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Interaction effect of starter physical form and alfalfa hay on growth performance, ruminal fermentation, and blood metabolites in Holstein calves

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Abstract The present study was conducted to evaluate the effects of physical form of starter and alfalfa hay (AH) provision on growth performance, ruminal fermentation, and blood metabolites of Holstein dairy calves. Forty-four 3d-old Holstein dairy calves with a mean starting BW of 39.9 ± 1.1 kg were used in a 2 × 2 factorial arrangement. The factors were dietary forage level (0 or 150 g kg^{-1} AH; DM basis) and physical form of starter feed (coarsely mashed vs. pelleted). Individually housed calves were randomly assigned to 4 treatments: 1) a coarsely mashed starter feed without AH provision (MS-NAH), 2) a coarsely mashed starter feed with AH provision (MS-AH), 3) a pelleted starter feed without AH provision (PS-NAH), and 4) a pelleted starter feed with AH provision (PS-AH). The calves were weaned on d 60 and remained in the study until d 70. The results showed that feeding pelleted starter decreased starter intake significantly compared with coarsely mashed groups during the post-weaning ($P < 0.01$) and the entire period ($P = 0.026$).The interaction between AH inclusion and physical form of starter feed tended to be significant for both average daily gain ($P = 0.092$) and feed efficiency ($P = 0.086$). Inclusion of AH in the starter feeds increased rumination time and body barrel in calves. Blood urea nitrogen concentration in calves fed AH increased during post-weaning stage. Blood aspartate aminotransferase concentration was greater in calves fed PS form than those fed MS form. Although AH inclusion prevented rumen pH reduction during the pre-weaning period in calves fed PS form; ruminal pH decreased in PS groups in comparison with coarsely mashed groups during the post-weaning period. The pelleted form of starter increased total short chain fatty acids and butyric acid concentrations. However, coarsely mashed form and AH supplementation increased acetate concentration in the ruminal fluid. Overall, the results indicated that coarsely mashed form of starter could be recommended for Holstein dairy calves. Furthermore, AH inclusion in the starter diet may ameliorate the negative effects when pelleted starter is fed to dairy calves.

Keywords: diary calf nutrition, feed efficiency, starter processing

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Introduction

The starter processing and its physical form could have important role in the growth of young calves (Terré et al., 2015). Inappropriate processing of feedstuffs could even reduce feed intake and hence decrease its efficiency (Kazemi-Bonchenari et al., 2017). Data on the optimal physical form of the starter are inconsistent (Lassiter et al., 1955; Bach et al., 2007; Terré et al., 2015). Pelleting improved feed efficiency through reducing weaning age, increasing palatability, and resulted in greater feed intake compared with un-pelleted feeds (Lassiter et al., 1955; Bach et al., 2007); however, others reported that pelleted form reduced intake or feed efficiency (Porter et al., 2007). Although recently, Pazoki et al. (2017) evaluated the effects of different physical form of starter in dairy calves; however, their experimental diets were not supplemented with forages which could have affected their results.

Controversy exists regarding the negative or positive effects of forage inclusion in the starter feed. The advantages of forage inclusion in diets are promoting the

Abbreviations:
AH: alfalfa hay,
MS: coarsely mashed starter,
PS: pelleted starter,
SCFA: short chain fatty acids,
ADG: average daily gain,
AST: aspartate aminotransferase,
ALT: alanine aminotransferase.

rumination, stimulating the muscular layer of the rumen and increasing absorption in the rumen, and maintaining ruminal integrity and health. (Phillips, 2004; Beiranvand et al., 2014). Forage inclusion has some disadvantages such as displace concentrate intake and shifting the ruminal fermentation in favor of acetate rather than propionate and butyrate production which delays papillary development, reduced starter feed intake, and decreased body weight and dry matter (DM) digestibility (Nocek and Kesler, 1980; Zitnan et al., 1998; Phillips, 2004). Therefore, in addition to the uncertainties concerning the starter physical form and forage inclusion in the starter feed, their interactions is not well understood and requires more research.

Despite several advantages in feeding pelleted starter in animal nutrition, lower ruminal fluid pH could be a concerning issue with pelleted diets (Porter et al., 2007) such as hyperkeratinization of ruminal papillae (Zitnan et al., 1998) which negatively affects nutrient absorption(Nocek and Kesler, 1980). Forage inclusion improved rumen pH as well as reducing hyperkeratinization process of rumen papillae (Beiranvand et al., 2014; Mirzaei et al., 2016). We hypothesized whether forage inclusion could attenuate the negative effect of pelleted starter form in dairy calves. Therefore, the objective of this study was to examine the effects of, and interactions between, physical forms of starter feed (coarsely mashed vs. pelleted) and alfalfa hay supplementation (no forage or 150 g kg^{-1} of dietary DM) on starter feed intake, growth performance, feeding behavior, ruminal fermentation parameters, and some blood metabolites in dairy calves both before and after weaning.

