Growth performance, feeding behavior, and selected blood metabolites of Holstein dairy calves fed restricted amounts of milk: No interactions between sources of finely ground grain and forage provision

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ABSTRACT

The objective of this study was to investigate the effects of grain sources and forage provision on growth performance, blood metabolites, and feeding behaviors of dairy calves. Sixty 3-d-old Holstein dairy calves (42.2 ± 2.5 kg of body weight) were used in a 2×3 factorial arrangement with the factors being grain sources (barley and corn) and forage provision (no forage, alfalfa hay, and corn silage). Individually housed calves were randomly assigned ($n = 10$ calves per treatment: 5 males and 5 females) to 6 treatments: (1) barley grain (BG) without forage supplement, (2) BG with alfalfa hay (AH) supplementation, (3) BG with corn silage (CS) supplementation, (4) corn grain (CG) without forage supplement, (5) CG with AH supplementation, and (6) CG with CS supplementation. All calves had ad libitum access to water and starter feed throughout the experiment. All calves were weaned on d 49 and remained in the study until d 63. Starter feed intake and average daily gain (ADG) was greater for calves fed barley than those fed corn during the preweaning and overall periods. Calves supplemented with CS had greater final body weight and postweaning as well as overall starter feed intake than AH and non-forage-supplemented calves. During the preweaning and overall periods, feeding of CS was found to increase ADG compared with feeding AH and nonforage diets. However, feed efficiency was not affected by dietary treatments. Calves supplemented with CS spent more time ruminating compared with AH and control groups; nonnutritive oral behaviors were the greatest in non-forage-supplemented calves. Regardless of the grain sources, the rumen pH value was greater for AH calves compared with CS and

non-forage-supplemented calves. Blood concentration of BHB was greater for CS-supplemented calves compared with AH and non-forage-supplemented calves. Furthermore, body length and heart girth were greater for calves fed barley compared with those fed corn, and also in forage-supplemented calves compared with non-forage-supplemented calves. These results showed no interactions between grain sources and forage provision on calf performance; however, the inclusion of CS and barley in starter diets could enhance the growth performance of Holstein calves during the transition from liquid to solid feed.

Key words: forage, grain, dairy calf

INTRODUCTION

Physical and metabolic development of the reticulorumen is important both for easing the transition from preruminant to mature ruminant state and improving calf health (Drackley, 2008; Khan et al., 2011). The preweaning growth of calves is a complex process involving interactions among nutritional components and physiological signals that highly depends on dietary strategies and feeding management (Baldwin et al., 2004). For example, variations in fermentable carbohydrate (Hill et al., 2008; Khan et al., 2008) and forage sources (Castells et al., 2012) of starter feeds can affect the performance of dairy calves. Cereal grains are the primary source of starch in ruminant diets, and corn and barley are commonly used worldwide as starch sources in calf feeds. Although feeding concentrate diets in calves in early life stimulates rumen microbial proliferation and VFA production, and initiates rumen development, forage, or high-fiber feedstuffs, increases rumen muscularization (Beiranvand et al., 2014), volume, and motility (Žitnan et al., 1998).

Sources of fermentable carbohydrates such as grains may differ in their rate of starch ruminal fermentation and therefore in their effects on ruminal pH and short-chain fatty acids (Kiran and Mutsvangwa, 2007), which

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could affect starter feed intake, growth performance, and rumen development in dairy calves. Corn grain is more slowly fermented than barley and slower rates of digestion increase the amount of starch bypassing the rumen (Khan et al., 2008). Differences in starch shape, granule size, and interactions between amylose and surface compounds can alter the rate of enzymatic digestion of corn and barley starches (Kotarski et al., 1992). Previous studies have reported that starter feeds high in ruminal degradable starch (e.g., barley vs. corn) decrease nutrient intake and rumen development (Khan et al., 2008); however, it is not still clear how different starch sources affect ruminal fermentation and contribute to meeting the energy requirements of developing calves (Khan et al., 2016).

Provision of forage to young calves has been recommended because it has been shown to increase starter feed intake, ADG, as well as improve rumen fermentation (Daneshvar et al., 2015; Mirzaei et al., 2016), stimulate the muscular layer of the rumen (Suárez et al., 2007; Beiranvand et al., 2014; Mirzaei et al., 2015), promote rumination (EbnAli et al., 2016; Hosseini et al., 2016), and reduce behavioral problems (Phillips, 2004). In previous studies, the positive effect of alfalfa hay (AH) on stimulating starter feed intake observed when AH was supplemented to the finely ground starter feed (Beiranvand et al., 2014; Daneshvar et al., 2015) to young calves; however, this was not the same in the textured starter feed (Porter et al., 2007; Hill et al., 2008, 2010). Castells et al. (2012) reported that free-choice provision of forage sources (with the exception of AH) to young calves improved both starter feed intake and total DMI compared with nonforage supplementation.

