Providing calves different feed source early in life would not affect feed sorting and rumen fermentation for a long term

Jianxin Xiao (1016249009@qq.com)
China Agricultural University https://orcid.org/0000-0002-3074-5973
Muhammad Zahoor Khan
China Agricultural University
Gibson Maswayi Alugongo
China Agricultural University
Wei Wang
China Agricultural University
Yajing Wang
China Agricultural University
Zhijun Cao
China Agricultural University
Shengli Li
China Agricultural University

Research

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Abstract

This study aimed to investigate the short and long-term effect of early in life exposure to different feed sources on feed sorting and rumen fermentation of calves. Forty newborn female Holstein calves were randomly divided based on supplementation of concentrate (CON) or hay (HAY) only during the milk-feeding stage (d 1 to 56). After that, all calves were offered a total mixed ration (TMR) containing 43 % of forage and 57 % of concentrate until the end of the experiment (d 57 to 196). Rectal temperature, feed intake, body weight, body structural measurement, fecal and cough score were recorded ranges from day 1 to 196. Fresh and orts feed were sampled daily at the first two weeks (d 57-70) once TMR was offered and at the last week of the experiment (d 190 to 196) for analysis of feed sorting. Rumen fluid samples were collected at ranges from d 14-190 for detecting rumen pH and volatile fatty acids (VFA) concentrations. Our findings showed that early feed exposure did not influence calf health and growth performance but affected feed sorting in a short period. Upon transition to the same mixed diet, differences were found between treatments in the sorting of the different particle fractions. At week 9 and 10, calves early exposed to concentrates began to sort for fine particle fraction, and against the long particle fraction, whereas calves early fed hay sorted for the long particle fraction, and against the fine particle fraction. However, no carryover effect was found, the differences between treatments fade away when detecting feed sorting at the last week, all calves sorted for short and fine particle fractions, and sort against long and medium particle fractions. Although the pattern of feed sorting initially differed between treatments, the rumen pH and VFA concentration were not altered once all calves transitioned to a TMR. These results suggested that the feed familiarity established early in life would not affect diet selection and rumen fermentation in heifer later in life, the dietary experience after transition to same TMR would eventually override the effect of early feed experience.

Introduction

Dairy cattle are commonly fed a balanced ration containing concentrates and forages in order to meet their nutritional requirements. Concentrates are normally high in sugars which can supply the cattle with adequate energy. Meanwhile, forages are high in physically effective fiber that stimulates rumination and thus improve saliva production to maintain an appropriate rumen pH [1]. Since, cows mainly prefer sweet flavor [2], it has been observed that both growing heifers [3, 4] and adult cows [5] are likely to consume first the concentrate rather than the hay component. To avoid the preferential consumption of the concentrate component, total mixed ration (TMR) is often formulated and used on most commercial dairy farms by blending several feedstuffs into a complete ration. However, when TMR is fed, cows are inclined to “sorting” behavior, whereby they tend to select smaller grain particles over long forage particles [6], resulting in an unbalanced nutrient intake with overconsumption of the rapidly fermentable concentrate and refusal of physically effective fiber, which is expected to decrease rumen pH through increased acid production and reduced chewing activity [7]. Consequently, the risk of subacute ruminal acidosis (SARA) might be increased [8].

In order to address the sorting problem, various nutritional strategies may be implemented on the dairy farm, such as decreasing forage inclusion rate [6, 9], controlling forage particle size [10, 11], and altering ration dry matter (DM) content [12, 13]. Although these strategies can prevent sorting behavior at the herd level, the feed preference and behavior of some individual animals might not be fully transformed with sorting recurring especially under poor feed management. It has been postulated that early life feed experience may affect the eating behavior in human [14] and animal [15]. In children, early exposure to both the taste and texture of different foods in the pre-weaning period is the most salient way to improve acceptance of diverse food patterns and prevent picky eating [14]. In ruminants, a previous study found that adult sheep exhibited preferences for flavors they were exposed to in the first months of life [15]. The above studies suggest that exposure to different feed source during calfhood might also affect the feed preference of cows later in life, fundamentally solving the sorting issues. Therefore, the first objective of this study was to determine whether calves exposed to either CON or HAY would prefer, one over the other when fed as a TMR post-weaning. The second objective was to determine whether, the feed components fed pre-weaning would influence the sorting behavior and the rumen fermentation both in the short- and long-term. We hypothesized that calves would prefer feed components they had been exposed to early in life resulting in long lasting changes in feed sorting and the rumen environment.

Materials And Methods

Treatments, Animals and Feeding

This study was conducted at the Modern Farming Co. Ltd. (Baoji, Shanxi, P. R. China), which has approximately 18,500 Holstein dairy cattle, of which 2,500 are calves and 9,200 are milking cows. The experiment was performed as a randomized complete block design with 40 female calves. Calves were selected based on the birth weight (ranging from 34 to 42 kg) and serum total protein (≥ 5.5 g/dL). They were enrolled in the experiment immediately after birth and blocked by date of birth. Calves within each block were randomly assigned to one of the two feed exposure treatments: concentrate (CON), and hay (HAY). The calves remained on their respective solid feed source (Table 1) throughout the milk-feeding stage (d 1 to 56). Immediately post-weaning, calves were introduced to a novel TMR (Table 1) from d 57 to 196. During the first 56 d, concentrate grain (Modern Farming Co. Ltd., Baoji, Shanxi, P. R. China) and chopped mixed hay (50% of alfalfa and 50% of oat hay at < 2.5 cm) were offered separately once every morning for ad libitum intake. During d 57 to 196, fresh TMR was offered once daily at 1000 h immediately after orts disposal. Feed was offered in sufficient amounts and adjusted daily to ensure at least 10% orts such that feed was always available. The actual percentage of orts was similar between concentrate and hay treatments (15.6 vs. 14.2%, P = 0.68).