Materials and methods

Animals, management and treatments

The experiment was conducted at a commercial dairy farm (Goldasht-Nemune Sepahan, Isfahan, Iran). Forty-four (24 males and 20 females) Holstein calves aged 3 d with an average BW of 39.9 ± 1.1 kg were randomly assigned to the treatments (6 male and 5 female calves per treatment). The calves were separated from their dams immediately after birth, weighed, and moved to individual pens (1.2 × 2.5 m) bedded with sand, which was renewed every 24 h. The animals were fed 4 L/meal of colostrum for the first 2 d of life. The calves were allotted to the treatments on d 3 of age. The calves received 4 L/d milk in steel buckets twice daily at 0830 and 1630 from d 3 to 10, 6 L/d from d 11 to 50, and 5 L/d from d 51 to 59 of age. The calves were weaned at d 59 of age and fed the experimental diets until d 70 of age. The first 8 wk (d 3-59) was considered as the pre-

Table 1. Ingredients and chemical composition of experimental diets (g kg^{-1} dry matter; unless otherwise stated)

	Diets[1]			
Ingredients	MS-NAH	MS-AH	PS-NAH	PS-AH
Alfalfa hay, chopped	0	150	0	150
Corn grain, ground	650	523	650	523
Soybean meal	250	230	250	230
Molasses, beet	35	35	35	35
Soybean seed	25	25	25	25
Energizer RP-10[2]	10	10	10	10
Vitamin-mineral mix[3]	3	3	3	3
Calcium carbonate	15	12	15	12
Sodium bicarbonate	5	5	5	5
Dicalcium phosphate	2	2	2	2
Salt	5	5	5	5
Chemical composition				
Metabolizable energy[3], Mcal kg^{-1}	2.76	2.71	2.76	2.71
CP	198	198	198	198
NDF	119	172	119	172
NFC[4]	565	487	565	487
Ether extract	39	39	39	39
Ca	7	7	7	7
P	4	4	4	4

[1]Diets were; 1) coarsely mashed starter without alfalfa hay provision (MS-NAH), 2) coarsely mashed starter with alfalfa hay provision (MS-AH), 3) pelleted starter without alfalfa hay provision (PS-NAH), and 4) pelleted starter with alfalfa hay provision (PS-AH).

[2]Prilled saturated free fatty acids (99% fatty acid weight basis); Cargill Animal Nutrition proprietary blend (ELK River, MN)

[3]Contained per kilogram of supplement: 15,000 IU vitamin A, 10 IU vitamin D_3, 10 mg vitamin E, 1 g Mn, 30 g Ca, 1 g Zn, 5 g P, 9 g Mg, 18 g Na, 20 mg Fe, 10 mg S, 14 mg Co, 20 mg Cu, 11 mg I, and 4 mg Se.

[4]Calculated from NRC (2001); [Non-fiber-carbohydrate was calculated as DM - (NDF + CP + ether extract + ash)].

weaning, and the last 10 days (d 60-70) as the post-weaning periods. Treatments were 1) mashed starter without alfalfa hay provision (MS-NAH), 2) mashed starter with alfalfa hay provision (MS-AH), 3) pelleted starter without alfalfa hay provision (PS-NAH), and 4) pelleted starter with alfalfa hay provision (PS-AH). Alfalfa hay was fed at 150 g kg^{-1} (DM basis) and pellet diameter was 3 mm. The diets (Table 1) were formulated to meet dairy calf nutrient requirements (NRC, 2001). The calves had free access to water and *ad libitum* starter intake was achieved by offering an amount that resulted in the residue of 50-100 g kg^{-1} of offered feed after 24 h. The amount of feed offered and refused was weighed daily for determination of total starter daily intake calculated to determine individual daily feed intakes throughout the study. Vaccination and rearing procedures were followed as carried out on the farm.

Experimental procedures and chemical analyses

Starter intake was measured daily, and individual BW was recorded at 10-day intervals. The amounts of feed offered and refused were recorded daily for each individual calf. Pre- and post-weaning, and overall means of the average daily gain (ADG) and feed efficiency (kg BW gain/kg total DM intake) were calculated. Samples of feed and orts were dried at 60°C using a forced-air oven for 48 h. Subsamples of feed and refusals were mixed thoroughly, dried, and ground to pass a 1 mm screen in a mill (Ogaw Seiki CO., Ltd., Tokyo, Japan) before chemical analysis for crude protein (method 988.05; AOAC, 1990) and ether extract (method 920.39; AOAC, 1990). Neutral detergent fiber (NDF) was analyzed in the absence of sodium sulfite and in the presence of α-amylase (Van Soest et al. 1991).

Data on feeding behavior attributes (standing, ruminating, lying, eating and non-nutritional behavior) monitored by direct observations were collected once per week 2 wk before (d 35 and 42 of the trial) and 2 wk after weaning (d 62 and 69 of the trial). Calves were observed at 1 h after the solid feed was offered (at 0800 h) and 2 h immediately after the morning milk feeding (at 0900 h) before weaning. After weaning, the calves were observed 1 h before and 2 h after the solid feed was offered at 0800 h.