Limited information is available on how changes in the dietary content of ruminal fermentable carbohydrate and source of supplemental dietary fiber might influence the performance of dairy calves during the pre- and postweaning periods. In this study, we tested the hypothesis that providing a diet supplemented with fiber can alleviate the negative effects associated with rapidly degradable starch such as barley grain, which may modify rumen conditions and improve calf performance. Further, we assume that increasing ruminal fermentation of starch can improve dairy calves' performance if acidosis is avoided with a proper source of forage in the diet.

MATERIALS AND METHODS

Animals, Management, and Treatments

The experiment was conducted between March 17 and June 22, 2014, on a local dairy farm (Ghiam Dairy

Co.), Isfahan, Iran. All the calves were cared and managed according to the guidelines of Iranian Council of Animal Care (1995). Sixty 3-d-old Holstein dairy calves (42.2 ± 2.5 kg of BW) were randomly assigned ($n = 10$ calves per treatment: 5 males and 5 females) in a 2×3 factorial arrangement with the factors of dietary grain sources (barley vs. corn) and forage provision (without forage, alfalfa hay, or corn silage). 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 3.0 L of colostrum at each of the first 2 feedings (i.e., within 1.5 h of life and at 12 h after the first feeding). Colostrum feeding was carried out for the first 2 d of life.

Calves received 4 L/d of milk containing $3.14 \pm 0.13\%$ fat, $3.02 \pm 0.08\%$ CP, $4.73 \pm 0.06\%$ lactose, and $11.45 \pm 0.17\%$ TS in steel buckets twice daily at 0900 and 1800 h from d 3 to 46 of the study followed by feeding 2 L/d milks until d 49 of the study. Calves were assigned to 1 of 6 dietary treatments: (1) barley grain (BG) without forage supplement, (2) BG with AH supplementation, (3) BG with corn silage (CS) supplementation, (4) corn grain (CG) without forage supplement, (5) CG with AH supplementation, and (6) CG with CS supplementation. All calves were weaned on d 49 of study and the study was terminated on d 63. From d 3 to 63 of the study, all the calves had free access to fresh water and the starter ration formulated according to the current NRC (2001). All diets were formulated to be isonitrogenous. The ingredient and nutrient composition of the starter feed are given in Table 1.

Starter feeds were fed ad libitum to permit at least 10%orts (i.e., the portion of the starter not consumed over a 24-h period), and feed refusal from each individual calf was collected at 0800 h. The forage-supplemented calves received a starter feed containing 15% chopped AH (particle size distribution: $1.0 \pm 0.2\%$ greater than 18 mm, $26.0 \pm 1.9\%$ between 8 and 18 mm, $35.1 \pm 0.9\%$ between 1.18 and 8 mm, and $37.8 \pm 2.3\%$ less than 1.18 mm and geometric mean particle 2.9 ± 0.1 mm) or CS (particle size distribution: $21.3 \pm 1.5\%$ greater than 18 mm, $62.6 \pm 2.0\%$ between 8 and 18 mm, $14.6 \pm 1.1\%$ between 1.18 and 8 mm, and $1.3 \pm 0.58\%$ less than 1.18 mm and geometric mean particle size 12.07 ± 1.98 mm) as a TMR mixed with concentrates throughout the study. Corn and barley grain were ground using a hammer mill with 3 mm screen size (model 5543 GEN, Isfahan Dasht, Isfahan, Iran). All the calves were healthy with no clinical symptoms of systemic disease or mortality other than diarrhea and few pneumonia occurrences throughout the experiment.

Sampling

Intakes of calf starter (starter feed or TMR) were measured daily and individual BW was recorded using an electronic balance every week throughout the experiment. Amounts of feed offered and refused were recorded daily for each individual calf. Preweaning, postweaning, and overall means of ADG and feed efficiency (kg of BW gain/kg of total DMI) were also calculated. Feed samples were collected every 7 d throughout the study and stored at -20°C until chemical analysis. To calculate feed intake, the offered and refused amounts of TMR were recorded daily on an individual basis.

Blood samples were collected 3 h after the morning meal on d 35 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. Tubes were centrifuged at $2,000 \times g$ for 20 min at 4°C to separate the plasma, which was then stored at -20°C for subsequent analysis. Rumen fluid was sampled on d 35 by a stomach tube fitted to a vacuum pump at 3 to 4 h postfeeding, and rumen pH was measured im-

mediately (HI 8318, Hanna Instruments, Cluj-Napoca, Romania).

Body measurements at the end of the study (d 63) including body length (distance between the points of shoulder and rump), heart girth (circumference of the chest), withers height (distance from base of the front feet to the withers), body barrel (circumference of the belly before feeding), hip width (distance between the points of hook bones), and hip height (distance from base of the rear feet to hook bones) of the calves were performed according to the method described by Khan et al. (2007).