The animals were managed according to practices outlined in the Guide for the Care and Use of Agriculture Animals in Agriculture Research and Teaching [16]. Calves were separated from their dams and weighed immediately after birth, and then transferred to individual hutches before receiving 4 L of colostrum within 1 h of life. As an indicator of health, blood serum total protein was measured at 24 h after birth to determine whether the calf had achieved passive immunity transfer after consuming

colostrum. Birth weight (38.5 ± 2.4 vs. 38.4 ± 2.3 kg, respectively; P = 0.97) and serum total protein (6.9 ± 0.4 vs. 6.7 ± 0.2 g/dL, respectively; P = 0.25) were similar for both groups of calves exposed to either concentrate or hay. Calves were offered pasteurized whole milk twice daily at 0700 and 1500 h at a fixed amount of 6 L/d from d 2 to 14, 8 L/d from d 15 to 42, 6 L/d from d 43 to 49, and 4 L/d from d 50 to 56. After weaning at d 56, calves remained in the hutch until d 70. The hutches were cleaned and sterilized before introducing calves according to the standard operating procedures of this farm. Sand was used as the bedding material and was replenished weekly. Two buckets hanging inside the hutches were used to provide solid feed (CON or HAY), and another two buckets hanging on the fence outside of the hutch were used to feed water and milk. Solid feed and water were offered by bucket after 30 min of milk feeding from d 2 until weaning on d 56. Calves were offered the novel TMR once daily at 0800 from d 57 to 70. All buckets were removed for cleaning before each feeding. At d 71, calves were transferred to a barn and housed within treatment in pairs (2 calves/pen; n = 20) until the end of the experiment (d 196), the pens consisted of a lying area bedded with sawdust, an automatic water trough, and a feeding lane equipped with an automatic cable scraping system. The bedding material was replaced and renewed as needed, with fresh bedding added weekly at minimum. The daily ration was offered in feed bunks which were located in front of the lane. The calves raised in the pens had free access to feed and water at any time.

Sample collection

Feed Sampling and Analysis. Feed intake was calculated every day according to the amount of solid feed offered and refused by each calf from d 2 to 70 and by each pen during last week of the study (d 190 to 196). Representative feed samples of concentrate, hay and TMR were collected and immediately stored at − 20 °C until further analyses. The DM, crude protein (CP), neutral detergent fiber (NDF), acid detergent fiber (ADF), ether extract, and ash were analyzed following the methods of AOAC International [17].

Feed Sorting Analysis. For determination of feed sorting behavior of the calves, fresh TMR offered and orts were sampled daily from each calf on week 9 and 10 (d 57 to 70), and from each pen on week 27 (d190 to 196). Fresh feed and orts were collected immediately after delivery of new TMR and 1 h before removal of refusals, respectively. Samples for particle size analysis were immediately separated into 4 fractions (long: >19 mm; medium: <19 and > 8 mm; short: <8 and > 4 mm; fine: <4 mm) [18] using a 3-sieve (19, 8, and 4 mm) Penn State Particle Separator (PSPS) [19]. Feed sorting behavior for each fraction was assessed through actual intake of each fraction expressed as a percentage of the predicted intake of that fraction [6]. The actual intake of each individual fraction was calculated as the difference between the as-fed amount of each fraction in the feed offered and that in the orts. The predicted intake of individual fractions was calculated as the product of the as-fed intake of the total diet multiplied by the as-fed percentage of that fraction in the TMR. Values less than 100% indicate selective refusals (sorting against), values equal to 100% indicate no sorting, and values greater than 100% indicate preferential consumption (sorting for). The daily data were summarized for each calf on week 9 and 10, and for each pen on week 28 before analysis.

Body Weight and Structural Measurements. All calves were weighed by a digital scale every 2 week for the first 10 week (d 1, 28, 56, 70) and finally at week 28 (d 190). Body structural measurements including

body length (distance between the points of shoulder and point of pin bone), heart girth (circumference of the chest measured directly behind the front leg), withers height (distance from base of the front feet to the withers), and hip height (distance from base of the rear feet to hook bones) measurements were also recorded at d 1, 28, 56 and 70. Only heart girth and withers height were recorded at d 190.

Table 1
Chemical composition and particle size distributions of concentrate, hay and TMR (mean ± SD)[1]

Item	CON[2]	HAY[3]	TMR[4]
Chemical composition			
Dry matter, %	89.8 ± 0.4	88.7 ± 0.7	56.0 ± 1.4
Crude protein, % of DM	18.7 ± 0.4	13.2 ± 0.3	16.8 ± 0.6
NDF, % of DM	15.7 ± 0.3	47.4 ± 0.9	27.4 ± 1.4
ADF, % of DM	5.9 ± 0.2	29.8 ± 0.7	18.3 ± 1.0
ASH, % of DM	4.8 ± 0.1	6.8 ± 0.2	6.4 ± 1.0
EE, % of DM	3.6 ± 0.3	2.3 ± 0.1	3.3 ± 0.3
Particles,[5] %			
Long	0	32.0 ± 4.6	11.7 ± 3.3
Medium	18.3 ± 2.5	26.9 ± 3.5	30.1 ± 5.5
Short	53.0 ± 2.8	26.5 ± 2.9	26.8 ± 3.6
Fine	28.6 ± 1.4	14.5 ± 2.8	31.4 ± 2.6

[1]Calves were fed either concentrate (CON), or hay (HAY) during the pre-weaning period (d 2 to 56). After weaning, all calves were fed same TMR from d 71 to 196.