Body measurements, including the heart girth, body length (distance between the points of shoulder and rump), body barrel (circumference of the belly before feeding), wither height (distance from the base of the front feet to wither), hip width (distance between two hip bones) and hip height (distance from the base of the rear feet to the hook bones) were recorded at the start of the experiment (d 3), at weaning (d 59) and at the end of the study (d 70) according to the method described by Khan et al. (2007).

Heparinized blood samples were collected at 4 h after morning feeding from the jugular vein into 10 mL tubes on d 34 (pre-weaning sample) and 68 (post-weaning sample) of the study. Blood samples were centrifuged (3,000 × g 4°C, 20 min) and plasma samples stored at –20°C. Plasma concentrations of glucose (Kit No. 9308), blood urea nitrogen (BUN) (Kit No. 93013), albumin (Kit No. 9307), and total protein (Kit No. 9304) were determined using commercial (Pars Azmoon Co., Tehran, Iran). Aspartate aminotransferase (AST) (Kit No. 11840) and alanine aminotransferase (ALT) (Kit No. 11940) were measured using ELISA (Auto Analyzer Hitachi 717, Japan). Beta-hydroxybutyrate (BHBA) concentration was measured using a commercial kit (Randox Laboratories, Ardmore, UK.

Ruminal fluid samples (50 mL) were collected before (d 35) and after (d 70) weaning, using a stomach tube fitted to a vacuum pump at 3-4 h post morning feeding; the first 10 mL were discarded because of possible saliva contamination, and rumen pH was measured immediately (HI 8314 membrane pH meter, Hanna Instruments, Villafranca, Italy). The samples were squeezed through 4-layers of cheesecloth and a 10 mL aliquot was preserved with 2 mL of 25% meta-phosphoric acid and frozen at −20°C until analysis. After thawing at room temperature, ruminal fluid samples were analyzed for SCFA using gas chromatography (model CP-9002, Chrompack, Delft, the Netherlands) as previously described (Kazemi-Bonchenari et al., 2016).

Statistical analysis

Statistical analyses were conducted for 3 periods: pre-weaning, post-weaning and the entire period using PROC MIXED (version 9.1; SAS Institute, Cary, NC) with the individual calf as the experimental unit. Starter intake, ADG, and feed efficiency were analyzed as repeated measures with week as repeated variable using the following model:

$$Y_{ijkl} = \mu + SF_i + AH_j + W_k + (SF\times W)_{ik} + (AH\times W)_{jk} + (SF\times AH)_{ij} + (SF\times AH\times W)_{ijk} + \beta(Xi- \bar{X}) + \varepsilon_{ijkl}$$

where Y_{ijk} is the dependent variable; μ is the overall mean; SF_i is the effect of starter physical form (mashed vs. pelleted), AH_j is the effect of alfalfa hay provision (NAH vs. AH), W_k is the effect of week, $(SF\times W)_{ij}$ is the effect of the interaction between starter physical form and week; $(AH\times W)_{ik}$ is the effect of the interaction between alfalfa hay provision and week; $(SF\times AH)_{jk}$ is the interaction between starter physical form and alfalfa hay provision; $(SF \times AH \times W)_{ijk}$ is the tripartite effect of starter form, alfalfa hay provision and week; $\beta(Xi-\bar{X})$ is the covariate variable (used only for BW with initial BW as covariate) and ε_{ijk} is the overall error term. The sex effect was not significant, and it was removed from the model. For growth variables, the initial measurements were included as a covariate in the statistical analysis of body measurements. The differences among treatment means were determined using Tukey's multiple range tests. Effects were considered to be significant when $P < 0.05$, and a tendency was considered when $0.05 \leq P \leq 0.10$. Data are reported as least squares means.

Results

Calf performance, feeding behavior and growth

The results of the experimental diets on feed intake, BW, ADG and feed efficiency are presented in Table 2. Milk DM intake was not changed among treatments (Table 2). The intake of starter feed was lower in calves fed pelleted than those fed mashed starter after weaning ($P < 0.01$) and during the entire period of the experiment ($P = 0.026$). There was no negative effect of AH inclusion in the diet on starter intake. The weaning and final BW and ADG were not affected by the starter physical form and alfalfa hay inclusion and their interaction. The starter form did not affect the feeding behavior (Table 3). Regardless of the starter form, alfalfa provision caused greater rumination time ($P < 0.01$). Heart girth, body length, and hip height did not differ among the treatments (Table 4). Alfalfa hay supplementation increased ($P < 0.01$) the body barrel after weaning and over the whole experimental period. The structural growth indicators were not affected by the physical form of the starter diet or its interaction with alfalfa hay.

Blood metabolites and enzymes

Concentrations of plasma glucose, BHBA, urea nitrogen, albumin, and total proteins, before weaning were not influenced by the diet (Table 5). However, plasma BUN concentration after weaning was higher in calves receiving AH ($P = 0.036$). Plasma ALT concentration was not affected by treatments, however, AST levels was higher in calves receiving the pelleted starter ($P = 0.039$) compared with the coarsely mashed starter. No interaction was found between the starter physical form and alfalfa hay provision on blood metabolites.

