Rumen fermentation, blood metabolites, and growth performance of calves during transition from liquid to solid feed: Effects of dietary level and particle size of alfalfa hay

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ABSTRACT

This study evaluated the effects of particle size (PS) and dietary level of alfalfa hay (AH) on rumen fermentation parameters, blood metabolites, eating behavior, and growth performance in dairy calves during transition from liquid to solid feed. Sixty newborn dairy calves (41 ± 2.5 , kg of body weight) were used in a 2×2 factorial arrangement with the factors dietary AH level (medium, 12.5%, or high, 25%, on DM basis) and PS (fine = 1 mm or medium = 3 mm, as geometric means) of AH. Hence, the dietary treatments were (1) medium level of AH with fine PS (M-FPS), (2) medium level of AH with medium PS (M-MPS), (3) high level of AH with fine PS (H-FPS), and (4) high level of AH with medium PS (H-MPS). Particle size of AH did not affect total DMI (TDMI) during the preweaning period, although TDMI was greater for calves fed MPS than in those fed FPS during the postweaning and overall periods. Calves fed MPS spent more time eating solid feed and ruminating and less time on nonnutritive oral behaviors compared with FPS calves. The dietary level of AH did not affect behavioral parameters. Average daily gain of calves was not affected by dietary treatment before weaning. During the postweaning and overall periods, average daily gain was greater in calves fed MPS than in those fed FPS at the 25% AH level, but this effect was absent with 12.5% AH. Furthermore, the rumen pH values on d 35 and 70 of the study were greater for MPS than for FPS, regardless of the dietary level of AH. Effects of AH level, PS, and their interaction did not affect blood glucose concentrations in developing calves. These results indicate that feed intake, feeding behavior, rumen fermentation parameters, and blood β -hydroxybutyrate concentration may be affected by

rations differing in forage PS; thus, providing calves with MPS can improve calf performance and reduce their nonnutritive oral behaviors.

Key words: alfalfa hay, particle size, dairy calf

INTRODUCTION

Physical and metabolic development of the reticulorumen in young calves is the prerequisite to their transition from a preruminant milk diet to a solid diet based on grain forage (Drackley, 2008). Although rumen development in young calves is affected by many factors (Baldwin et al., 2004), it is mainly dictated by the amount and nature of the solid feed offered (Khan et al., 2011). Development of the rumen epithelium is triggered by the VFA produced during microbial fermentation of ingested solid feed in the rumen (Baldwin et al., 2004). Development of rumen musculature, rumen movements, and the capacity of the rumen are influenced by the physical stimuli provided by the bulk of the ingested material (Beharka et al., 1998).

Cereal-based starter diets generally contain higher levels of fermentable carbohydrates than forages, which provide greater amounts of VFA to stimulate rumen papillae when they ferment in the rumen (Lesmeister and Heinrichs, 2004; Khan et al., 2008). Ingestion of forage provides bulk in the rumen and thereby promotes rumen musculature and helps maintain the integrity of the rumen epithelium in developing calves (Beharka et al., 1998; Beiranvand et al., 2014b). Forage provision to young calves is generally discouraged because forage is less energy intensive and digestible compared with starter diets, and could, therefore, delay rumen epithelial development and suppress growth in young ruminants (Stobo et al., 1966; Hill et al., 2008, 2009). Furthermore, the guidelines of National Research Council (NRC, 2001) do not include any recommendations for fiber provision for developing calves. However, recent studies have demonstrated beneficial effects of

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feeding forage (grasses and legumes) early in life on rumen fermentation (Castells et al., 2013; Terré et al., 2013), rumen pH (Laarman and Oba, 2011), rumen development, feed consumption, and growth performance (Khan et al., 2011; Castells et al., 2012; Montoro et al., 2013; Beiranvand et al., 2014b). Furthermore, Castells et al. (2013) reported increased expression of a VFA transporter (mono-carboxylate transporter 1) in the rumen epithelium of calves that had access to forage compared with calves fed pelleted starter without forage. Previous studies (Castells et al., 2013; Beiranvand et al., 2014a,b) have shown enhanced concentrate intake and growth performance in young calves supplemented with forage. Particle size (**PS**) of the dietary forage affects the rumen environment, with high amounts of long and coarse forage resulting in decreased intake because of gut fill in dairy cows (Mirzaei et al., 2015). This may also apply to dairy calves, especially because of the limited capacity of their reticulorumen (Beharka et al., 1998; Godden et al., 2009).

