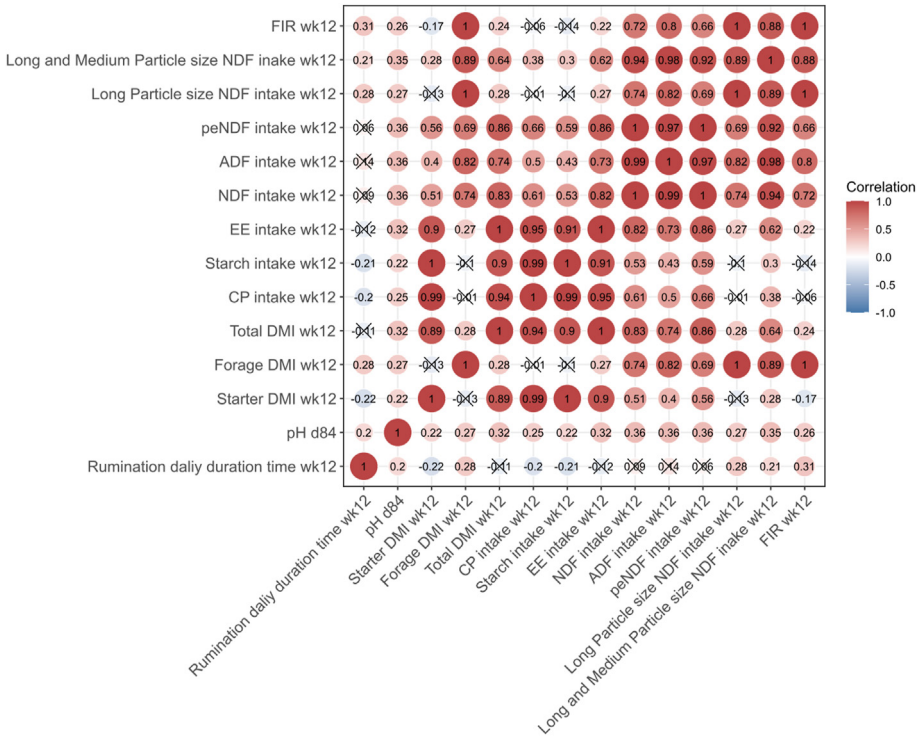

Fig. 2. Spearman correlations between rumination, feed and nutrient intake in calves at week 12 of age. DMI = dry matter intake; CP = crude protein; NDF = neutral detergent fiber; peNDF = physically effective NDF; ADF = acid detergent fiber; EE = ether extract; FIR = forage to total solid intake ratio. Only significant correlations ($P \leq 0.05$) were shown with their correlation coefficients in circles. Non-significant correlations ($P > 0.05$) were shown as crosses. The red circle means a positive relationship, while the blue circle means a negative relationship.

HAY: 415.8 vs. 410.2 min/d, $P = 0.54$). Thus, although forage consumption could stimulate the development of rumination behavior in the short term, the feed type might play a much more important role than early forage experience. More studies using different rations and feeding different amounts of milk are encouraged to test whether the neonatal diet could affect the long-term development of rumination.

The data on ruminal pH and VFA concentration at d 1, 7, 35, 84 and 196 of age are shown in Table 6. Treatment differences were not found for ruminal pH and VFA during the pre-weaning (d 1, 7 and 35) and TMR feeding periods (d 196). Unsurprisingly, ruminal pH (6.32 vs. 6.03, $P < 0.01$) and acetate-to-propionate ratio (1.67 vs.1.48, $P = 0.05$) were higher, while acetate (45.0% vs. 43.3%, $P = 0.10$) and propionate (28.7% vs. 30.8%, $P = 0.07$) concentrations

Table 6
Effect of early forage feeding on ruminal pH and fermentation in calves at d 1, 7, 35, 84 and 196 of age.