Behavioral data (standing, lying, eating, drinking, and nonnutritive oral behavior) were monitored by direct observations of all the calves over the total time (min) devoted to each monitored behavior once per wk for 2 wk before weaning (d 35 and 42 of the trial) as well as 2 wk after weaning (d 56 and 63 of the trial). Calves were observed 1 h after the solid feed was offered (at 0800 h) and 2 h immediately after the morning milk feeding (at 0900 h) during the preweaning week. During the postweaning week, calves were observed 1 h before and 2 h after the solid feed was offered at 0800 h. Thus,

Table 1. Ingredient and chemical composition of experimental diets (NF = with no forage; AH = 15% of DM alfalfa hay; CS = 15% of DM corn silage)

Item	Treatment					
	Barley			Corn		
	NF	AH	CS	NF	AH	CS
Ingredient (% of DM basis)						
Alfalfa hay	—	15.0	—	—	15.0	—
Corn silage	—	—	15.0	—	—	15.0
Corn grain	—	—	—	66.7	53.5	50.2
Barley grain	69.2	56.0	52.4	—	—	—
Soybean meal (45% CP)	20.5	18.9	22.0	27.7	23.6	28.9
Extruded soybean	6.0	6.0	6.0	2.0	4.5	2.0
Energizer RP-10 ¹	1.2	1.5	1.5	0.5	0.8	0.8
Calcium carbonate	1.5	1.0	1.5	1.5	1.0	1.5
Dicalcium phosphate	0.3	0.3	0.3	0.3	0.3	0.3
Vitamin and mineral premix ²	1.0	1.0	1.0	1.0	1.0	1.0
Salt	0.3	0.3	0.3	0.3	0.3	0.3
Chemical composition (% of DM, unless otherwise indicated)						
NE _G ³ (Mcal/kg)	1.43	1.33	1.37	1.43	1.33	1.36
NDF	18.8	23.4	23.6	10.9	17.2	17.6
NFC ⁴	52.3	47.1	46.8	60.4	53.3	52.9
CP	20.7	20.7	20.6	20.4	20.5	20.6
Ether extract	4.2	4.4	4.5	4.1	4.5	4.1
Ca ³	0.8	0.8	0.8	0.8	0.8	0.8
P ³	0.5	0.5	0.5	0.5	0.5	0.5

¹Prilled saturated free fatty acids (99% fatty acid weight basis); Cargill Animal Nutrition proprietary blend (Elk River, MN). Contained 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.

²Prilled saturated free fatty acids.

³Calculated from NRC (2001).

⁴Nonfiber carbohydrate was calculated as DM – (NDF + CP + ether extract + ash) (NRC, 2001).

the total time for each calf's behavior was equal to 12 h (6 h before and 6 h after weaning).

Laboratory Analyses

Subsamples of feeds and refusals were mixed thoroughly and were dried at 55°C for 48 h and then ground to pass a 1-mm screen in a Wiley mill (Ogaw Seiki Co. Ltd., Tokyo, Japan) before chemical analyses for DM content (method 934.01; AOAC, 1990), CP (method 988.05; AOAC, 1990), lipids (AOAC, 1990; method 920.39), and NDF contents using a heat stable α -amylase (100 μ L/0.5 g of the sample) and sodium sulfite (Van Soest et al., 1991). Milk samples were analyzed for fat, CP, lactose, and TS content by Milkoscan (Foss Electric, Hillerød, Denmark; AOAC International). Blood metabolite concentrations were spectrophotometrically (UNICCO, 2100, Zistchemi Co., Tehran, Iran) determined using commercially available kits [Pars Azmoon Company, catalog numbers: glucose (1–500–017), urea nitrogen (BUN; 1–400–029), Tehran, Iran] according to the manufacturer's instructions. Plasma BHB concentrations were analyzed using a commercial assay kit (BHBA kit 310-A UV, Sigma Chemical, St. Louis, MO).

Statistical Analysis

Starter intake, ADG, and feed efficiency values are analyzed for 3 discrete periods as preweaning (wk 1 to 7), postweaning (wk 7 and 9), and overall (wk 1 to 9) periods. Data of starter feed intake, ADG, and feed efficiency were analyzed as repeated measures with week as a repeated variable using PROC MIXED in SAS (version 9.2, SAS Institute, Cary, NC). The following model was adopted:

$$Y_{ijkl} = \mu + G_i + F_j + W_k + (G \times F)_{ij} + (G \times W)_{ik} + (F \times W)_{jk} + (G \times F \times W)_{ijk} + \beta(X_i - \bar{X}) + \epsilon_{ijkl},$$

where Y is the dependent variable; μ is the overall mean; G_i is the effect of grain source i ; i is barley and corn; F_j is the effect of forage source j ; j is no forage supplementation, alfalfa hay, and corn silage; W_k is the effect of week k ; k is 9 wk; $(G \times F)_{ij}$ is the effect of the interaction between grain and forage sources; $(G \times W)_{ik}$ is the effect of the interaction between grain sources and week; $(F \times W)_{jk}$ is the effect of interaction between forage source and week; $(G \times F \times W)_{ijk}$ is the tripartite effect of grain sources, forage provision, and week; $\beta(X_i - \bar{X})$ is the covariate variable; and ϵ_{ijkl} is the