We hypothesized that PS of alfalfa hay (**AH**) in the diet with rapidly degradable starch, as in barley grain, may have the potential to modify rumen conditions and to improve the performance of dairy calves. Hence, 2 levels of PS (fine and medium) were combined with 2 levels of AH (12.5 and 25% of dietary DM) in forage-supplemented diets to investigate their interaction on feed intake, rumen fermentation parameters, feeding behavior, and growth performance in dairy calves.

MATERIALS AND METHODS

The experiment was conducted on a local dairy farm (Ghiam Esfahan Plantation and Domesticated Co., Isfahan, Iran). All the calves were cared and managed according to the guidelines of Iranian Council of Animal Care (1995).

Calves, Management, and Treatments

Sixty 3-d-old Holstein male dairy calves (41 ± 2.5 kg of BW) were separated from their dams, weighed, and moved to individual pens (1.2×2.5 m) bedded with sawdust that was renewed every 48 h. The animals were fed 3 to 4 L of colostrum within 2 to 6 h after birth. Colostrum feeding continued for the first 2 d of life. Calves were fed whole milk (4 L/d), at a rate of 10% of BW, containing $3.22 \pm 0.11\%$ fat, $2.96 \pm 0.07\%$ CP, $4.92 \pm 0.05\%$ lactose, and $11.77 \pm 0.15\%$ TS in steel buckets twice daily at 0900 and 1700 h from d 3 to 47, followed by morning feeding (2 L/d) from 47 to 50 d of age before being weaned on d 51 of age.

The calves were randomly assigned ($n = 15$ per treatment) in a 2×2 factorial arrangement to a medium

(12.5% on DM basis) and a high dietary level of AH (25% on DM basis) with a fine (1 mm; geometric mean) or medium PS (3 mm; geometric means). Calves were assigned to 1 of 4 treatments: (1) medium level of AH with fine PS (**M-FPS**), (2) medium level of AH with medium PS (**M-MPS**), (3) high level of AH with fine PS (**H-FPS**), and (4) high level of AH with medium PS (**H-MPS**). The ingredient and chemical composition of the experimental diets are shown in Table 1. All the diets were formulated and to be isoenergetic and isonitrogenous according to the Cornell Net Carbohydrate and Protein System version 5.1 (Fox et al., 2000). For the first 16 d, all calves received the starter diet without forage provision. From d 16 onward, AH was mixed with finely ground concentrates and provided as a TMR. Starter containing AH (TMR) was fed ad libitum to permit at least 10%orts (i.e., the portion of the starter not consumed over a 24-h period), and TMR feed refusal from each individual calf was collected at 0800 h. Calves had ad libitum access to water and starter throughout the study. Before the experiment, AH was chopped (Golchin Trasher Hay Co., Isfahan, Iran) to obtain fine (1 mm, geometric mean) and medium (3 mm, geometric mean) PS. At least 6 representative samples were collected from each PS and used for analyzing PS distribution of AH by the Penn State Particle Separator (PSPS; Table 2). Geometric mean PS was calculated as described by the American Society of Agricultural Engineers (ASAE, 1996; method S424.1).

Sampling

Intakes of calf starter (forage and starter feed, as TMR) and total DMI (milk, starter feed, and forage) were measured daily and BW were recorded weekly during the preweaning (wk 1 to 7), postweaning (wk 8 to 10), and overall periods (wk 1 to 10). Amounts of feed offered and refused were recorded daily for each individual calf. Preweaning, postweaning, and overall means of total DMI (milk solids, starter feed), ADG, and feed efficiency (kg of BW gain/kg of total DMI) were also calculated.

Body measurements at weaning (d 51) and at the end of the study (d 71), including body length (distance between the points of shoulder and rump), body girth (the measurement of the distance around the belly over the part of the back before morning feeding), withers height (distance from base of the front feet to the withers), heart girth (circumference of the chest), hip height (distance from base of the rear feet to hook bones), and hip width (distance between the points of hook bones), of the calves were performed according to the method described by Khan et al. (2007).