Ruminal fermentation parameters

The ruminal fluid pH before weaning was increased with AH inclusion in the starter feed during the pre-

Table 2. Least squares means of the starter intake, ADG, and feed efficiency in dairy calves fed different starter physical forms (coarsely mashed vs. pellet) and alfalfa hay level (0 vs. 150 g kg^{-1} DM) (n = 11 calves per diet)

Item	Diet[1]					P-value[2]		
	MS		PS					
	NAH	AH	NAH	AH	SEM	SF	AH	SF×AH
Milk intake, g DM day^{-1}	686.1	685.0	686.1	686.9	1.34	0.202	0.856	0.191
Starter feed intake, g day^{-1}								
Pre-weaning (d 3-59)	634	610	479	595	55.6	0.154	0.438	0.234
Post-weaning (d 60-70)	2500[ab]	2660[a]	1940[b]	2106[ab]	174.7	<0.01	0.346	0.985
Entire period (d 3-70)	902	935	678	783	74.9	0.026	0.377	0.647
Average daily gain, g day^{-1}								
Pre-weaning (d 3-59)	514	457	421	462	30.8	0.186	0.909	0.148
Post-weaning (d 60-70)	1024	1047	770	1007	95.2	0.124	0.170	0.263
Entire period (d 3-70)	585	541	466	545	34.0	0.118	0.633	0.092
Body weight								
Initial	39.5	40.1	40.1	39.6	0.68	0.98	0.96	0.41
Weaning	67.2	69.4	67.1	63.3	2.1	0.13	0.68	0.15
Final	81.9	83.1	81.8	74.2	3.3	0.15	0.31	0.17
Feed efficiency[3]								
Pre-weaning (d 3-59)	0.41	0.38	0.39	0.37	0.023	0.537	0.285	0.934
Post-weaning (d 60-70)	0.40	0.39	0.36	0.46	0.031	0.634	0.156	0.086
Entire period (d 3-70)	0.41	0.38	0.38	0.39	0.026	0.652	0.607	0.527

[1]Treatments were; 1) coarsely mashed starter without alfalfa hay provision (MS-NAH), 2) coarsely mashed starter with alfalfa hay provision (MS-AH), 3) pelleted starter without alfalfa hay provision (PS-NAH), and 4) pelleted starter with alfalfa hay provision (PS-AH).
[2]Statistical comparisons: SF = starter physical form; AH; alfalfa hay provision; SF×AH= SF by AH interaction.
[3]kg of body weight gain/kg of total dry matter intake
Within rows, means with common superscripts do not differ (P > 0.05).

Table 3. Least squares means of feeding behavior attributes (min) in dairy calves fed different starter physical form (coarsely mashed vs. pellet) and alfalfa hay level (0 vs. 150 g kg^{-1}) (n = 11 calves per diet)

Item	Diets[1]				SEM	P-value[2]		
	MS		PS					
	NAH	AH	NAH	AH		SF	AH	SF×AH
Standing	110.4	90.5	109.2	113.6	11.43	0.343	0.496	0.292
Lying	191.8	208.5	198.0	177.2	22.82	0.584	0.925	0.414
Eating	48.6	25.8	14.7	31.2	6.87	0.363	0.486	0.092
Ruminating	32.6[b]	88.3[a]	37.3[b]	74.7[a]	9.30	0.181	<0.01	0.892
Non-nutritional behaviors	68.1	67.3	87.0	82.7	14.75	0.244	0.865	0.906

[1]Diets were; 1) coarsely mashed starter without alfalfa hay provision (MS-NAH), 2) coarsely mashed starter with alfalfa hay provision (MS-AH), 3) pelleted starter without alfalfa hay provision (PS-NAH), and 4) pelleted starter with alfalfa hay provision (PS-AH).
[2]Statistical comparisons: SF = starter physical form; AH; alfalfa hay provision; SF×AH= SF by AH interaction.
Within rows, means with common superscripts do not differ (P > 0.05).

Table 4. Least squares means (cm) of skeletal growth measures in dairy calves fed different starter physical forms (coarsely mashed vs. pellet) and alfalfa hay level (0 vs. 150 g kg^{-1}) (n = 11 calves per diet)

Item	Diet[1]				SEM	P-value[2]		
	MS		PS					
	NAH	AH	NAH	AH		SF	AH	SF×AH
Heart Girth								
Initial	84.4	83.2	82.7	82.2	0.89	0.143	0.312	0.704
Weaning	101.7	100.6	100.1	100.8	1.30	0.613	0.884	0.513
Final	106.7	105.6	106.1	106.4	1.50	0.926	0.782	0.626
Body Barrel								
Initial	83.9	82.8	83.7	83.1	0.90	0.967	0.373	0.774
Weaning	109.2[b]	114.5[a]	110.0[b]	114.0[a]	1.80	0.915	0.015	0.717
Final	118.7[ab]	123.7[a]	117.4[b]	124.9[a]	2.10	0.977	<0.01	0.538
Body Length								
Initial	46.7	46.8	47.8	45.0	0.94	0.708	0.156	0.121
Weaning	56.4	56.0	55.6	56.1	1.10	0.773	0.987	0.711
Final	60.7	59.0	58.9	59.7	1.10	0.635	0.706	0.283
Hip Height								
Initial	81.4	80.8	79.9	81.1	0.63	0.327	0.616	0.106
Weaning	90.1	90.2	89.6	89.7	0.61	0.382	0.835	0.967
Final	93.0	91.8	92.2	92.1	0.70	0.723	0.384	0.448
Hip Width								
Initial	19.6	19.3	19.0	19.6	0.22	0.384	0.376	0.119
Weaning	23.7	22.8	23.5	23.4	0.41	0.596	0.198	0.347
Final	25.2	24.5	25.4	24.7	0.40	0.687	0.120	0.945
Wither Height								
Initial	78.5	77.9	77.2	78.9	0.63	0.679	0.339	0.125
Weaning	87.9	87.3	87.3	86.4	0.52	0.137	0.135	0.764
Final	90.6	89.4	89.5	89.2	0.62	0.282	0.226	0.514