error term. The model contained calf within treatment as a random effect and a covariance structure was chosen based on best Akaike information criterion and Bayesian information criteria indexes. Autoregressive (1) was found to be the most appropriate for all variables. Initial BW was used as a covariate for weaning weight and final weight. Blood parameters, behaviors, and rumen pH data were analyzed using the above model without the effect of time. Skeletal growth for d 63 (at the end of trial) were analyzed separately with a model without the effect of time; however, the initial values were included in the model as a covariate. The means were compared using least squares means adjusted for Tukey, and the significant differences and tendencies were stated at $P \leq 0.05$ and $0.05 < P < 0.10$, respectively.

RESULTS

Calf Performance

Starter feed intake, ADG, and feed efficiency data are reported in Table 2. No interaction was detected between the grain sources and forage provision with respect to starter feed intake, ADG, and feed efficiency during the preweaning, postweaning, and overall periods. The preweaning (399 vs. 320 g/d; $P = 0.05$) and overall starter feed intake (700 vs. 607 g/d; $P = 0.08$) tended to be greater for calves fed barley compared with those fed corn, but intake of starter feed was not changed among treatments during the postweaning period. The overall ADG (535 vs. 479 g/d) were greater ($P < 0.01$) and preweaning ADG tended ($P = 0.08$) to be greater for calves fed barley compared with those fed corn; however, ADG were not changed among treatments during the postweaning period. The postweaning (1,970 vs. 1,609 and 1,484 g/d, respectively; $P < 0.01$) and overall intake of starter feed (725 vs. 594 and 501 g/d, respectively; $P < 0.05$) were greater for CS-supplemented calves compared with AH-supplemented calves and non-forage-supplemented group; however, they were not changed among treatments during the preweaning period. The preweaning (467 vs. 396 g/d; $P < 0.05$), postweaning (922 vs. 672 g/d; $P < 0.05$), and overall ADG (569 vs. 457 g/d; $P < 0.01$) were greater for CS-supplemented calves compared with non-forage-supplemented calves. Moreover, CS-supplemented calves had greater ($P < 0.05$) ADG compared with AH-supplemented calves during the preweaning and overall periods; however, forage source did not affect ADG during the postweaning period. No difference was observed in feed efficiency among treatments.

Table 2. Effects of grain source (barley versus corn) and forage provision (no forage, alfalfa hay, and corn silage) on starter intake, ADG, and feed efficiency in dairy calves (n = 10 calves per treatment)

Item	Treatment ¹						SEM	P-value ²		
	Barley			Corn				G	F	G × F
	NF	AH	CS	NF	AH	CS				
Starter intake, g/d										
Preweaning (d 3 to 49)	465	301	432	266	311	383	50	0.05	0.13	0.11
Postweaning (d 50 to 63)	1,603	1,613	2,031	1,366	1,604	1,910	128	0.26	<0.01	0.69
Overall (d 3 to 63)	718	594	787	501	597	725	64	0.08	0.02	0.22
ADG, g/d										
Preweaning (d 3 to 49)	430	403	498	362	407	436	28	0.08	0.03	0.38
Postweaning (d 50 to 63)	720	842	999	624	777	846	86	0.15	0.02	0.88
Overall (d 3 to 63)	494	501	610	419	489	527	29	0.02	<0.01	0.44
Feed efficiency ³										
Preweaning (d 3 to 49)	0.44	0.52	0.50	0.46	0.46	0.45	0.03	0.31	0.49	0.41
Postweaning (d 50 to 63)	0.51	0.53	0.46	0.44	0.51	0.45	0.05	0.43	0.43	0.91
Overall (d 3 to 63)	0.50	0.52	0.44	0.45	0.47	0.46	0.03	0.21	0.27	0.41

¹NF = without forage; AH = 15% DM alfalfa hay; CS = 15% DM corn silage.

²Statistical comparisons: G = grain source; F = forage provision; G × F = grain source by forage provision interaction.

³Feed efficiency was calculated from dividing ADG (g) by average daily DMI.

Structural Growth and Animal Behavior

The data of BW and body measurements are presented in Table 3. No interaction was detected between the grain sources and forage provision with respect to BW, body length, heart girth, body barrel, withers height, hip height, and hip width. The BW at weaning tended to be greater ($P = 0.06$) for CS-supplemented calves compared with non-forage-supplemented calves. In addition, final BW ($P < 0.05$) was greater in calves that received CS compared with AH-supplemented and non-forage-supplemented calves (81.7 vs. 75.2 and 73.2, respectively). Body length and heart girth were greater ($P < 0.05$) for calves fed barley compared with those fed corn. Body length and heart girth were greater (P

< 0.05) for forage-supplemented calves compared with non-forage-supplemented calves.