Table 1. Ingredients and chemical compositions of experimental diets with different (12.5 and 25%) levels of alfalfa hay (AH)

Item	Concentration in the diet ¹	
	12.5% AH	25% AH
Ingredient (% of DM)		
Alfalfa hay	12.5	25.0
Barley grain	57.5	45.0
Soybean meal (45% CP)	25	20.8
Extruded soybean	1.6	5.0
Calcium carbonate	1.0	1.0
Energy booster 100 ²	0.4	1.2
Vitamin and mineral premix ³	1.5	1.5
Salt	0.5	0.5
Chemical composition (% of DM)		
ME ⁴ (Mcal/kg)	2.82	2.85
NE _G ⁴ (Mcal/kg)	1.25	1.25
DM	90	92
CP	19.61	19.73
NDF	23.0	24.5
Ether extract	3.96	3.87
Ash	11.2	11.4
Ca ⁴	0.75	0.78
P ⁴	0.42	0.47

¹Control = diet without alfalfa hay; 12.5% AH = diet with 12.5% alfalfa hay; 25% AH = diet with 25% alfalfa hay.

²Prilled saturated FFA (Milk Specialties, Dundee, IL).

³Contained per kilogram of supplement: 250,000 IU of vitamin A, 50,000 IU of vitamin D, 1,500 IU of vitamin E, 2.25 g of Mn, 120 g of Ca, 7.7 g of Zn, 20 g of P, 20.5 g of Mg, 186 g of Na, 1.25 g of Fe, 3 g of S, 14 mg of Co, 1.25 g of Cu, 56 mg of I, and 10 mg of Se.

⁴Calculated from NRC (2001).

Behavioral data were monitored by direct observations of all calves as the total time (min) devoted to each monitored behavior for a duration of 4 d, at 2 wk before weaning (d 37 and 44 of the experiment) and 2 wk after weaning (d 58 and 56 of the experiment). Calves were observed 1 h after the solid feed was offered and 2 h immediately after the morning milk feeding during the preweaning week. During the postweaning week, calves were observed for 1 h before and 2 h after the solid feed was offered. Thus, the total time for observing the behavior of each calf was equal to 12 h (6 h before and 6 h after weaning). Calves were observed every minute, and the following behaviors were recorded: standing, lying, eating, ruminating, and non-nutritive oral behaviors (when the animal licked any surface, tongue rolled, or consumed wood shavings).

Feces were scored daily for physical shape and consistency according to the procedure described in Heinrichs et al. (2003): 1 = normal; 2 = soft to loose; 3 = loose to watery; 4 = watery, mucous, slightly bloody; and 5 = watery, mucous, and bloody.

On d 35 and 71 of the study period, rumen fluid samples were obtained by using a stomach tube 4 h after the morning feeding, and rumen pH was measured immediately (HI 8318, Hanna Instruments, Cluj-Napoca,

Table 2. Distribution of alfalfa hay with fine and medium particle sizes (PS; mean $\pm$ SD) measured as DM retained on sieve (%)

Item	Alfalfa hay	
	Fine PS	Medium PS
19.0 mm	0	1.0 $\pm$ 0.2
8.0 mm	6.5 $\pm$ 0.6	26.0 $\pm$ 1.9
1.18 mm	50.5 $\pm$ 1.2	35.1 $\pm$ 0.9
<1.18 mm	42.9 $\pm$ 2.1	37.8 $\pm$ 2.3
GMPL ¹ (mm)	2.1 $\pm$ 0.1	2.9 $\pm$ 0.1

¹GMPL = geometric mean particle size calculated as described by ASAE (1996; method S424.1).

Romania). Rumen contents were squeezed through 4 layers of cheesecloth and the fluid was stored at -20°C pending analysis. For stabilization, 10 mL of the rumen fluid was acidified with 3 mL of 25% metaphosphoric acid and stored (-20°C) until analysis for VFA by gas chromatography.

Blood samples were collected 3 h after the morning meal on d 21, 35, and 74 of the study period. Blood was withdrawn from the jugular vein into evacuated tubes containing K₂ EDTA (Becton Dickinson Vacutainer Systems, Franklin Lakes, NJ) and immediately placed on ice. The tubes were then centrifuged at $2,000 \times g$ (4°C) for 20 min within 2 h of sampling. The plasma was separated and stored at -20°C for subsequent analysis.