[2]Concentrate starter was supplied by Modern Farming Co., Ltd, containing (on as-fed basis) 36.0% corn, 10.0% wheat bran, 25.0% rolled barley, 16.0% soybean meal, 8.0% Canola meal, 2.0% molasses, 1.5% sodium bicarbonates, 1.0% sodium chloride, and 0.5% Premix compound.

[3]Alfalfa and oat hay was mixed (50:50) and cut less than 2.5 cm using a stationary mixer (20 m^3, Trioliet Co., Ltd., Holland).

[4]The total mixed ration (TMR) was composed of 33.3% steam flaked corn, 21.5% alfalfa hay, 21.5% oat hay, 18.3% soybean meal and 5.4% premix compound on dry matter basis.

[5]Particle size was determined using a 3-sieve Penn State particle separator, which separates the particles into 4 fractions (long: >19 mm; medium: <19 and > 8 mm; short: <8 and > 4 mm; fine: <4 mm).

Blood and Rumen Samples. Blood was sampled after 24 h of birth from the jugular vein in evacuated tubes containing no anticoagulant for serum separation. Serum was used to determine total serum protein using optical refractometer (Honneur Nutritional Technology Co. Ltd., Beijing, P. R. China). Rumen fluid was sampled via a flexible esophageal tube (2 mm wall thickness, 6 mm internal diameter; Anscitech Co. Ltd., Wuhan, Hubei, P. R. China) from all calves 2 h after the morning feeding on d 28, 56, 58, 63, 70 and 190 of age. The first 10 ml of rumen fluid was discarded to avoid saliva contamination. Rumen fluid was filtered through 4 layers of cheesecloth and collected into two 15 ml containers. The one was used to measure rumen pH immediately with a glass electrode pH meter. The other was stored at –20 °C for later analysis of VFA [20].

The Risk of SARA

Subacute ruminal acidosis is a disorder of rumen fermentation that is characterized by extended periods of depressed rumen pH below 5.8 in both mature cattle [21] and young calf [22]. Based on the analysis of rumen pH data, calves are thought to suffer from SARA when rumen pH was lower than 5.8. The number of animals with or without SARA was recorded per treatment on d 28, 56, 58, 63, 70 and 190 for further analysis.

Body Temperature, Fecal and Cough Scoring. Calves rectal temperature was measured using a digital thermometer (MC-347, Omron Co. Ltd., Beijing, P. R. China), fecal consistency and cough were scored by the research team based on the scoring system designed by researchers at the University of Wisconsin at Madison [23]. Fecal consistency and cough scoring were done using a 1 to 4 scale. Fecal consistency was scored as 1 when firm, 2 when soft or of moderate consistency, 3 when runny or mild diarrhea and 4 when watery and profuse diarrhea. For cough scoring, calves were categorized as 1 when no cough, 2 when single cough, 3 when repeated coughs or occasional spontaneous cough and 4 when repeated spontaneous coughs induced. All these parameters were recorded daily for individual calves before the morning milk feeding from d 1 to 70. Weekly averages of these data were generated for individual calves for further statistical analyses. Calves with fecal consistency score > 2 were considered diarrheic, cough score > 2 were considered having severe coughing, and with temperatures ≥ 39.5 °C were considered having a fever. The number of days calves had diarrhea, severe coughing or fever was recorded for each calf.

Statistical Analyses

The calf was the experimental unit for data of health status (temperature, fecal consistency and cough score), growth performance (body weight and structural measurement) and rumen fermentation (pH and VFA) throughout the experiment. For data of feeding (feed intake and sorting behavior), the calf was also the experimental unit before d 70 when they were fed in the hutches. Upon transition to group feeding, data for feed intake and sorting behavior was obtained for each pen. Hence, the pen was used as the experimental unit.

Data obtained daily, such as feed intake, sorting behavior, temperature, fecal consistency and cough score, were summarized for each calf or each pen by week and analyzed separately by time period (Pre-

weaning, week 1 to 8; Post-weaning, week 9 to 10; Heifer, week 27). Data for body weight and structural growth at d 1, 28, 56, 70, and 190, and data for pH and VFA at d 28, 56, 58, 63, 70, and 190 were collected for each calf and were also analyzed separately by time period. All data were analyzed as a randomized complete block design using the MIXED procedure of SAS (SAS 9.2, SAS Institute Inc., Cary, NC) with time as a repeated measure. The model included the fixed effects of treatment, time and their interactions (treatment × time), and the random effect of calf or pen within the treatment. A covariance structure was chosen based on the lowest Akaike and information criterion used. Categorical responses (number of days calf within each treatment) related to temperature, fecal score and cough score were tested using the GLIMMIX procedure of SAS (SAS 9.2, SAS Institute Inc., Cary, NC), with the Poisson distribution. To gain a deeper understanding of the effect of early feed exposure on rumen health, the number of calves with SARA (pH < 5.8) and without SARA (pH ≥ 5.8) was analyzed for each rumen sample collection day (d 28, 56, 58, 63, 70 and 190), using the χ^2 test in FREQ procedure of SAS (SAS 9.2, SAS Institute Inc., Cary, NC). For all analyses, significant differences were declared at $P \leq 0.05$, with trends indicated at $0.05 < P \leq 0.10$.

Results

Feed Intake and Growth Performance

Calves consumed all the milk offered to them during the milk-feeding period (d 1–56) and were also provided different solid feeds (concentrate or hay) which differed both in nutrient composition and particle size (Table 1). The results for dry matter intake (DMI), body weight (BW), averaged daily gain (ADG) and structural growth are presented in Tables 2. Calves offered concentrate had a numerically higher DMI than calves fed hay before weaning (121.8 vs. 96.2 g/d, P = 0.14). After weaning, all calves were fed a novel TMR and no treatment effect was found on DMI in these stages (immediately post-weaning and as heifers).