Table 4 presents the total time devoted to each behavior of the experimental animals during 12 h of observations conducted during 2 wk before and 2 wk after weaning. No interaction was detected between the grain sources and forage provision with respect to time devoted to standing, lying, eating, ruminating, and non-nutritional behaviors. Standing, lying, and eating times (min/12 h) were not affected by treatments. Regardless of the grain sources, CS-supplemented calves devoted greater ($P < 0.01$) time to ruminating compared with AH-supplemented calves and calves offered no forage (83 vs. 53 and 40 min/12 h, respectively). Moreover, non-forage-supplemented calves spent more time ($P <$

Table 3. Effects of grain source (barley versus corn) and forage provision (no forage, alfalfa hay, and corn silage) on BW and structural growth of dairy calves (n = 10 calves per treatment)

Item	Treatment ¹						SEM	<i>P</i> -value ²		
	Barley			Corn				G	F	G × F
	NF	AH	CS	NF	AH	CS				
BW, kg										
Initial (d 1)	43.6	43.4	42.2	42.7	42.5	42.0	0.7	0.11	0.13	0.57
At weaning (d 49)	61.8	63.4	66.9	59.5	62.7	64.0	1.6	0.16	0.06	0.31
Final (d 63)	75.2	76.1	83.8	70.2	75.1	79.6	2.6	0.12	0.01	0.51
Heart girth	103.5	102.5	103.9	99.2	103.4	101.6	1.1	0.03	0.27	0.11
Body barrel	121.0	118.3	120.6	118.0	118.7	119.1	1.9	0.15	0.63	0.27
Body length	52.0	53.7	54.3	52.8	52.3	53.0	0.7	0.04	0.01	0.12
Hip height	91.5	91.9	91.7	91.2	92.5	91.6	0.7	0.11	0.15	0.20
Hip width	22.3	22.0	20.7	21.8	22.3	21.6	0.4	0.51	0.12	0.29
Withers height	86.5	88.0	90.3	87.8	87.9	87.7	0.9	0.53	0.12	0.11

¹NF = without forage; AH = 15% DM alfalfa hay; CS = 15% DM corn silage.

²Statistical comparisons: G = grain source; F = forage provision; G × F = grain source by forage provision interaction.

Table 4. Effects of grain source (barley versus corn) and forage provision (no forage, alfalfa hay, and corn silage) on total time devoted to performing different behaviors during 12 h of observation in dairy calves (n = 10 per treatment)

Item ³	Treatment ¹						SEM	P-value ²		
	Barley			Corn				G	F	G × F
	NF	AH	CS	NF	AH	CS				
Standing (min)	245	275	240	280	294	240	23	0.33	0.19	0.72
Lying (min)	239	204	174	171	174	190	24	0.17	0.60	0.22
Eating (min)	92	98	123	96	115	111	11	0.76	0.16	0.46
Ruminating (min)	43	53	85	38	53	81	9	0.72	<0.01	0.95
Nonnutritional behaviors (min)	101	90	98	135	84	98	11	0.34	0.03	0.18

¹NF = without forage; AH = 15% DM alfalfa hay; CS = 15% DM corn silage.

²Statistical comparisons: G = grain sources; F = forage provision; G × F = grain sources by forage provision interaction.

³Observations were conducted between 2 wk before and 2 wk after weaning by calves offered the experimental diets.

0.05) performing nonnutritive oral behaviors compared with AH-supplemented calves.

Rumen pH and Blood Metabolites

The data for rumen pH and blood metabolites are presented in Table 5. No interaction was detected between the grain sources and forage provision with respect to blood glucose, BUN, BHB levels, and ruminal pH. Regardless of the grain sources, forage supplemented calves had greater ruminal pH compared with non-forage-supplemented calves. Blood concentrations of glucose and BUN were not affected by grain sources, forage provision, or their interaction. Regardless of the grain sources, BHB concentration was greater ($P = 0.05$) for CS-supplemented calves compared with AH-supplemented calves and non-forage-supplemented calves.

DISCUSSION

In the current study, starter feed intake was improved during the preweaning period when barley was fed as a grain source. The type of grain in starter feed has been

reported to have controversial effects on starter feed intake as some studies reported an increase (Latrille et al., 1983; Bush, 1989; Guertin et al., 1995) whereas another study reported a decrease (Khan et al., 2007) in starter feed intake of calves fed the barley diet compared with those fed corn diets. These opposing results may be explained by differences in ruminal pH. In the current experiment, no difference was observed in ruminal pH between calves fed different type of grains as opposed to the results observed by Khan et al. (2007) where ruminal pH was lower in calves fed barley diets than those fed corn diet in the pelleted physical forms. The variable responses in ruminal pH across studies could be due to several factors, including grain variety, type, and extent of grain processing (Khorasani et al., 2001).