Chemical and Biochemical Analyses

Total mixed rations were sampled weekly at the time of feeding and frozen in plastic bags. Samples were thawed and composited into 1 sample per month. The composited samples were mixed thoroughly and ground to pass a 1-mm screen in a Wiley mill (Ogaw Seiki Co. Ltd., Tokyo, Japan) before chemical analyses. Feed and refusal samples were analyzed for their DM content according to the procedures of AOAC (1990). Crude protein (method 988.05; AOAC, 1990) and NDF without the use of sodium sulfite and with α -amylase included (Van Soest et al. 1991) were also analyzed.

Rumen samples were analyzed for VFA by gas chromatography (model CP-9002, Chrompack, Middelburg, the Netherlands) with a 50-m (0.32 mm i.d.) silica-fused column (CP-Wax Chrompack Capillary Column, Varian, Palo Alto, CA). Helium was used as the carrier gas and the oven initial and final temperatures were 55 and 195°C , respectively. The detector and injector temperatures were set at 250°C . Crotonic acid (1:7, vol/vol) was used as the internal standard. Plasma BHBA was determined by autoanalyzer using a laboratory kit (Randox Laboratories Ltd, Ardmore, UK). Plasma concentration of glucose was measured using a serum

spectrophotometer (UNICCO, 2100, Zistchemi Co., Tehran, Iran).

Statistical Analyses

Data of DMI, ADG, and gain-to-feed ratio were subjected to ANOVA for a complete randomized design with repeated measurements using PROC MIXED in SAS (version 9.2; SAS Institute, Cary, NC) with the individual calf as the experimental unit. The model included level, PS of AH, the interaction between level and PS of AH, and the interactions of level $\times$ week, PS $\times$ week, and level $\times$ PS $\times$ week for DMI, ADG, and gain-to-feed ratio data. An autoregressive (order 1) covariance structure was chosen based on the Akaike and Bayesian information criteria. Statistical analyses were conducted over 3 periods: preweaning (wk 1 to 7), postweaning (wk 8 to 10), and overall (wk 1 to 10) periods. Rumen fermentation, behaviors, fecal score, and structural growth variables were analyzed using a similar model but without the effect of time. For structural growth variables, the initial measurements were included as a covariate for the statistical analysis of body measurements. When differences for dietary treatments or interactions were detected, means separation was conducted using a Tukey's adjustment for the probability. Significance was declared at $P \leq 0.05$ and trends were considered when $0.05 < P < 0.10$.

RESULTS

All the calves were healthy with no clinical symptoms of systemic disease or mortality throughout the experiment.

Calf Performance

Medium PS promoted total DMI (TDMI) when AH was supplemented at 25% of dietary DM compared with the 12.5% level (Figure 1; Table 3). Total DMI was not affected by AH PS during the preweaning period, although TDMI was greater for calves fed MPS than for those fed FPS during the postweaning and overall periods ($P < 0.05$; Table 3). Final BW (d 70) increased ($P < 0.01$) from 75.18 to 84.1 kg when PS changed from fine (1 mm) to medium (3 mm; Figure 2; Table 3). Mean ADG and gain-to-feed ratio of the calves did not differ across the treatments before weaning. Regardless of the AH level, ADG was greater in calves fed MPS than in those fed FPS during the postweaning and overall periods ($P < 0.01$; Figure 3). No interaction was found in this study between AH level and PS regarding their effects on TDMI, BW, or ADG. In general, gain-to-feed ratio and fecal score

were not affected by AH level, PS, or their interaction ($P > 0.05$; Table 3). No significant 2-way interactions (week $\times$ level and week $\times$ PS of AH) were found for gain-to-feed ratio, indicating that the effect of level and PS of AH on gain-to-feed ratio was similar throughout the experiment. No significant 3-way interactions (week $\times$ level $\times$ PS of AH) were found for DMI, ADG, and gain-to-feed ratio (Table 3).

Rumen Fermentation Parameters and Blood Metabolites

Regardless of the level of AH, ruminal pH was greater for MPS than for FPS on d 35 and 70 of the study ($P < 0.01$; Table 4). No interaction was observed between forage PS and the level of AH on ruminal pH (Table 4).