Calf performance, body weight and structural growth were not influenced by treatment throughout the experiment. Likewise, average daily gain was similar between treatments during pre-weaning (d 1 to 56) and post-weaning (d 57 to 70) periods. Only significant time effects (P < 0.01) were found for feed intake and calf performance before and after weaning off milk, with DMI, BW and structural growth increasing as calves grew older.

Table 2

Effect of early feed exposure on DMI, BW, ADG and structural growth before and after weaning[1]

Item	Treatment[2]		SEM	P value		
	CON	HAY		Trt	Time	Trt*Time
Pre-weaning (d 1 to 56)						
DMI (g/d)	121.8	96.2	11.8	0.14	< 0.01	0.84
BW (kg)	62.3	62.3	0.60	0.99	< 0.01	0.28
ADG (kg/d)	0.79	0.81	0.01	0.35	< 0.01	0.54
Withers Height (cm)	82.6	82.9	0.37	0.21	< 0.01	0.18
Heart Girth (cm)	89.6	89.3	0.40	0.51	< 0.01	0.19
Body Length (cm)	77.7	77.1	0.40	0.30	< 0.01	0.33
Hip Girth (cm)	85.9	85.3	0.46	0.37	< 0.01	0.33
Post-weaning (d 57 o 70)						
DMI (g/d)	1359.3	1360.9	81.2	0.84	< 0.01	0.21
BW (kg)	92.9	93.0	1.25	0.97	-	-
ADG (kg/d)	0.72	0.63	0.07	0.33	-	-
Withers Height (cm)	90.1	90.6	0.59	0.52	-	-
Heart Girth (cm)	105.6	104.8	0.62	0.39	-	-
Body Length (cm)	87.1	89.6	3.18	0.33	-	-
Hip Girth (cm)	120.2	117.7	1.32	0.19	-	-
Heifer (d 190 to 196)						
DMI (kg/d)	5.61	5.65	0.18	0.85	-	-
BW (kg)	228.2	230.5	3.90	0.35	-	-
Withers Height (cm)	110.1	111.4	0.56	0.16	-	-
Heart Girth (cm)	143.8	145.1	0.77	0.26	-	-

[1]Sample size = 20 female calves per treatment.

[2]Treatment: Calves were offered either concentrate (CON) or hay (HAY) during pre-weaning period (d 1 to 56). After weaning, all calves were fed novel TMR. SEM = Standard error mean, Trt = treatment.

Temperature, fecal and cough score

Daily rectal temperature, fecal consistency and cough score of calves before (d 1 to 56) and after (d 57 to 70) weaning off milk were recorded, and no treatment differences were found for these three parameters. Likewise, the average number of days calves had diarrhea (fecal score > 2), severe cough (cough score > 2), or fever (temperature > 39.5°) were similar between groups. The significant difference ($P < 0.01$) of time effect for fecal score and cough score were noticed in pre-weaning period. In addition, the highest fecal score and cough score for calves were observed at week 3 and week 8 of age, respectively. No treatment * time effect was detected throughout the experiment.

Table 3
Effect of early feed exposure on selected health parameters before and after weaning[1].

Item	Treatment[2]		SEM	P value		
	CON	HAY		Trt	Time	Trt*Time
Pre-weaning (d 1 to 56)						
Temperature	38.78	38.76	0.04	0.72	0.20	0.49
Fecal score	1.17	1.20	0.03	0.32	< 0.01	0.95
Cough score	1.22	1.14	0.04	0.17	< 0.01	0.36
Post-weaning (d 57 to 70)						
Temperature	38.60	38.71	0.07	0.24	0.42	0.81
Fecal score	1.68	1.65	0.11	0.83	0.35	0.59
Cough score	1.10	1.20	0.07	0.35	0.89	0.22
Number of days (d 1 to 56)						
Temperature ≥ 39.5	2.50	1.95	0.67	0.57	-	-
Fecal score > 2	2.50	3.00	0.43	0.42	-	-
Cough score > 2	2.15	1.65	0.53	0.51	-	-
Number of days (d 57 to 70)						
Temperature ≥ 39.5°	0.65	0.85	0.23	0.56	-	-
Fecal score > 2	2.35	2.25	0.48	0.88	-	-
Cough score > 2	0.55	0.70	0.32	0.50	-	-

[1]Sample size = 20 female calves per treatment.

[2]Treatment: Calves were offered either concentrate (CON) or hay (HAY) during pre-weaning period (d 1 to 56). After weaning, all calves were fed novel TMR. SEM = Standard error mean, Trt = treatment.

Feed Sorting

After weaning off milk, diets were changed from solely solid feed (concentrate or hay) to a novel TMR (containing around 43% of forage and 57% of concentrate). Upon transition to TMR, calves were raised in hutches before d 70 and then transferred to a barn and raised in pens till the end of the experiment (d 196). To investigate the short- and long-term effects of early feed exposure, feed intake and feed sorting were recorded in the first two weeks of offering TMR (from d 57 to 70) and in the last week of the

experiment (from d 190 to 196). After the transition to the same mixed diet, calves that were originally fed concentrate had similar DMI to calves fed hay immediately post-weaning (1359.3 vs. 1360.9 g/d, P = 0.84) and as heifers (5.61 vs. 5.65 kg/d, P = 0.85). Although no treatment effect was observed in feed intake, differences were found in the sorting behavior of the different particle fractions. Immediately following transition to the mixed ration (week 9), calves exposed to concentrates early began to sort TMR for fine particle fractions (102.0%), and against the long particle fractions (97.5%). Similarly, calves fed hay early sorted for the long particle fraction (112.0%), and against the fine particle fractions (95.9%). After one week of transitioning to the TMR (week 10), calves previously fed concentrate still had lower preference for long particle fraction (98.1%) and higher preference for fine particle fraction (101.6%) compared to those fed hay early (Long particle: 109.3%, P = 0.01; Fine particle: 96.6%, P = 0.02). However, in the long-term the effect of early feed exposure on feed sorting, fade away between treatments, with all calves sorting for short (CON:101.7%, HAY: 102.3%, P = 0.31) and fine (CON: 103.4%, HAY: 103.4%, P = 0.45) particle fractions, rather than long (CON: 98.0%, HAY: 98.1%, P = 0.43) and medium (CON: 97.6%, HAY: 97.4%, P = 0.25) particle fractions (Table 4).