It has been widely recognized that forage supplementation in pelleted or finely ground starter feeds increase live weight and postweaning DMI of dairy calves (Terré et al., 2013; Nemati et al., 2016). An improved rumen environment and enhanced the growth of the muscular layer of the rumen may have contributed to the increased calf starter feed intake during the postweaning period (Coverdale et al., 2004; Castells et al.,

Table 5. Effects of grain sources (barley versus corn) and forage provision (no forage, alfalfa hay, and corn silage) on ruminal pH and selected blood metabolites of dairy calves (n = 10 calves per treatment)

Item	Treatment ¹						SEM	P-value ²		
	Barley			Corn				G	F	G × F
	NF	AH	CS	NF	AH	CS				
Ruminal pH	5.26	5.61	5.41	5.37	5.71	5.42	0.11	0.41	<0.01	0.84
Blood metabolite										
Glucose (mg/dL)	100.0	100.4	90.4	90.0	85.8	93	6.2	0.17	0.63	0.52
BUN (mg/dL)	28.0	27.0	26.4	27.2	21.5	28.0	2.2	0.32	0.21	0.29
BHB (mmol/L)	0.09	0.07	0.14	0.11	0.07	0.13	0.02	0.91	0.05	0.90

¹NF = without forage; AH = 15% DM alfalfa hay; CS = 15% DM corn silage.

²Statistical comparisons: G = grain sources; F = forage provision; G × F = grain sources by forage provision interaction.

2012; Nemati et al., 2016). In the current study, finely ground starter feed intake was increased when a forage source was offered compared with non-forage-supplemented calves, which is in line with previously reported data that used pelleted or finely ground starter feeds (Terré et al., 2013; Daneshvar et al., 2015; Nemati et al., 2016). Results regarding starter feed intake herein are in agreement with those reported by Castells et al. (2012) who showed that starter feed intake was greater in calves fed CS than those fed AH in the pelleted starter feeds. In the current experiment, the increased starter feed intake in calves fed CS diets might have been due to the lesser dustiness of the finely ground grains in the starter feed and the greater palatability of the starter feed with increased moisture, so that calves were encouraged to consume more starter feed. Recently, Beiranvand et al. (2016) reported that starter feed intake linearly increased with increasing dietary moisture content of calf starter feed during the pre-weaning and overall periods.

In the current study, we found higher ADG when finely ground barley was given to dairy calves. The greater ADG observed with calves fed finely ground barley may be explained by higher starter feed intakes. Guertin et al. (1995) reported that calves on the whole barley diet increased their DMI to compensate for lower energy concentration when compared with whole corn-based diets. From the present study, it can be envisaged that CS provision at the rate of 15% in the starter feed enhances ADG compared with AH and nonforage diets during the preweaning and postweaning periods. Data obtained from the current study are in line with results of Mirzaei et al. (2016) where CS-supplemented calves had greater pre- and postweaning ADG compared with non-forage-supplemented calves. However, there is a common perception that an increase in BW by forage feeding can be due to greater gut fill and increased the weight of gastrointestinal tissues (Hill et al., 2008). Castells et al. (2013) reported that with a high level of forage provision to dairy calves (14% of AH), differences in BW could be a consequence of gut fill. Therefore, further euthanasia studies are needed to measure the gut fill of calves fed forage.

Despite the differences observed in starter feed intake and ADG with the provision of forages, no differences were observed in feed efficiency among treatments, which is in agreement with previous studies (Terré et al., 2013; Mirzaei et al., 2015). Although these results indicated that forage consumption within the levels reported herein does not compromise nutrient utilization in dairy calves, supplementary studies are warranted to investigate the confounding effects of gut fill on nutrient utilization of calves supplemented with a high level of forage.

In agreement with previous reports (Castells et al., 2012; Nemati et al., 2016), calves fed forage spent more time ruminating and devoted less time to performing nonnutritive oral behaviors compared with non-forage-supplemented calves. Nonnutritive 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 suggested that forage, especially CS, could play an important role in increasing rumination time and reducing nonnutritive oral behaviors of dairy calves in the finely ground starter feeds; therefore, forage supplementation may have some benefits on calf welfare.

In agreement with previous reports on pelleted or finely ground starter feeds (Castells et al., 2012; Mirzaei et al., 2016), CS-supplemented calves had a greater final BW compared with calves without access to a forage source in the present study. Results regarding body measurements herein are consistent with those reported by Mirzaei et al. (2015), who did not find differences in withers height, hip height, or hip width of calves fed 8 and 16% alfalfa hay in comparison with control calves. In the current experiment, the greater body length and heart girth as the structural growth indexes in CS-supplemented calves might be attributed to greater nutrients intake in calves supplemented with CS than those fed other diets.