Ruminal pH was not affected by AH level, PS, or their interaction (Table 4). Regardless of the AH level, acetate concentrations were greater on d 70 of the study in calves fed MPS than in those fed FPS ($P < 0.05$; Table 4). Regardless of PS, propionate concentration tended to be greater at 12.5% inclusion of AH than at 25% ($P = 0.08$; Table 4). The concentration of butyrate was not affected by AH level, PS, or their interaction. Furthermore, regardless of the AH level, the acetate-to-propionate ratio was greater ($P < 0.05$) on d 35 and 70 of the study in calves fed MPS than in those fed FPS (Table 4). Blood glucose concentration was not affected by AH level, PS, or their interaction (Figure 4). However, regardless of the AH level, blood BHBA concentrations were greater on d 70 of the study period in calves fed MPS than in those fed FPS ($P < 0.05$; Figure 5).

Animal Behavior and Structural Growth

Regardless of AH level, calves receiving the MPS treatment devoted greater time to eating starter feed and ruminating than those receiving FPS ($P < 0.01$; Table 5). Moreover, calves on the MPS diet spent less time performing non-nutritive oral behaviors compared with those on the FPS diet (Table 5).

Body length, withers height, heart girth, hip width, and hip height were not affected by AH level, PS, or their interaction (Table 6). However, body girth at the end of experiment increased with increasing PS from fine to medium, regardless of the AH level ($P < 0.05$; Table 6).

DISCUSSION

Calf Performance

It has been widely recognized that feeding forage increases postweaning TDMI (Castells et al., 2012; Terré

Figure 1. Weekly mean ($\pm$ SEM) total DMI (kg) in calves fed different diets. M-FPS = medium level (12.5%) of alfalfa hay (AH) with fine particle size (PS; ■), M-MPS = medium level (12.5%) of AH with medium PS (▲), H-FPS = high level (25%) of AH with fine PS (■), H-MPS = high level (25%) of AH with medium PS (●). For each time point, * denotes significant difference at $P < 0.05$; ** denotes significant difference at $P < 0.01$.

et al., 2013). It seems that forage particle size may affect postweaning DMI in young calves. Providing AH with a fine PS at medium and high supplementation levels may not have provided sufficient mechanical stimuli to increase the physical capacity and muscular development of the reticulorumen (Tamate et al., 1962), or rumen motility (Nocek, 1997; Owens et al., 1998), resulting in similar values of DMI, BW, ADG, and gain-to-feed ratio compared with non-forage-supplemented calves. In the present study, the greater DMI with MPS diets may be explained by the increased capacity and development of the reticulorumen (Khan et al., 2011; Montoro et al., 2013). This is in line with the results of a recent study (Montoro et al., 2013), which showed that the greater PS of grass hay with a DM inclusion level of 10% improved starter feed intake by dairy calves after weaning. The improved rumen environment and enhanced muscular development of the rumen may, in turn, have contributed to the increased intake of solid feed during the postweaning period (Coverdale et al., 2004; Khan et al., 2011; Castells et al., 2012). Montoro et al. (2013) reported that calves fed 10% coarsely chopped (3 to 4 cm) grass hay tended to have a greater gain-to-feed ratio than those fed 0% finely ground (2

mm) grass hay (0.68 vs. 0.63 ± 0.02 kg of gain/kg of DMI).

The results obtained for fecal scores are in agreement with those reported by Coverdale et al. (2004) who did not find differences in the fecal scores of calves fed different inclusion levels of brome grass hay compared with their control group.

Rumen Fermentation Parameters and Blood Metabolites

In the current study, ruminal pH ranged from 5.05 to 5.72 in calves supplemented with higher levels of forage after weaning (5.22 to 5.72) compared with before weaning (5.05 to 5.51), which is consistent with the findings of Mirzaei et al. (2015). Anderson et al. (1987) also reported that pH in the rumen of dairy calves did not exceed 6 during the first 10 wk of life. Krause et al. (2002) reported that mean ruminal pH was positively correlated with the time spent on ruminating and chewing. Our results concerning forage PS are in agreement with those of other authors (Krause et al., 2002; Teimouri Yansari et al., 2004), confirming that forage PS plays a major role in maintaining opti-

Table 3. Effects of forage level (12.5 vs. 25%) and particle size (fine vs. medium) on BW, DMI, ADG, gain-to-feed ratio, and fecal score in dairy calves (n = 15 per treatment)