Table 4
Effect of early feed exposure on feed sorting after the transition to TMR[1]

Item[2]	Treatment[3]		SEM	P value
	CON	HAY		
Post-weaning				
Week 9 (d 57 to 63)				
Long, %	97.5	112.0	1.50	< 0.01
Medium, %	100.1	102.3	0.87	0.09
Short, %	102.5	101.9	0.62	0.52
Fine, %	102.0	95.9	1.48	0.01
Week 10 (d 64 to 70)				
Long, %	98.1	109.3	1.25	0.01
Medium, %	101.0	102.5	0.47	0.03
Short, %	100.4	99.4	0.80	0.09
Fine, %	101.6	96.6	0.80	0.02
Heifer				
Week 27 (d 190 to 196)				
Long, %	98.0	98.1	0.13	0.43
Medium, %	97.6	97.4	0.12	0.25
Short, %	101.7	102.3	0.42	0.31
Fine, %	103.4	103.4	0.09	0.45

[1]Sample size = 20 female calves per treatment during post-weaning period (week 9 and 10), and 10 groups (20 heifers; 2 heifer/group) are included in each treatment during the heifer period (Week 27)

[2]Particle fraction was determined by a Penn State Particle Separator, which separates the particles into long (> 19 mm), medium (< 19, > 8 mm), short (< 8, > 4 mm), and fine (< 4 mm) fractions.

[3]Treatment: Calves were offered either concentrate (CON) or hay (HAY) during pre-weaning period (d 1 to 56). After weaning, all calves were fed novel TMR. SEM = Standard error mean, Trt = treatment.

Rumen Fermentation and the risk of SARA

Calves rumen fluid samples were collected at d 28, 56, 58, 63, 70 and 190 of age for analysis the rumen pH and rumen volatile fatty acid (VFA). The least-square mean for those data were listed in Table 5. Most of the treatment differences were observed before weaning off milk (d 56), which was also known as the date changing sole feed component to TMR. Compared with calves fed concentrate, calves fed hay had higher rumen pH (6.08 vs. 6.79, $P < 0.01$), lower total VFA concentration (108.2 vs 59.1 mmol/L, $P < 0.01$) during the pre-weaning period. A greater acetate ($P < 0.01$), and a lower propionate ($P < 0.01$), butyrate ($P = 0.08$) and valerate ($P < 0.01$) proportions were obtained in Hay group than Con group. After calf diet transitioned to TMR, a diverse variation rapidly occurred in each treatment on d 58; no treatment effects were found in rumen pH and VFA from then on (Table 5 and Fig. 1). In similar with the data of rumen pH, the risk of SARA (the number of calves had rumen pH < 5.8) was higher in Con group. However, calves previous fed concentrates had a numerically higher number of calves suffered SARA than those fed only hay at d 58 (Con: 4/20 vs. Hay: 0/20, $P = 0.11$) and 63 (Con: 8/20 vs. Hay: 3/20, $P = 0.16$), respectively.

At d 190, no calf owned a rumen pH less than 5.8, and no treatment effects were found in rumen pH and VFA concentration.

Table 5

Effect of early feed exposure on pH and VFA before and after weaning off milk[1]

Item	Treatment[2]		SEM	P value		
	CON	HAY		Trt	Time	Trt*Time
Pre-weaning (d 28, 56)						
pH	6.08	6.79	0.09	< 0.01	0.03	0.21
Total VFA (mmol/L)	108.2	59.1	5.25	< 0.01	< 0.01	0.01
Acetate (mol/100 mol)	48.0	63.5	0.73	< 0.01	< 0.01	< 0.01
Propionate (mol/100 mol)	34.7	22.7	0.74	< 0.01	0.17	< 0.01
Butyrate (mol/100 mol)	8.82	7.06	0.69	0.08	< 0.01	0.01
Valerate (mol/100 mol)	5.32	3.34	0.38	< 0.01	0.01	0.20
Aetate/Propionate	1.45	2.90	0.07	< 0.01	0.22	0.01
Post-weaning (d 58, 63, 70)						
pH	6.02	6.09	0.78	0.52	0.01	0.21
Total VFA (mM)	112.5	117.6	5.82	0.54	< 0.01	0.15
Acetate (mM/100 mM)	48.3	48.6	0.93	0.83	< 0.01	0.03
Propionate (mM/100 mM)	32.6	30.5	1.16	0.21	0.83	0.03
Butyrate (mM/100 mM)	12.3	13.4	0.59	0.20	0.07	0.12
Valerate (mM/100 mM)	4.67	4.45	0.32	0.63	0.61	0.09
Aetate/Propionate	1.54	1.74	0.10	0.15	0.23	0.03
Heifer (d 190)						
pH	6.68	6.72	0.07	0.70	-	-
Total VFA (mM)	108.0	117.8	7.86	0.46	-	-
Acetate (mM/100 mM)	54.1	53.8	0.39	0.49	-	-
Propionate (mM/100 mM)	23.4	23.3	0.37	0.75	-	-

[1]Sample size = 20 female calves per treatment. Rumen fluid sample was collected at d 28 and 56 as a pre-weaning stage, at d 58, 63 and 70 as post-weaning period, and at d 190 as heifer period.