In the current study, calves supplemented with forage in the finely ground starter feeds presented higher rumen pH compared with unsupplemented calves. Similar to our results, an increase in rumen pH was reported by Mirzaei et al. (2016) in dairy calves when CS was included in their diets during the preweaning period. Moreover, recent studies have demonstrated beneficial effects of feeding AH forage early in life on rumen pH of dairy calves fed finely ground starter feed (Nemati et al., 2016). Feeding finely ground starter feeds with high levels of readily fermentable carbohydrates may reduce ruminal pH of dairy calves (Ghassemi-Nejad et al., 2012); therefore, supplementing forage could reduce the risk of ruminal acidosis. In the current study, ruminal pH improvement with supplementation of AH due to greater dietary cation-anion difference in comparison with CS could be related to higher inherent buffering capacity of AH compared with corn silage (Krause and Oetzel, 2006). In immature ruminants, such as young calves, improved ruminal pH with forage supplementation could be attributed to the greater removal of the keratinized layer and the dead cells from the rumen wall (Bull et al., 1965), which can increase the ability of rumen wall to absorb SCFA and maintain normal rumen pH. In addition, the greater gene expression of the SCFA transporters, such as mono-carboxylate transporter-1 (MCT-1; Castells et al., 2013), in rumen wall

and higher SCFA absorption could be related mechanisms for greater ruminal pH in forage-supplemented calves.

In the current study, no differences were observed in the levels of plasma glucose, BUN, and BHB in preweaned calves. This lack of grain source effects on the concentration of plasma glucose, BUN, and BHB in our study was inconsistent with the results of Khan et al. (2007), who found lesser serum glucose and greater BUN and BHB in calves fed corn diet than those fed barley. These opposing results may be related to differences in processing method of starter feeds in these studies (mashed vs. pelleted, respectively). Glucose data for forage effect in the current study is consistent with the results of Terré et al. (2013), who observed similar serum glucose concentrations between calves supplemented and not supplemented with forage. Similarly, Daneshvar et al. (2015) reported that supplementation of AH (15% DM) did not affect plasma glucose and BUN concentrations in dairy calves. In contrast to previous studies (Khan et al., 2011; Terré et al., 2013), forage provision to dairy calves increased plasma BHB concentrations. The greater plasma BHB levels (an indicator of metabolic function of the rumen wall) for CS-supplemented calves compared to other treatments could be due to an increase in starter intake.

CONCLUSIONS

In the current study, no interactions were observed between grain sources and forage provision on starter feed intake, ADG, skeletal growth, or rumen pH, feeding behavior, and blood metabolites, which was in contrast to our hypothesis. During the preweaning period, barley diets resulted in greater starter feed intake and ADG in calves. Furthermore, the improvement in starter feed intake, ADG, and final BW was evident for calves offered CS compared with AH; however, the improvement reported in weight gain by forage supplementation could be due to increased gut fill. Greater rumination time and plasma BHB concentrations were also noted in calves fed CS than those fed AH. Overall, barley grain and CS would be recommended for dairy calves during the pre- and postweaning periods.

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REFERENCES

AOAC. 1990. Official Methods of Analysis. 15th ed. Association of Official Analytical Chemists, Arlington, VA.