Item	Trt[1]		SEM	*P*-value
	CON	HAY		Trt
pH				
d 1	5.16	5.14	0.055	0.80
d 7	6.45	6.34	0.052	0.13
d 35	6.35	6.27	0.056	0.28
d 84	6.03[b]	6.32[a]	0.053	<0.01
d 196	6.53	6.56	0.069	0.88
Total VFA, mmol/L				
d 1	14.1	13.0	2.30	0.76
d 7	28.6	24.2	2.12	0.15
d 35	39.2	43.1	4.24	0.52
d 84	97.7	96.6	6.16	0.90
d 196	53.9	59.1	3.10	0.25
Acetate, %				
d 1	43.2	47.3	3.69	0.38
d 7	52.3	52.7	1.25	0.82
d 35	50.9	51.1	0.95	0.93
d 84	43.4	45.0	0.67	0.10
d 196	62.4	61.8	0.46	0.32
Propionate, %				
d 1	32.1	30.0	3.20	0.66
d 7	31.3	32.1	1.04	0.62
d 35	31.8	31.6	0.73	0.79
d 84	30.8	28.7	0.79	0.07
d 196	21.0	21.5	0.35	0.30
Butyrate, %				
d 1	22.1	19.2	0.15	0.21
d 7	12.8	12.8	0.95	0.97
d 35	14.1	14.3	0.65	0.83
d 84	19.0	20.6	0.75	0.15
d 196	10.9	11.1	0.19	0.52
Acetate-to-propionate ratio				
d 1	1.51	1.82	0.255	0.40
d 7	1.79	1.78	0.083	0.94
d 35	1.67	1.72	0.063	0.57
d 84	1.48[b]	1.67[a]	0.060	0.05
d 196	3.05	2.93	0.068	0.20

Trt = treatment; SEM = standard error of the mean.
[a, b]Different letters indicate a significant difference ($P \leq 0.05$).
[1] CON = calves fed starter without forage from d 1 to 84, HAY = starter and forage were fed to calves as free choices from d 1 to 84. From d 85 to 196, calves were fed the same diet.

tended to be higher and lower, respectively, in calves consuming forage than those feeding solely on starter at d 84. In line with the present results, most studies have reported increased ruminal pH and acetate-to-propionate ratios when young calves were offered forages (Hosseini et al., 2019; Mirzaei et al., 2016). Forage consumption increases the intake of physically effective fiber, stimulating rumination activity and subsequently improving saliva flow into the rumen and rumen buffering in calves (Nemati et al., 2016). Hence, after weaning, the observed positive relationship between the daily rumination duration time and ruminal pH ($r = 0.2$, $P = 0.01$, Fig. 3A) corroborated with the aforementioned results. Besides, the lower acetate and acetate-to-propionate ratio observed in CON compared to HAY calves was most likely due to the dietary forage inclusion increasing the ruminal pH. Similar to our study, a previous study found that dietary forage inclusion increased the abundance of cellulolytic bacteria that produce more acetate and less propionate in the rumen (Lin et al., 2018). However, the results did not last for a longer period of time, as when all the calves were transferred to the TMR diet, the ruminal pH and VFA at 6 months (d 196) of age were similar. Since rumination plays an important role in regulating the ruminal pH and fermentation, a similar rumination pattern observed in the current study could partially explain the ruminal pH and VFA results. These results agreed with our previous study investigating the effect of early feed exposure on long-term rumen fermentation, whereby no treatment differences were found after the same TMR diet was offered to the calves (Xiao et al., 2018). Therefore, there is a high probability that early forage experience might not affect rumination and rumen fermentation in the long term. Rather, the characteristics of the feed might play a much more important role in regulating rumination and rumen fermentation. Interestingly, although the early forage inclusion could not affect the long-term rumination behavior and rumen fermentation, the relationship between rumination duration in week 28 and ruminal pH at d 196 was significantly positive ($r = 0.23$, $P = 0.02$, Fig. 3B), indicating that no matter the kind of diet, rumination affects the ruminal pH in calves. However, differences in the rumination in week 28 could have been subject to regulation by DMI and inherent variability among animals (Beauchemin, 2018). Hence, further investigation is required to understand the factors regulating rumination and the related biological changes in the rumen environment.