[2]Treatment: Calves were offered either concentrate (CON) or hay (HAY) during pre-weaning period (d 1 to 56). After weaning, all calves were fed novel TMR. SEM = Standard error mean, Trt = treatment.

Item	Treatment[2]		SEM	P value		
	CON	HAY		Trt	Time	Trt*Time
Butyrate (mM/100 mM)	14.2	14.4	0.26	0.56	-	-
Valerate (mM/100 mM)	2.81	2.78	0.07	0.75	-	-
Aetate/Propionate	2.32	2.32	0.05	0.96	-	-
[1]Sample size = 20 female calves per treatment. Rumen fluid sample was collected at d 28 and 56 as a pre-weaning stage, at d 58, 63 and 70 as post-weaning period, and at d 190 as heifer period.						
[2]Treatment: Calves were offered either concentrate (CON) or hay (HAY) during pre-weaning period (d 1 to 56). After weaning, all calves were fed novel TMR. SEM = Standard error mean, Trt = treatment.						

[1]Sample size = 20 female calves per treatment. Calves were offered either concentrate (CON) or hay (HAY) during pre-weaning period (d 1 to 56). After weaning, all calves were fed novel TMR.

Table 6
Effect of early feed exposure on a number of calves who had rumen pH less than 5.8 before and after weaning off milk[1]

Item	Treatment[2]		P value
	CON	HAY	
Number of calves owned pH < 5.8			
d 28	4/20	0/20	0.11
d 56	11/20	1/20	< 0.01
d 58	4/20	0/20	0.11
d 63	8/20	3/20	0.16
d 70	9/20	8/20	0.74
d 190	0/20	0/20	1.00
[1]Sample size = 20 female calves per treatment.			
[2]Calves were offered either concentrate (CON) or hay (HAY) during pre-weaning period (d 1 to 56). After weaning, all calves were fed novel TMR.			

Discussion

Feed Intake and Growth Performance

During the milk-feeding period, calves that were offered hay consumed a numerically lower DMI (96.2 g/d) compared to calves fed concentrate only (121.8, P = 0.14). Similarly, it has been reported that calves are likely to consume more concentrate than hay when provided as the sole feed [24, 25] or offered separately as free choice diet [26]. Xiao et al. [25] showed that calves that fed hay only consumed a negligible amount (6 g/d) of hay before weaning, which was lower than calves consuming concentrate (34.1 g/d). Castells et al. [26] claimed that when calves were offered concentrates along with different forages (straw, hay, and silages, respectively) ad libitum and separately, the consumption of forage was only 4 to 15% of total solid intake, while concentrate accounted for 85 to 96% before weaning. Previously, studies on effect of the flavor of feeds demonstrated that cattle are highly sensitive and have a strong preference for sweet tastes [2, 27], which might partially explain the bias towards higher consumption of DM in calves offered concentrate as opposed to hay in the current study. On the other hand, given that newborn calves have a small and nonfunctional rumen [28], the bulkier forage is more likely to fill the rumen rather than the highly fermented concentrate, which might limit the forage consumption and resulting in further intake of hay before weaning.

Numerous studies have claimed that concentrate, high in rapidly fermentable carbohydrates, have a greater ability to improve the rumen papillae development than hay [29, 30]. Therefore, compared with offering hay as solid feed, feeding concentrate during the pre-weaning period is more likely to increase BW and ADG as a result of greater nutrient absorption in rumen [31, 32]. In contrast to previous studies, calves offered concentrate had similar BW and ADG to calves offered hay in current study. It was noteworthy that the DMI was only 121.8 and 96.2 g/d before weaning for calves offered concentrate and hay, respectively, which was quite low when compared with other studies [30, 33]. Hill et al. [33] fed their calves a total of 120 L of milk (3.9 L/d) and 850 g/d of concentrate starter during the milk-feeding period. Castells et al. [30] observed that calves consumed 214 L (3.8 L/d) of milk and 600 g/d of solid feed. The lower DMI of solid feed in current study was probably due to relatively high levels of milk (376 L in total, 6.7 L/d) fed to calves, which met the daily nutritional and energy requirements hence limiting the solid feed consumption [34], thus, the effect of different feed sources on BW and ADG might have been masked.

Temperature, fecal and cough score

Temperature and days with fever did not differ across treatments. Furthermore, similar body temperatures were recorded in pre-weaning and post-weaning. These findings are in line with Jahanimoghadam et al. [35] who did not notice any detectable effects of feeding forage on body temperature. In similar with current study, most studies claimed that early inclusion of forage in the diet at various levels did not affect the fecal consistency and cough score [36–38]. However, a few studies reported that increasing dietary fiber intake decreased calves fecal score [39] and cough score [35], which might be related to fewer abnormal behaviors when forage is supplemented in calves diet [40, 41]. Although controversy remains on whether different solid feed affect fecal score or not, however, it has been well documented that time significantly influenced fecal score. In agreement with previous studies [42, 43], we documented that the fecal score increased from birth to week 3 before declining and reaching nadir before weaning.

This is probably because calf's passive immunity obtained from colostrum gradually decreased and its own immune system was not yet well developed [44], and thus reduced the calf's resistance to disease during this stage.