Item	Treatment ¹				SEM	P-value ²					
						Treatment			Interaction ³		
	M-FPS	M-MPS	H-FPS	H-MPS		L	PS	L × PS	1	2	3
DMI, kg/d											
Prewaning	0.80	0.82	0.79	0.83	0.03	0.98	0.46	0.86	0.52	0.72	0.58
Postweaning	1.49	1.69	1.45	2.02	0.08	0.23	0.01	0.11	0.08	0.01	0.15
Overall	1.01	1.08	0.99	1.19	0.05	0.51	0.04	0.35	0.05	0.01	0.13
BW, kg											
Initial, d 1	42.25	42.63	43.18	42.7	1.16	0.23	0.43	0.64	—	—	—
At weaning, d 51	63.10	65.12	63.02	64.99	1.31	0.99	0.15	0.94	—	—	—
Final, d 70	76.52	80.22	75.18	84.10	2.22	0.52	0.01	0.25	—	—	—
ADG, kg/d											
Prewaning	0.41	0.45	0.41	0.45	0.02	0.99	0.13	0.94	0.35	0.20	0.13
Postweaning	0.64	0.72	0.58	0.89	0.07	0.37	0.01	0.08	0.86	0.01	0.47
Overall	0.48	0.53	0.46	0.58	0.03	0.50	0.01	0.23	0.72	0.01	0.47
Gain-to-feed ratio ⁴											
Prewaning	0.47	0.47	0.46	0.49	0.03	0.72	0.61	0.58	0.81	0.65	0.79
Postweaning	0.42	0.42	0.40	0.45	0.04	0.87	0.35	0.51	0.98	0.12	0.86
Overall	0.45	0.46	0.44	0.48	0.03	0.83	0.34	0.34	0.95	0.57	0.91
Fecal score ⁵	2.46	2.64	2.59	2.67	0.15	0.59	0.39	0.74	—	—	—

¹Treatment: M-FPS = medium level (12.5%) of alfalfa hay (AH) with fine particle size (PS); M-MPS = medium level (12.5%) of AH with medium PS; H-FPS = high level (25%) of AH with fine PS; H-MPS = high level (25%) of AH with medium PS.

²Statistical comparisons: L = level of alfalfa hay; PS = particle size of alfalfa hay; L × PS = L by PS interaction.

³Interactions 1, 2, and 3 = level of AH × week; PS of AH × week; level of AH × PS of AH × week.

⁴Gain-to-feed ratio was calculated by dividing ADG (g) by average daily DMI.

⁵Scoring was as follows: 1 = normal; 2 = soft to loose; 3 = loose to watery; 4 = watery, mucous, slightly bloody; 5 = watery, mucous, and bloody.

Figure 2. Weekly BW ($\pm$ SEM; kg) in calves fed different diets. M-FPS = medium level (12.5%) of alfalfa hay (AH) with fine particle size (PS; ■), M-MPS = medium level (12.5%) of AH with medium PS (▲), H-FPS = high level (25%) of AH with fine PS (■), H-MPS = high level (25%) of AH with medium PS (●).

Table 4. Effects of forage level (12.5 vs. 25%) and particle size (fine vs. medium) on rumen fermentation parameters in dairy calves (n = 15 per treatment)

Item	Treatment ¹				SEM	P-value ²		
	M-FPS	M-MPS	H-FPS	H-MPS		L	PS	L × PS
Rumen pH								
d 35	5.05	5.45	5.13	5.51	0.04	0.12	0.01	0.83
d 70	5.22	5.48	5.23	5.72	0.07	0.13	0.01	0.15
Total VFA, mmol/L								
d 35	101.10	94.65	91.72	98.19	5.88	0.63	0.99	0.29
d 70	110.1	105.7	96.1	103.8	4.54	0.11	0.72	0.21
Individual VFA, mol/100 mol								
Acetate								
d 35	52.11	50.89	46.23	54.66	3.84	0.78	0.36	0.23
d 70	49.95	53.99	46.06	56.24	2.46	0.74	0.01	0.24
Propionate								
d 35	35.68	32.27	33.41	31.49	1.95	0.45	0.20	0.71
d 70	39.66	37.04	34.81	34.92	1.86	0.08	0.51	0.48
Butyrate								
d 35	13.29	11.48	12.08	12.03	0.88	0.72	0.32	0.34
d 70	20.5	14.68	15.25	12.68	2.43	0.16	0.11	0.52
Acetate:propionate								
d 35	1.46	1.57	1.37	1.74	0.06	0.54	0.01	0.08
d 70	1.26	1.48	1.33	1.62	0.09	0.30	0.02	0.69

¹Treatment: M-FPS = medium level (12.5%) of alfalfa hay (AH) with fine particle size (PS); M-MPS = medium level (12.5%) of AH with medium PS; H-FPS = high level (25%) of AH with fine PS; H-MPS = high level (25%) of AH with medium PS.