3.3. Apparent total-tract digestibility

The least square means for apparent nutrient digestibility are shown in Table 7. The digestibility percentages observed herein were within the ranges previously reported in weaned calves (Castells et al., 2012; Daneshvar et al., 2015; Ebnali et al., 2016). Similar to a previous study (Castells et al., 2012), the apparent total-tract digestibility of DM, NDF and EE did not differ across treatments. However, the digestibility of OM, CP and starch was higher in CON compared with HAY calves.

The nutrient source largely affects nutrient digestibility along the gut in early life. Compared with oat hay, starter contains more digestible nutrients and less indigestible fiber, which may affect overall nutrient digestion. Generally, high-fiber diets (Porter et al., 2007; Zanton and Heinrichs, 2009) compromise nutrient digestibility. Thus, while CON calves consumed the starter only, a partial proportion of total DMI in HAY calves was derived from oat hay (10.0%), resulting in a higher NDF (20.0% vs. 17.0%) and ADF (12.0% vs. 10.5%) intakes in HAY than CON calves after weaning. The higher indigestible fiber may explain why calves in the HAY group had lower digestibility of OM, CP and starch. Similar to our results, Porter et al. (2007) indicated that the protein and energy (CP and TDN) digestibility in calves offered high-fiber diets (27% of NDF) was lower than that of calves consuming low-fiber diets (20% of NDF). We did a Spearman correlation analysis to understand further the relationship between nutrient digestibility, forage DMI and NDF intake. We observed that higher forage DMI, FIR, NDF intake and ADF intake were negatively correlated with DM, CP, starch and OM digestibility (Fig. 4), indicating that higher forage fiber intake could alter feed digestion in calves after weaning. In addition, greater starch intake in CON calves may partially explain the higher starch digestibility compared to HAY calves in the present study. A weak positive relationship between starch intake and starch digestibility was found in the current study, suggesting that increasing the starch intake could improve the digestibility of starch in calves, which is likely due to the induction of rumen development (Suarez-Mena et al., 2011; Terré et al., 2007). Similarly, a previous study has shown that the natural logarithm of cumulative starch intake was the best predictor of total-tract starch digestion, such that the higher the cumulative starch intake, the higher the digestion of starch in young calves (Quigley et al., 2018). Starch is primarily digested enzymatically in the small intestines of monogastric animals (Lim et al., 2021), but majorly fermented in the rumen of ruminants (Harmon and Swanson, 2020). The composition and activity of rumen microbial populations, including the abundance of amylolytic bacteria, can greatly influence starch digestion (Nagaraja and Titgemeyer, 2007). Higher starch intake