Feed Sorting

Given that the feed particles of concentrate are mainly observed in short and fine fractions and the feed particles of hay are mostly distributed in long and medium fractions, the long and fine particle fractions of TMR to greater extent represent hay and concentrate, respectively [6]. After transitioning to a mixed diet, differences between treatments were observed in feed sorting. Calves fed concentrate early were in favor of selecting short and fine particle fractions of novel TMR, which were primarily concentrate, while those fed hay preferred long particle fractions, which were mainly hay. These results indicate that calves begin to sort the feed immediately following transition to a mixed diet, and they prefer the feed component they were initially familiar with. In agreement with current study, previous studies also revealed that feed sorting behavior was observed in both milk-feeding [45] and weaned calves [46], suggesting that feeding and sorting behaviors are established early in life even though the brain and digestive system are not fully developed at this stage. It was not surprising to find that calves were in favor of feed components which they had been exposed to earlier. Previous evidence demonstrated that early feed experience with different feed flavor [15] or physical shapes [47] could influence the feeding behavior in ruminants later in life. On the other hand, food neophobia which is defined as avoidance of unfamiliar foods, and helps animals to avoid toxic foods [48] is well known in animals [49] and humans [50]. Recently, Costa et al. [51] observed that food neophobia to unfamiliar feed in calves was initially present, whereby calves were hesitant and reluctant to taste a novel feed and consumed only small amounts. Therefore, a period of neophobia might encourage calves to sort against the novel feed component for which they have not been exposed to early in life. Similar to our results, Miller-Cushon et al. [24] showed in their study that the early feed experiences would affect the feed selection in later stages, calves previously exposed to concentrate sorted against long particle fractions (56%), while those exposed to hay sorted against fine particle fractions (89%). A similar trend was noticed in the current study for calves offered concentrate and hay sorting against long and fine particle to the extent of 97.5 and 95.9%, respectively. A more severe sorting behavior was found in Miller-Cushon's study compared to this study, which was probably because of the differences in TMR. It is well documented that ration characteristics greatly influence feed sorting [52]. The higher DM content (90.7 vs. 56.0%) [12, 13] and higher forage particle size (Long particle fraction: 67.9 vs. 32.0%) [6] in ration of Miller-Cuhson's study than ours might aggravate the degree of feed sorting. Besides, the area occupied by feed might also affect ability of cattle to sort. While cows are more capable to pushing feed far and wide on an open and flat space, it is more difficult for them to increase sorting behavior in a more enclosed feed bunk [52]. One rectangular bin (diameter = 80.6 cm) was used in the study done by Miller-Cushon et al. [24], while we offered feed in two separate circular buckets (diameter = 25.0 cm) for each calf in the current study. Hence, less feeding space might have limited the extent of sorting. However, no empirical data of calves are available to support this point of view; further research is encouraged to investigate the effect of feeding space on feed sorting.

In contrast to a previous study [24], no carryover effects on feed sorting were found in this study. After feeding on TMR for a long period, treatment effects seemed to disappear during d 190 to 196. All calves began to sort for short and fine particle fractions, and sort against long and medium particle fractions. To our knowledge, this is the first study to investigate the effect of early feed experience on feed sorting beyond 6 month of heifer life. It is possible that dietary experience would eventually override previous feed experience. Given that only a small sample size (n = 4) was used in the previous study [24], it was worth noting that a greater number of replicates per treatment were used in the current study (n = 20 calves/treatment during d 57 to 70; n = 10 pairs/treatment during d 190 to 196), which gives us more statistical power to support the long-term observations made. However, our results should also be interpreted with caution because calves were grouped in pairs after d 70. Group-feeding system might increase the feed sorting behavior because of the increased competition and social pressure [52]. Leonardi and Armentano [53] found that cows fed in a group free-stall setting had a more severe extent of feed sorting than cows individually fed in a tie-stall setting. Hosseinkhani et al. [54] reported no differences in the extent of feed sorting when comparing cows fed in a non-competitive (1 cow/bin) to those fed in a competitive group (2 cows/bin). These results suggest that the effect of social environment on feed sorting is still a controversial area of study and to the best of our knowledge, there are no calf-based studies in this area. Hence, further research is encouraged to determine whether social environment has an effect on the extent of feed sorting in calves.

Rumen Fermentation and the incidence of SARA

It was not surprising to observe that pre-weaning calves fed concentrate had a lower rumen pH and a higher total VFA concentration than calves fed hay early. In line with the current study, previous studies claimed that concentrate, high in rapidly fermentable carbohydrates, stimulated the rumen fermentation and produced more VFA, especially propionate and butyrate [55]. It is well documented that rumen total VFA has a linear negative correlation with rumen fluid pH [40], indicating that higher VFA concentrations might result in lower pH value. Inclusion of forage in calf diet increases the ruminating and chewing activity [56], which further enhances saliva production that can neutralize VFA, and consequently improve the buffering effect resulting in higher rumen pH [1, 57]. When all calves were transitioned their respective pre-weaning feed to the same TMR, a variation occurred between treatments, whereby the rumen pH increased in CON group and decreased in HAY group from d 56 to 58, respectively. These changes could be attributed to the consumption of hay and concentrate by CON and HAY groups, respectively when TMR (containing 43% of hay and 57% of concentrate) were introduced to calves post-weaning.

Previous studies demonstrate that feed sorting (for fine particles and against long particles) was often observed in dairy cows [52], which resulted in greater consumption of fermentable carbohydrates and lesser consumption of physical fiber than intended [6], and this type of sorting was associated with reduced rumen pH [7] and the risk of SARA [8]. As stated above, calves were found to sort for the different feed particle fraction when TMR was offered. However, in contrast to our hypothesis, the treatment difference in feed sorting did not bring about any differences in rumen fermentation. After changing the diet to same TMR, no treatment effect on rumen pH was found at d 58, 63 and 70 of age. This could be