[1]Diets were; 1) coarsely mashed starter without alfalfa hay provision (MS-NAH), 2) coarsely mashed starter with alfalfa hay provision (MS-AH), 3) pelleted starter without alfalfa hay provision (PS-NAH), and 4) pelleted starter with alfalfa hay provision (PS-AH).
[2]Statistical comparisons: SF = starter physical form; AH; alfalfa hay provision; SF×AH= SF by AH interaction.
Within rows, means with common superscripts do not differ (P > 0.05).

weaning period (Table 6, P < 0.01), however, after weaning pH in calves feeding on the pelleted starter in comparison with coarsely mashed form (P = 0.018). Provision of AH in the diet decreased the runinal SCFA concentration before weaning (P < *0.01*). The pelleted starter increased the concentration of total SCFA in the ruminal fluid both before and after weaning (P = *0.012*). Before weaning, the proportion of acetate in total SCFA increased by AH inclusion in the starter feed and decreased by the pelleted starter (P < *0.01*). On d 35 of the study, the propionate was greater in calves fed coarsely mashed starter compared with the pelleted form (P = *0.044*). Inclusion if AH in the starter feed was accompanied by reduced ruminal butyrate proportion (P = *0.014*)

Table 5. Least squares means for blood metabolites and liver enzymes in dairy calves fed different starter physical forms (coarsely mashed vs. pellet) and alfalfa hay level (0 vs. 150 g kg^{-1}) (n = 11 calves per diet)

Item	Diets[1]					P-value[2]		
	MS		PS					
	NAH	AH	NAH	AH	SEM	SF	AH	SF×AH
Glucose, mg dL^{-1}								
Pre-weaning	84.5	83.6	85.5	92.0	5.53	0.391	0.632	0.532
Post-weaning	98.6	94.8	85.8	91.4	5.80	0.173	0.873	0.421
BHBA, mmol L^{-1}								
Pre-weaning	0.16	0.20	0.17	0.22	0.034	0.665	0.204	0.915
Post-weaning	0.27	0.32	0.31	0.32	0.043	0.606	0.506	0.606
BUN, mg dL^{-1}								
Pre-weaning	21.5	23.5	24.1	20.8	2.05	0.895	0.747	0.207
Post-weaning	19.8	23.8	21.6	23.2	1.29	0.622	0.036	0.358
Albumin, mg dL^{-1}								
Pre-weaning	3.0	3.0	3.1	3.1	0.12	0.753	0.843	0.842
Post-weaning	3.0	3.0	2.8	2.8	0.13	0.121	0.824	0.691
Total protein, mg dL^{-1}								
Pre-weaning	5.6	5.7	5.9	6.1	0.18	0.114	0.303	0.861
Post-weaning	6.0	5.9	5.6	6.0	0.22	0.598	0.601	0.424
ALT, IU L^{-1}								
Pre-weaning	9.8	10.1	10.1	12.6	1.75	0.439	0.549	0.437
Post-weaning	21.1	19.1	20.3	17.8	2.77	0.690	0.423	0.926
AST, IU L^{-1}								
Pre-weaning	41.2^{ab}	36.5^{b}	54.8^{a}	46.0^{ab}	4.98	0.039	0.194	0.683
Post-weaning	60.6	68.3	62.0	62.5	5.48	0.682	0.465	0.524

[1]Diets were; 1) coarsely mashed starter without alfalfa hay provision (MS-NAH), 2) coarsely mashed starter with alfalfa hay provision (MS-AH), 3) pelleted starter without alfalfa hay provision (PS-NAH), and 4) pelleted starter with alfalfa hay provision (PS-AH).
[2]Statistical comparisons: SF = starter physical form; AH; alfalfa hay provision; SF×AH= SF by AH interaction.
Within rows, means with common superscripts do not differ (P > 0.05).

before weaning when butyrate concentration was higher in calves feeding on the pelted starter (*P < 0.01*). Before weaning, the acetate: propionate ratio was increased by forage provision (*P = 0.043*). The proportion of valerate in the ruminal fluid was positively influence by feeding the pelleted starter (*P < 0.01*) and negatively with AH inclusion in the diet before weaning (*P 0.029*). Except for valerate production in after weaning (*P = 0.036*), no interaction was observed between AH provision and the starter physical on the ruminal fermentation pattern.