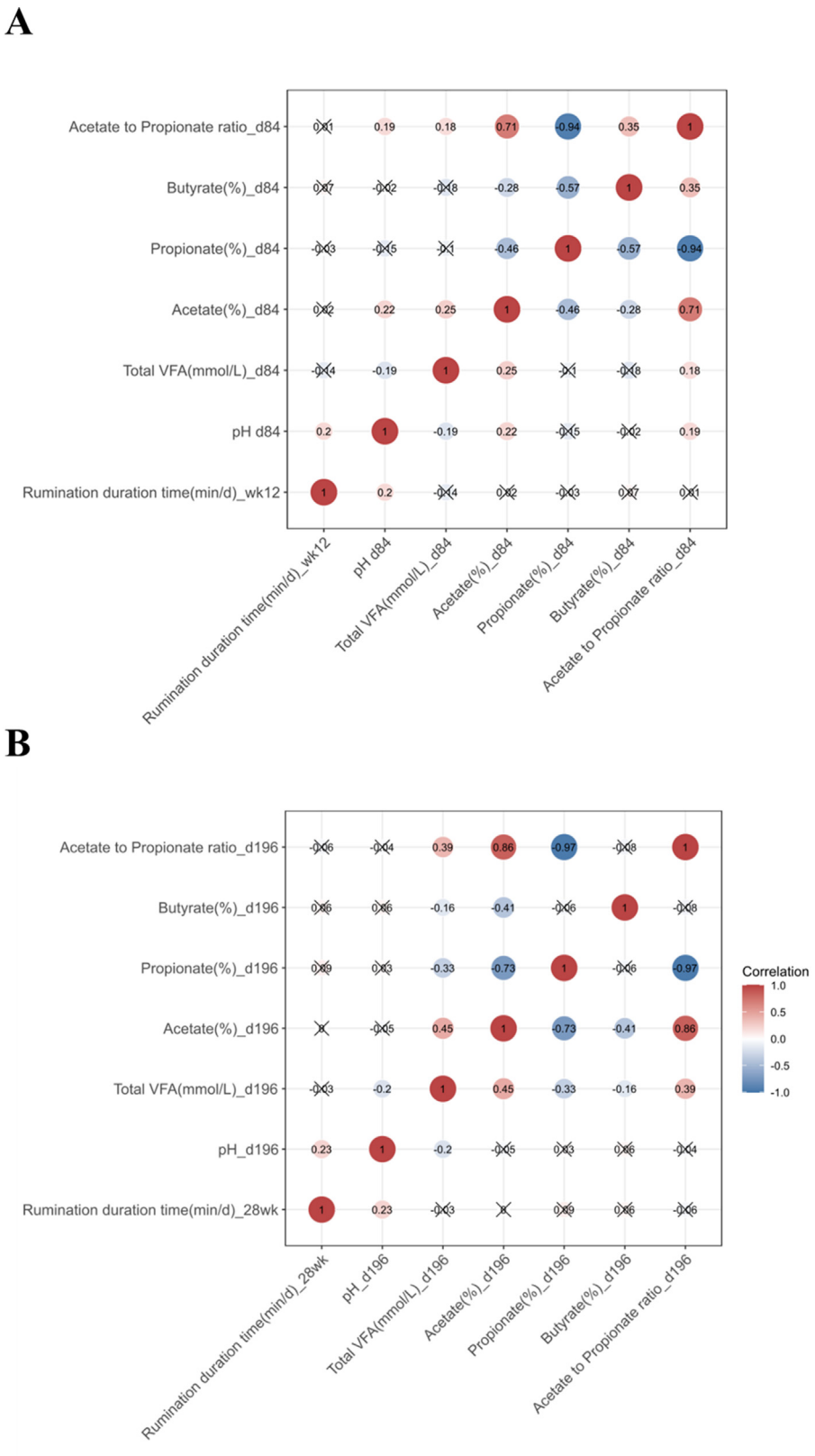

Fig. 3. Spearman correlations between rumination and VFA concentration at (A) d 84 and (B) d 196 of age. VFA = volatile fatty acids. Only significant correlations ($P \leq 0.05$) were shown with their correlation coefficients in circles. Non-significant correlations ($P > 0.05$) were shown as crosses. The red circle means a positive relationship, while the blue circle means a negative relationship.

Table 7
Effect of forage supplementation on apparent nutrient digestibility in calves at week 12 of age.

Item	Trt[1]		SEM	*P*-value
	CON	HAY		
Fecal DM content, %	22.7	21.6	0.45	0.11
Nutrient digestibility, %				
DM	75.1	74.6	0.26	0.28
OM	79.7[a]	76.1[b]	1.14	0.03
CP	81.5[a]	76.8[b]	1.17	<0.01
NDF	53.4	53.8	2.28	0.89
Starch	98.8[a]	98.2[b]	0.15	<0.01
EE	64.7	61.3	3.04	0.43

DM = dry matter; OM = organic matter; CP = crude protein; NDF = neutral detergent fiber; EE = ether extract; Trt = treatment; SEM = standard error of the mean.
[a, b]Different letters indicate a significant difference ($P \leq 0.05$).
[1] CON = calves fed starter without forage from d 1 to 84, HAY = starter and forage were fed to calves as free choices from d 1 to 84. From d 85 to 196, calves were fed the same diet.

might promote the growth of amylolytic bacteria in the rumen, thus affecting its digestibility. Future studies should incorporate microbial analysis to deduce their role in dietary starch digestibility. Furthermore, the lower OM, CP and starch digestibility may partially explain the higher feed-to-gain ratio in HAY than in the CON calves in the present study.