partially ascribed to the limited sorting behavior observed in the current study, which might result in small differences in the intake of concentrate or physical fiber between treatments. However, no empirical data is available to support this hypothesis; further research is needed to further explore the relationship between feed sorting and rumen pH in calves. Furthermore, the lack of difference in rumen pH might relate to the method of feed presentation. In our previous study, Xiao et al. [25] observed concurrent differences in feed preference and rumen pH, when concentrate and hay were offered separately after transitioning to new feed to allow calves to leisurely choose between feed components. Whereas, total mixed ration was used to test the effect of early feed experience in the current study, given that the main purpose of feeding TMR is to encourage the consumption of a balanced ration and prevent feed selection, sorting could be more easily accomplished in a free-choice diet as opposed to a mixed diet [3], which might minimize the effects. Although no differences were found in rumen pH, it was worth noting that a numerically higher number of calves might suffer from SARA (pH < 5.8) at d 58 and 63, probably due to the effect of feed sorting. In line with the current study, DeVries et al. [7] continuously tested the rumen pH and demonstrated that most cows selecting against long particles had the lowest maximum rumen pH, which might aggravate rumen acidosis. Feed digestion and fermentation in rumen is a dynamic process; the single time point rumen fluid sampling in the current study may mask the effect of different patterns of sorting. Continuous rumen pH measurements might allow for precise and accurate detection of duration and severity of SARA [22, 58]. For example, Yang et al. [58] claimed that the time that pH less than 5.8 (the threshold for SARA) almost doubled (7.9 vs. 4.1 hours/day) in cows fed finely rolled diet rather than coarsely diet even when mean pH (6.1 vs. 6.0) was not significantly different. Therefore, increasing sampling time points or utilizing an indwelling probe system to continuously measure rumen pH is encouraged.

After a long-term (6 months) transition to TMR, all calves sorted for fine particle fractions and against long particle fractions. Possibly because of the similar sorting pattern between treatments, calves fed concentrate early had similar rumen pH and VFA concentration at d 190, and no calf had rumen pH below 5.8. These results suggest that early feed exposure may have the capacity to regulate feed sorting in the short term, but long term the effects fade away, and rumen fermentation could not be affected even when a diverse variation in feed sorting occurred in both CON and HAY groups. These outcomes showed that the characteristics of the feed itself may play a much more important role in feed sorting and rumen fermentation than early feed experiences.

Conclusions

Altogether our study showed that early exposure to different feed sources affects the pattern of feed sorting once calves were transitioned from one sole feed component to TMR. Calves were likely to sort for familiar feed components immediately they are introduced, but over a short period of time. During the first 2 weeks after transition, calves offered hay early sorted for long particle fraction and calves offered concentrate early sorted for fine particle fractions, which closely represented hay and concentrate, respectively. Unexpected, feed sorting difference did not affect the rumen pH and fermentation, probably because of limited sorting of feed observed in the current study. While a numerical difference in the

number of calves with depressed rumen pH (< 5.8) was observed, further research with continuous measurement of rumen pH is encouraged to gain a better understanding of the relationship between feed sorting and SARA in calves. After 6 months, no long-lasting effect on feed sorting and rumen fermentation was found between treatments. All calves sorted for fine particles and against long particles, indicating that feed characteristics may have a more important role in feed sorting than early feed experience. Dietary experience would eventually override the effect of feed source early in life.

Declarations

–Ethics approval and consent to participate

The experimental design and procedures were executed according to the protocols approved by the Ethical Committee of the College of Animal Science and Technology, China Agricultural University (No. 2016DR07).

–Consent for publication

Not applicable.

–Availability of data and material

All data generated during this study are included in this article.

–Competing interests

The authors declare no conflict of interest in this study.

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–Authors' contributions

The paper was mainly conceived and designed by Jianxin Xiao and Shengli Li. Animal experiment was mainly conducted by Jianxin Xiao, Muhammad Zahoor Khan and Gibson Maswayi Alugongo. Data were collected and analyzed by Jianxin Xiao and Gibson Maswayi Alugongo. The manuscript was mainly written by Jianxin Xiao and Muhammad Zahoor Khan, and edited by Wei Wang, Yajing Wang and Zhijun Cao. All authors have read and agreed to the published version of the manuscript.

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Figures

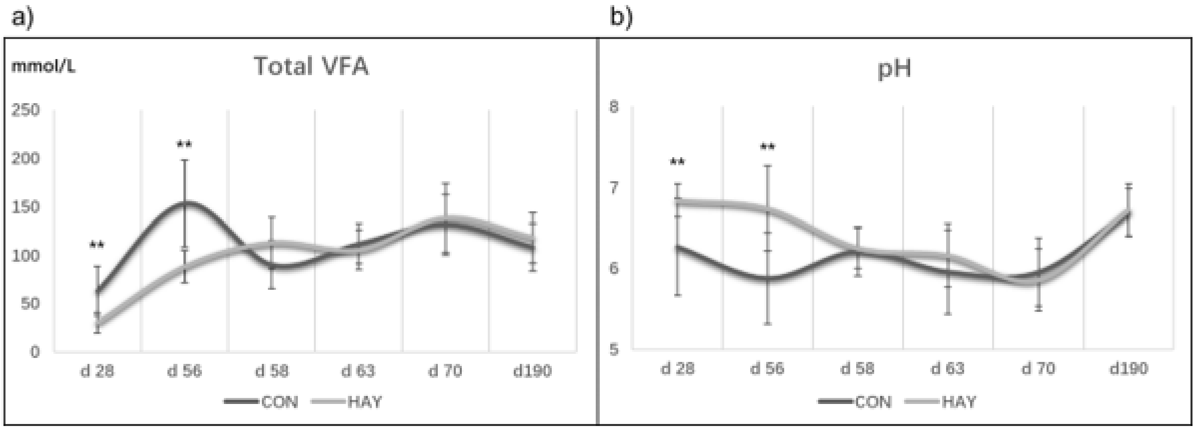

Figure 1

Effect of early feed exposure on total VFA and pH at d 28, 56, 58, 63, 70 and 190 of age1 1Sample size = 20 female calves per treatment. Calves were offered either concentrate (CON) or hay (HAY) during pre-weaning period (d 1 to 56). After weaning, all calves were fed novel TMR.