Discussion

In the current study, pelleted starter reduced feed intake during the post-weaning period. Bach et al. (2007) also stated that pelleted starter reduced intake in dairy calves in comparison to the multi-particle starter. In agreement with our results, Porter et al. (2007) showed that calves on coarse mashed diets consumed more starter than those on the pelleted starter. Increased the SCFA production in the rumen (Table 6) associated with decreased ruminal and blood pH negatively affect solid feed intake (Owens et al., 1998). Owens et al. (1998) also stated that accumulation of ruminal acids could increase ruminal fluid olality that stimulates the osmoreceptors leading to decreased feed intake. Although AH inclusion by itself did not influence feed intake in the current study but its inclusion in the starter could alleviate the negative effects of PS on intake in milk-fed calves. Regardless of the starter physical forms, researchers reported increased intake (Castells et al., 2013; Mirzaei et al., 2016) or decreased intake of the starter feed (Hill et al., 2010) in the presence of a forage source. Castells et al. (2013) stated that the performance response of pre-weaned calves to forage provision in the starter feed is related to the proportion of forage, and the acid detergent fiber content of the basal diet. The observed tendency for the interaction between the physical form of the starter and forage inclusion in the present study suggested that forage inclusion might improve the daily gain and feed efficiency in PS fed calves. The ADG in PS-NAH fed calves was 466 g d^{-1} and it was increased up to 545 g d^{-1} in the presence of alfalfa (i.e. PS-AH), accompanied by 27% improvement in the overall feed efficiency (0.36 vs. 0.46 for PS-NAH and PS-AH, respectively).

In agreement with previous reports, calves fed forage spent more time ruminating and devoted less time to

Table 6. Least squares means for rumen fermentation activities in dairy calves fed different starter physical form (coarsely mashed vs. pellet) and alfalfa hay level (0 vs. 150 g kg^{-1}) (n = 11 calves per diet)

Item	Diets[1]				SEM	P-value[2]		
	MS		PS					
	NAH	AH	NAH	AH		SF	AH	SF×AH
Rumen pH								
Pre-weaning	5.61^{ab}	6.10^{a}	5.40^{b}	5.95^{ab}	0.149	0.242	<0.01	0.822
Post-weaning	6.09^{ab}	6.36^{a}	5.52^{b}	5.87^{b}	0.180	0.018	0.123	0.841
Total SCFA, mmol L^{-1}								
Pre-weaning	79.3^{b}	67.6^{c}	106.4^{a}	76.5^{bc}	5.59	0.015	<0.01	0.140
Post-weaning	76.4^{ab}	70.9^{b}	90.2^{a}	89.0^{a}	5.45	0.012	0.548	0.709
Individual SCFA, mol/100 mol								
Acetate								
Pre-weaning	41.7^{bc}	48.2^{a}	36.7^{c}	42.2^{b}	1.14	<0.01	<0.01	0.693
Post-weaning	41.5	43.5	41.5	42.6	2.07	0.829	0.472	0.844
Propionate								
Pre-weaning	37.9^{a}	37.6^{a}	31.6^{b}	34.3^{b}	2.07	0.044	0.589	0.501
Post-weaning	39.3	37.0	36.6	35.7	1.58	0.243	0.33	0.673
Butyrate								
Pre-weaning	14.5^{b}	9.5^{c}	23.5^{a}	15.8^{b}	2.09	<0.01	0.014	0.546
Post-weaning	13.2	13.9	17.0	16.5	2.53	0.230	0.9813	0.817
Iso-valerate								
Pre-weaning	0.59	1.14	0.86	1.60	0.432	0.441	0.183	0.837
Post-weaning	1.11	2.03	0.95	0.97	0.301	0.064	0.149	0.162
Valerate								
Pre-weaning	5.17b^{c}	3.40^{c}	7.10^{a}	5.90^{b}	0.513	<0.01	0.029	0.631
Post-weaning	4.80^{a}	3.50^{b}	3.80^{b}	4.10^{ab}	0.339	0.602	0.210	0.036
Acetate/propionate (C2:C3)								
Pre-weaning	1.09^{b}	1.28^{a}	1.16^{b}	1.23^{ab}	0.032	0.417	0.043	0.294
Post-weaning	1.05	1.17	1.13	1.18	0.043	0.526	0.563	0.472

[1]Diets were; 1) coarsely mashed starter without alfalfa hay provision (MS-NAH), 2) coarsely mashed starter with alfalfa hay provision (MS-AH), 3) pelleted starter without alfalfa hay provision (PS-NAH), and 4) pelleted starter with alfalfa hay provision (PS-AH).
[2]Statistical comparisons: SF = starter physical form; AH; alfalfa hay provision; SF×AH= SF by AH interaction.
Within rows, means with common superscripts do not differ (P > 0.05).

non-nutritional behaviors compared to calves receiving no forage (Castells et al., 2013). Previous reports (Redbo and Nordbald, 1997) suggested that low chewing and rumination times were indicative of welfare which could have been improved by alfalfa inclusion in the starter feed in the present experiment. Contrary to previous reports of greater ruminating time recorded for mashed starter compared with the pelted form (Porter et al., 2007) we did not observed any difference between the two physical forms of the starter, although increased rumination was observed with AH inclusion in both forms of the starter feeds.