Theoretically, longer rumination time could contribute to more effective particle size reduction and improved digestibility (Aikman et al., 2008). No study has analyzed the relationship between rumination and feed digestion, probably due to the difficulty of rumination data acquisition, which could be time-consuming and laborious. However, using the rumination data acquired via an ear-attached accelerometer, we tried to gain a deeper understanding of the rumination's function by conducting a correlation analysis between rumination time or rumination time per kilogram NDF and nutrient digestibility. Unexpectedly, there was no relationship between rumination time and nutrient digestibility. These results were contrary to the assumptions held on the role rumination plays in improving feed digestion, as a physiological process that promotes the further breakdown and decomposition of herbivorous feed and stimulates digestion (Heinrichs and Lesmeister, 2005). Most likely, the greater forage intake could have increased the rumination time, as forage is less digestible compared to the starter, masking the effects of rumination on nutrient digestibility. Therefore, we further analyzed the relationship between rumination time and feed digestion by focusing on either CON or HAY calves separately, hence, excluding the dietary effects. Even then, there was no positive relationship between rumination time and feed

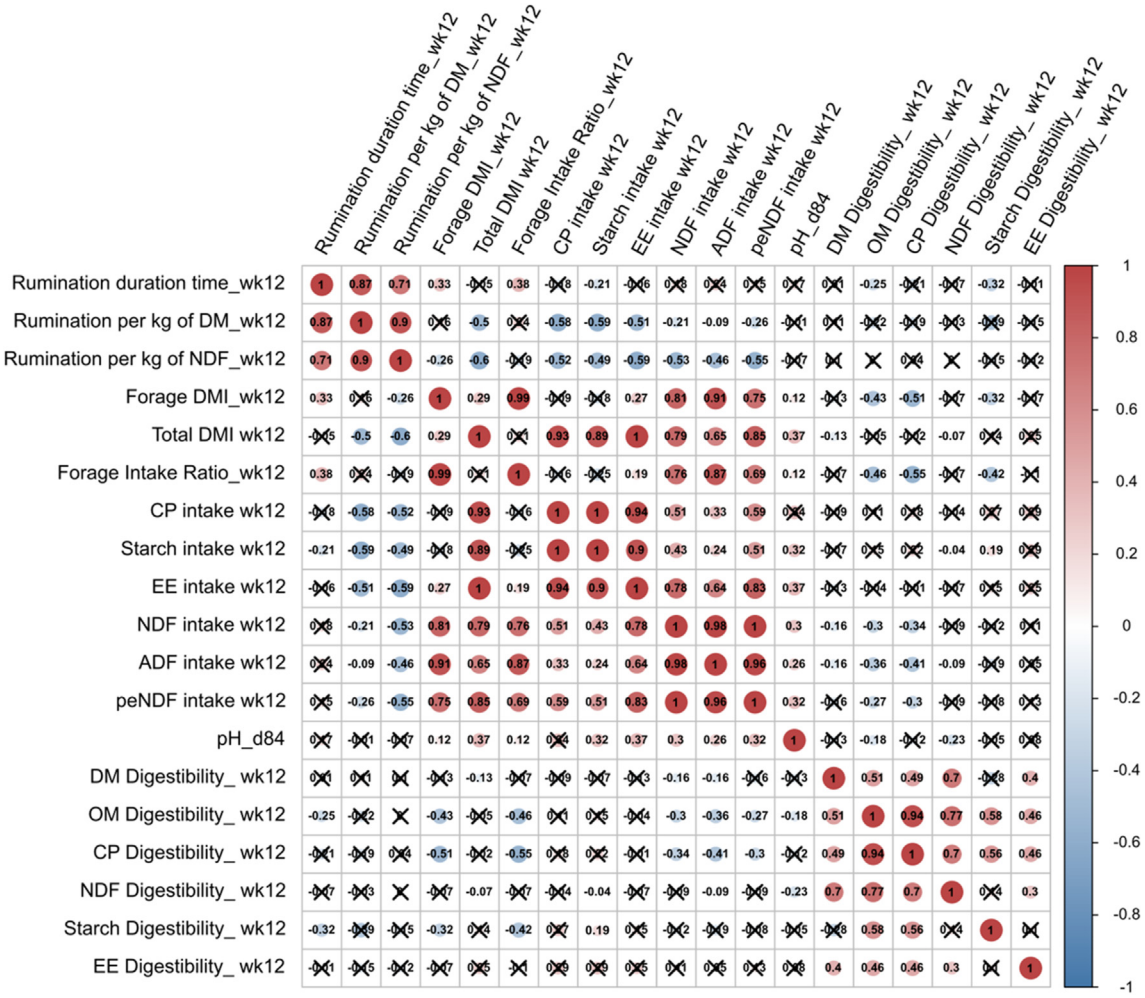

Fig. 4. Spearman correlations between rumination, feed intake and nutrient digestibility. DMI = dry matter intake; CP = crude protein; NDF = neutral detergent fiber; peNDF = physically effective NDF; ADF = acid detergent fiber; EE = ether extract; OM = organic matter. Only significant correlations ($P \leq 0.05$) were shown with their correlation coefficients in circles. Non-significant correlations ($P > 0.05$) were shown as crosses. The red circle means a positive relationship, while the blue circle means a negative relationship.

digestibility indicators (Figs. S1 and S2). These results suggested that feed digestion was not highly correlated to the occurrence and development of rumination behavior. Conversely, rumination is more of a behavioral response to solid feed ingestion, especially of the physically effective fiber, that helps reduce feed particle size. However, rumination cannot fully compensate for a deficiency in digestion induced by inherent differences in feed characteristics. Apart from the physical breakdown of feedstuff through rumination, the gut microbiome and enzymes are critical components in feed digestion. Future studies should determine the role gut microbiota and specific gut-derived metabolites play in regulating the development of the calf digestive system, as well as understand how rumination interacts with the microbiome during feed digestion.