²Statistical comparisons: L = level of alfalfa hay; PS = particle size of alfalfa hay; L × PS = L by PS interaction.

Figure 3. Weekly ADG ($\pm$ SEM; kg) in calves fed different diets. M-FPS = medium level (12.5%) of alfalfa hay (AH) with fine particle size (PS; ■), M-MPS = medium level (12.5%) of AH with medium PS (▲), H-FPS = high level (25%) of AH with fine PS (■), H-MPS = high level (25%) of AH with medium PS (●). For each time point, * = significant difference at $P < 0.05$; ** = significant difference at $P < 0.01$.

Figure 4. Blood glucose concentrations ($\pm$ SEM; mg/dL) in calves fed different diets. M-FPS = medium level (12.5%) of alfalfa hay (AH) with fine particle size (PS; ■), M-MPS = medium level (12.5%) of AH with medium PS (▲), H-FPS = high level (25%) of AH with fine PS cane (■), H-MPS = high level (25%) of AH with medium PS (●).

mal rumen pH and that increasing the PS of AH can improve ruminal pH, which may be the result of greater time spent ruminating in dairy calves. Nasrollahi et

al. (2012) reported that cows fed long AH had higher ruminal pH compared with those fed finely chopped AH diets (6.46 vs. 6.26). However, this observation is

Figure 5. Blood BHBA concentrations ($\pm$ SEM; mmol/L) in calves fed different diets. M-FPS = medium level (12.5%) of alfalfa hay (AH) with fine particle size (PS; ■), M-MPS = medium level (12.5%) of AH with medium PS (▲), H-FPS = high level (25%) of AH with fine PS (■), H-MPS = high level (25%) of AH with medium PS (●). For each time point, * = significant difference at $P < 0.05$.

Table 5. Effects of forage level (12.5 vs. 25%) and particle size (fine vs. medium) on total time devoted to performing different behaviors during 8 h of observation in dairy calves (n = 15 per treatment)

Item	Treatment ¹				SEM	P-value ²		
	M-FPS	M-MPS	H-FPS	H-MPS		L	PS	L × PS
Standing (min)	186.8	177.9	180.3	169.5	7.45	0.48	0.28	0.70
Lying (min)	217.5	213.9	225.3	212.6	6.59	0.58	0.25	0.45
Eating (min)	24.15	30.93	21.13	35.52	1.43	0.72	0.01	0.06
Ruminating (min)	21.17	33.91	20.37	39.38	2.1	0.24	0.01	0.11
Non-nutritive oral behaviors (min)	30.42	23.20	32.95	22.98	2.5	0.65	0.01	0.59

¹Treatment: M-FPS = medium level (12.5%) of alfalfa hay (AH) with fine particle size (PS); M-MPS = medium level (12.5%) of AH with medium PS; H-FPS = high level (25%) of AH with fine PS; H-MPS = high level (25%) of AH with medium PS.

²Statistical comparisons: L = level of alfalfa hay; PS = particle size of alfalfa hay; L × PS = L by PS interaction.

inconsistent with the report of Mirzaei et al. (2015), who found no significant differences in the ruminal pH of calves fed AH of different PS.

The total VFA concentrations in the current study were not affected by AH level, PS, or their interaction, which is in agreement with the results reported by Mirzaei et al. (2015). However, previous studies did not observe any differences in total rumen VFA concentrations between calves offered different forage levels and those not receiving any forage (Thomas and Hinks, 1982; Suárez et al., 2007). In the current study, insufficient particle size resulted in reduced ruminal pH and acetate-to-propionate ratio. The reduction in rumen pH impairs the growth of cellulolytic bacteria, resulting in declining acetate-to-propionate ratio (Grant et al., 1990).