Structural growth characteristics were not different between calves fed MS and PS diets. Expect for the barrel capacity, forage provision did not affect the other structural growth attributes. The interaction of starter form and forage inclusion was also statistically similar between experimental treatments for all measured growth indicators. This similarity is in part because of similar energy and protein consents among experimental mental treatments that caused similar growth rate in dairy calves. Forage inclusion in the starter feed increased the size of barrel. Mirzaei et al. (2015) reported a greater body barrel for calves fed corn silage in comparison with non-forage fed calves. They stated that gut fill could be a confounding factor in determining forage effects on body measurement of dairy calves as well. However body barrel did not differ when diets contained no forage (Kazemi-Bonchenari et al., 2016).

Plasma glucose, BHBA, and albumin as well as total protein concentrations were not affected by the starter physical form, forage inclusion or their interaction. Feeding pelleted starter caused a decrease in proportional ratio of propionate in the ruminal fluid (Table 6) and in plasma glucose concentration which was anticipated because propionate is the main glucose precursor in the liver (Bergman, 1990). However, similar glucose concentration was found for all treatments. Serum glucose concentration did not increase when calves were supplemented with calcium propionate (Ferreira and

Bittar, 2011). It seems that the greater glucose concentration in response to greater ruminal propionate concentration would be expected in mature animals with the developed rumen and not in dairy calves. BUN could be used as an indicator of rumen N capture and has high correlation with rumen ammonia concentration (Phuong et al., 2014). In addition, greater BUN concentration in the rumen could present the lower nitrogen efficiency in dairy calves (Kazemi-Bonchenari et al., 2018). Phuong et al. (2014) concluded lower nitrogen efficiency in higher fiber content diets in dairy cows. Consistent with Phuong et al. (2014), we found greater BUN concentration in AH fed calves after weaning indicating lower nitrogen efficiency with increased dietary fiber content.

Plasma AST but not ALT concentration was increased in PS fed calves in the pre-weaning period. Liver function is influenced by different factors such as reduced appetite, high fat deposition or when feeding high grain diets through reducing rumen pH (Nagaraja and Lechtenberg, 2007). The results showed that probably the liver function was influenced and caused an increase in AST concentration (Table 6) in these animals which might be due to the reduction in rumen pH. However, albumin and total protein concentrations were within normal ranges reported previously in diary calves (Kazemi-Bonchenari et al., 2016).

Ruminal fluid pH was lower in PS diet fed calves in comparison with MS fed calves. Feeding the pelleted starter increased total SCFA production in the ruminal fluid of calves before and after weaning (Table 6). The lower ruminal fluid pH found in PS form fed calves may be attributed to the greater production of SCFA in the ruminal fluid. Owens et al. (1998) stated that when the rate of acid production exceeds the rate of acid absorption, SCFA accumulates to higher concentrations. This status leads to chronic acidosis (Britton and Stock, 1987). Bramley et al. (2008). In the current study, the greater SCFA concentration in the ruminal fluid of PS fed compared with MS fed calves, as well as lower ruminal pH in these animals (Table 6), indicating that pelleted starter form has probably faster degradation compared to coarsely mashed starter. Offering forage in both MS and PS diets caused to reduce SCFA concentration in the rumen of pre-weaned calves. This was more relevant in PS fed calves. Two controversial mechanisms may contribute to the reduction of SCFA production in calves fed on the starter feed containing forages. First, lower digestibility of forage which could hinder the rumen development (Coverdale et al., 2004; Castells et al., 2013), and second, stimulatation of SCFA absorption by the forage particles via preventing the keratinization of rumen papilla that would cause greater absorption capacity (Beiranvand et al., 2014). Calves provided with forage had higher ruminal pH compared with control groups. Castells et al. (2013) reported increased ruminal pH in young calves when forage (chopped alfalfa hay or chopped oat hay) was included in their diets during the pre-weaning period. Laarman and Oba (2011) ascribed the forage benefits to rumen fermentation which subsequently affect ruminal pH. The results of the present study corroborates those of Suarez-Mena et al. (2016) who reported that the grain particle size and processing affected the fermentation rate of starch in the rumen. The greater time spent for rumination in forage fed calves could also be an important factor for ruminal fluid pH improvement via increased saliva secretion.

Mash form of the starter as well as the forage included diets positively influenced acetate concentration in the ruminal fluid before weaning. Results reported herein for ruminal acetate proportions are in accordance with Terré et al. (2015) who reported higher acetate proportions on forage inclusion (alfalfa hay or chopped oat hay) in the diet. Mirzaei et al. (2016) found that mashed starter feed produced more acetate in comparison with texturized starter in dairy calves. Our results showed that feeding coarsely mashed starter increased the molar proportion of propionate and decreased molar proportion of butyrate in the ruminal fluid before weaning. However, forage inclusion increased the molar proportion of ruminal acetate but decreased butyrate proportions in the ruminal fluid. As it has been shown previously, forage may displace concentrate intake and shift rumen fermentation in favor of acetate rather than butyrate production and, thus, delay rumen papillary development (Zitnan et al., 1998). In addition to the physical form of the starter, the discrepancy between studies might be attributed to the source and level of supplemented forage and hence their interaction with the physical form of the starter.