4. Conclusion

Providing hay to calves fed a high milk level stimulated the early onset of rumination, enhanced rumination time post-weaning and increased ruminal pH after weaning. The increase in rumination time appeared to be due to greater forage intake without necessarily improving feed digestibility. The post-weaning effects were lost in the long term when all the calves were transitioned to the same diet. Concomitantly, the growth performance of calves throughout the experiment was not affected, implying that providing hay in addition to calf starter does not have a detrimental effect in calves fed high quantities of milk. More studies are needed to determine how the interaction between different diets and milk volumes affects rumination behavior.

Author contributions

Jianxin Xiao designed the study, performed the animal trial and laboratory experiments, did the data collection and analysis, and wrote the original draft. **Tianyu Chen** and **Gibson Maswayi Alugongo** performed the animal trial and reviewed the manuscript. **Rong Peng, Hui Yang, Shuai Liu, Yulin Ma** and **Jingjun Wang** performed the animal trial and laboratory experiments. **Shengli Li** reviewed the manuscript. **Zhijun Cao** designed the study, consulted and reviewed. All authors read and approved the final manuscript.

Availability of data and materials

The datasets analyzed in the current study are available from the corresponding authors upon reasonable request.

Declaration of competing interest

We declare that we have no financial and personal relationships with other people or organizations that can inappropriately influence our work, and there is no professional or other personal interest of any nature or kind in any product, service and/or company that could be construed as influencing the content of this paper.

Acknowledgment

This study was supported by the National Natural Science Foundation of China (No. 32272902, 32302772), China Postdoctoral Science Foundation (No. 2023M732515), and the Postdoctoral Foundation from the Department of Human Resources and Social Security of Sichuan (No. 2322999007). We are grateful to Modern Farming (Baoji) Co., Ltd for allowing us to use their animals and facilities. We appreciate the help and technical assistance received from the following staff and students from China Agricultural University: Chunlin Han, Xiaoyong Li, Peng Ma, Yan Zhang, Bendong Zhang, Wenfei Li, Leilei Zhang, Jingjun Wang, Mo Sha, Jialin Wei, Wenli Guo, Hongxing Zhang, Yiming Xu, Xinjie Zhao, and Sirui Yang.

Appendix supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.aninu.2023.12.003.

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