Blood glucose concentration was also observed to decrease with calf age, which is consistent with the find-

ings of Khan et al. (2011). The reduced blood glucose concentration with age observed in calves regardless of treatment may be attributed to a physiological shift in the primary energy source from glucose to VFA when the rumen in young calves becomes functional (Hammon et al., 2002). In agreement with previous studies (Greenwood et al., 1997; Coverdale et al., 2004; Naeem et al., 2012), increasing blood BHBA concentration as calves aged was mainly due to a shift in the sources of physiological fuel during the transition from liquid to solid diets (Khan et al., 2011).

In the current study, the greater concentrations of blood BHBA (an indicator of metabolic function of the rumen wall) in calves fed MPS compared with FPS suggested that ruminal development was normal and that rumen walls were equally efficient in converting butyrate to BHBA (Khan et al., 2011).

Table 6. Effects of forage level (12.5 vs. 25%) and particle size (fine vs. medium) on structural growth in dairy calves (n = 15 per treatment)

Item	Treatment ¹				SEM	P-value ²		
	M-FPS	M-MPS	H-FPS	H-MPS		L	PS	L × PS
Body length								
Weaning	52.39	54.10	52.93	53.43	0.8	0.90	0.19	0.44
Final	57.06	57.33	57.24	58.43	0.8	0.46	0.41	0.58
Body girth								
Weaning	108.8	111.0	109.8	113.8	1.78	0.41	0.08	0.47
Final	116.1	119.5	117.8	123.8	2.00	0.14	0.02	0.51
Withers height								
Weaning	83.52	82.74	83.44	83.80	0.71	0.49	0.77	0.42
Final	85.39	84.96	85.41	85.46	0.44	0.81	0.85	0.47
Heart girth								
Weaning	94.27	95.77	94.37	95.30	0.92	0.85	0.22	0.63
Final	100.7	99.9	98.9	101.6	1.57	0.96	0.41	0.13
Hip width								
Weaning	19.57	20.02	19.73	20.31	0.51	0.85	0.23	0.49
Final	23.30	23.15	23.49	23.92	0.32	0.12	0.61	0.38
Hip height								
Weaning	89.40	88.94	89.41	88.85	0.56	0.95	0.56	0.11
Final	91.57	90.67	90.44	91.39	0.84	0.69	0.84	0.60

¹Treatment: M-FPS = medium level (12.5%) of alfalfa hay (AH) with fine particle size (PS); M-MPS = medium level (12.5%) of AH with medium PS; H-FPS = high level (25%) of AH with fine PS; H-MPS = high level (25%) of AH with medium PS.

²Statistical comparisons: L = level of alfalfa hay; PS = particle size of alfalfa hay; L × PS = L by PS interaction.

Animal Behavior and Structural Growth

The benefits of forage supplementation on stimulating rumination have been previously demonstrated (Phillips, 2004; Martin et al., 2006; Castells et al., 2012). In the current study, the greater DMI of MPS diets compared with FPS diets may have been due to the improved rumen function and the more stable pH achieved by the increased time spent on rumination and eating starter feed. These findings are in agreement with those reported by Nasrollahi et al. (2012), who also observed an increase in eating time with increasing AH PS in dairy cows. Calves fed the FPS diets, regardless of AH level, spent greater time performing non-nutritive oral behaviors than those fed the MPS diets. Non-nutritive oral behaviors are often considered an index of poor welfare because they are thought to be related to frustrated feeding activity (Redbo, 1990; Redbo and Nordblad, 1997). The present results suggest that forage PS plays an important role in reducing non-nutritive oral behaviors in young calves; therefore, providing calves with fine forage particles may have some adverse effects on welfare.

The role of forage feeding on the physical development of the rumen has been shown previously (Khan et al., 2011; Mirzaei et al., 2015). In the current study, providing forage, regardless of the level of AH, resulted in enhanced body girth, suggesting that anatomical development and digestive organ growth may be influenced by forage supplementation and the PS of AH in dairy calves. In contrast to our findings, Mirzaei et al. (2015) reported that the effect of AH PS on rumen development and growth characteristics of dairy calves depended on the level of supplementation.

CONCLUSIONS

The physical form of AH, in particular its particle size, plays a more important role than its chemical characteristics on performance, feeding behavior, and blood metabolites of dairy calves, especially during the postweaning period. It is possible to develop ruminant diets by altering the PS of AH from fine to medium to achieve high performance and reduced non-nutritive oral behaviors.

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