The ratio of acetate to propionate was not influenced by the physical form of the starter in the current study. However, increased molar proportion of ruminal acetate in AH included diets, caused to increase their ratio as well. The greater acetate: propionate ratio observed in forage provided calves could be attributed, in part, to the greater ruminal pH through improvement in buffering capacity. The reduction in ruminal pH impairs the growth of cellulolytic bacteria, resulting in the reduction of acetate: propionate ratio (Grant et al., 1990). Increased valerate proportion in pellet-fed animals and decreased SCFA in the ruminal fluid of forage-fed calves showed that valerate could be increased at lower ruminal pH. In fact, lower ruminal pH which was observed in calves on the pelleted starter resulted in greater valerate molar proportion. Bramley et al. (2008) also showed that molar proportion of valerate was greater in acidotic dairy cows.

Conclusion

Pelleted starter feed compared with coarsely-mash form reduced the starter feed intake. Providing alfalfa hay into starter feed did not affect the calf performance but alleviated the negative effects of the pelleted form but not mashed starter on the ruminal fermentation. Therefore, forage supplementation may be beneficial for dairy calves only when they are fed with pelleted starters.

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برهمکنش بین شکل فیزیکی استارتر و استفاده از علوفه یونجه بر عملکرد رشد، تخمیر شکمبه ای، و متابولیت‌های خون در گوساله های شیرخوار

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چکیده این پژوهش برای ارزیابی تاثیر شکل فیزیکی استارتر و علوفه خشک یونجه بر عملکرد رشد، تخمیر شکمبه ای و متابولیت‌های خون گوساله‌های شیرخوار انجام شد. ۴۴ گوساله شیرخوار سه روزه با میانگین وزن ۳۹ کیلوگرم و ۱/۱ کیلوگرم انحراف استاندارد در یک طرح فاکتوریل ۲ در ۲ با دو سطح علوفه (صفر و ۱۵۰ گرم در کیلوگرم خوراک)، و دو شکل فیزیکی استارتر (آسیاب زبر در برابر پلت) انجام شد. چهار تیمار آزمایشی شامل تیمارهای زیر بود؛ تیمار اول) استارتر زبر بدون علوفه یونجه (MS-NAH)، تیمار دوم) استارتر زبر دارای ۱۵۰ گرم در کیلوگرم یونجه (MS-AH)، تیمار سوم) استارتر پلت بدون یونجه (PS-NAH)، و تیمار چهارم استارتر پلت دارای ۱۵۰ گرم در کیلوگرم علوفه یونجه (PS-AH). گوساله ها در روز ۶۰ از شیرگیری شدند اما آزمایش تا روز ۷۰ طول کشید. تغذیه استارتر به صورت پلت سبب کاهش مصرف استارتر نسبت به استارتر زبر در دوره بعد از شیرگیری (P< ۰/۰۱) و در کل دوره آزمایشی (۰/۰۲۶ =P) شد. برهمکنش بین یونجه و شکل فیزیکی استارتر سبب تمایل به معنی داری در مورد افزایش وزن روزانه (۰/۰۹۲ =P) و بازدهی خوراک (P= ۰/۰۸۶) شد. حضور علوفه یونجه در جیره استارتر سبب افزایش مدت زمان نشخوار کردن و همچنین افزایش اندازه حجم شکم شد. نیتروژن اوره‌ای خون در گوساله هایی که علوفه یونجه استفاده کرده بودند در طول دوره بعد از شیرگیری افزایش یافت. غلظت آنزیم آسپارتات آمینوترانسفراز در خون کوساله‌هایی که استارتر پلت استفاده کرده بودند نسبت به آنهایی که استارتر زبر استفاده کرده بودند افزایش داشت. با وجودی که حضور علوفه یونجه از افت pH مایع شکمبه در طول دوره پیش از شیرگیری گوساله هایی که استارتر پلت دریافت کرده بودند پیشگیری کرد؛ اما pH مایع شکمبه در گوساله هایی که جیره پلت استفاده کرده بودند نسبت به گوساله هایی که جیره استارتر زبر استفاده کرده بودند در طول دوره بعد از شیرگیری کاهش نشان داد. فرم پلت استارتر سبب افزایش اسیدهای چرب کوتاه زنجیر در شکمبه به همراه افزایش اسید بوتیریک شد. اما استارتر زبر و علوفه یونجه سبب افزایش استات در مایع شکمبه شدند. به طور کلی، نتایج این پژوهش نشان داد که استارتر زبر می تواند برای گوساله‌های شیرخوار نسبت به استارتر پلت قابل توصیه باشد. فزون بر این، حضور علوفه یونجه در جیره استارتر می تواند اثر منفی فرم پلت را کاهش دهد.