- Baldwin, R. L., K. R. McLeod, J. L. Klotz, and R. N. Heitmann. 2004. Rumen development, intestinal growth and hepatic metabolism in the pre- and postweaning ruminant. *J. Dairy Sci.* 87(E Suppl.):E55–E65.
- Beiranvand, H., G. R. Ghorbani, M. Khorvash, A. Nabipour, M. Dehghan-Banadaky, A. Homayouni, and S. Kargar. 2014. Interactions of alfalfa hay and sodium propionate on dairy calf performance and rumen development. *J. Dairy Sci.* 97:2270–2280.
- Beiranvand, H., M. Khani, S. Omidian, M. Ariana, R. Rezvani, and M. H. Ghaffari. 2016. Does adding water to dry calf starter improve performance during summer? *J. Dairy Sci.* 99:1903–1911.
- Bull, L. S., L. J. Bush, J. D. Friend, B. Harris, and E. W. Jones. 1965. Incidence of ruminal parakeratosis in calves fed different rations and its relation to volatile fatty acid absorption. *J. Dairy Sci.* 48:1459–1466.
- Bush, R. S. 1989. Preference among different grains expressed by young Holstein calves. *Can. J. Anim. Sci.* 69:1099–1103.
- Castells, L., A. Bach, G. Araujo, C. Montoro, and M. Terré. 2012. Effect of different forage sources on performance and feeding behavior of Holstein calves. *J. Dairy Sci.* 95:286–293.
- Castells, L., A. Bach, A. Aris, and M. Terré. 2013. Effects of forage provision to young calves on rumen fermentation and development of the gastrointestinal tract. *J. Dairy Sci.* 96:5226–5236.
- Coverdale, J. A., H. D. Tyler, J. D. Quigley III, and J. A. Brumm. 2004. Effect of various levels of forage and form of diet on rumen development and growth in calves. *J. Dairy Sci.* 87:2554–2562.
- Daneshvar, D., M. Khorvash, E. Ghasemi, A. H. Mahdavi, B. Moshiri, M. Mirzaei, A. Pezeshki, and M. H. Ghaffari. 2015. The effect of restricted milk feeding through conventional or step-down methods with or without forage provision in starter feed on performance of Holstein bull calves. *J. Anim. Sci.* 93:3979–3989.
- Drackley, J. K. 2008. Calf nutrition from birth to breeding. *Vet. Clin. North Am. Food Anim. Pract.* 24:55–86.
- EbnAli, A., M. Khorvash, G. R. Ghorbani, A. Nargeskhani, M. Malekkehahi, M. Mirzaei, A. Pezeshki, and M. H. Ghaffari. 2016. Effects of forage offering method on performance, rumen fermentation, nutrient digestibility, blood metabolites, and nutritional behavior in Holstein dairy calves. *J. Anim. Physiol. Anim. Nutr. (Berl.)* 100:820–827.
- Ghassemi-Nejad, J., N. Torbatinejad, A. A. Naserian, S. Kumar, J. D. Kim, Y. H. Song, C. S. Ra, and K. I. Sung. 2012. Effects of processing of starter diets on performance, nutrient digestibility, rumen biochemical parameters and body measurements of brown Swiss dairy calves. *Asian-australas. J. Anim. Sci.* 25:980–987.
- Guertin, G., B. Lachance, G. Pelletier, G. J. St-Laurent, G. L. Roy, and D. Petitclerc. 1995. Effects of photoperiod and feeding whole maize, whole barley, or rolled barley on growth performance and diet digestibility in veal calves. *Livest. Prod. Sci.* 44:27–36.
- Hill, T. M., H. G. Bateman, J. M. Aldrich, and R. L. Schlotterbeck. 2008. Effects of the amount of chopped hay or cottonseed hulls in a textured calf starter on young calf performance. *J. Dairy Sci.* 91:2684–2693.
- Hill, T. M., H. G. Bateman, J. M. Aldrich, and R. L. Schlotterbeck. 2010. Roughage amount, source, and processing for diets fed to weaned dairy calves. *Prof. Anim. Sci.* 26:181–187.
- Hosseini, S. M., G. R. Ghorbani, P. Rezamand, and M. Khorvash. 2016. Determining optimum age of Holstein dairy calves when adding chopped alfalfa hay to meal starter diets based on measures of growth and performance. *Animal* 10:607–615.
- Iranian Council of Animal Care. 1995. Guide to the Care and Use of Experimental Animals, Vol. 1. Isfahan University of Technology, Isfahan, Iran.
- Khan, M. A., A. Bach, D. M. Weary, and M. A. G. von Keyserlingk. 2016. Invited review: Transitioning from milk to solid feed in dairy heifers. *J. Dairy Sci.* 99:885–902.
- Khan, M. A., H. J. Lee, W. S. Lee, H. S. Kim, S. B. Kim, K. S. Ki, S. J. Park, J. K. Ha, and Y. J. Choi. 2007. Starch source evaluation in calf starter: I. Feed consumption, body weight gain, structural growth, and blood metabolites in Holstein calves. *J. Dairy Sci.* 90:5259–5268.

- Khan, M. A., H. J. Lee, W. S. Lee, H. S. Kim, S. B. Kim, S. B. Park, K. S. Baek, J. K. Ha, and Y. J. Choi. 2008. Starch source evaluation in calf starter: II. Ruminal parameters, rumen development, nutrient digestibilities, and nitrogen utilization in Holstein calves. *J. Dairy Sci.* 91:1140–1149.
- Khan, M. A., D. M. Weary, and M. A. G. von Keyserlingk. 2011. Hay intake improves performance and rumen development of calves fed higher quantities of milk. *J. Dairy Sci.* 94:3547–3553.
- Khorasani, G. R., E. K. Okine, and J. J. Kennelly. 2001. Effects of substituting barley grain with corn on ruminal fermentation characteristics, milk yield, and milk composition of Holstein cows. *J. Dairy Sci.* 84:2760–2769.
- Kiran, D., and T. Mutsvangwa. 2007. Effects of barley grain processing and dietary ruminally degradable protein on urea-nitrogen recycling and nitrogen metabolism in growing lambs. *J. Anim. Sci.* 85:3391–3399.
- Kotarski, S. F., R. D. Waniska, and K. K. Thurn. 1992. Starch hydrolysis by the ruminal microflora. *J. Nutr.* 122:178–190.
- Krause, K. M., and G. Oetzel. 2006. Understanding and preventing subacute ruminal acidosis in dairy herds: A review. *Anim. Feed Sci. Technol.* 126:215–236.
- Latrille, L., J. P. Paré, G. St-Laurent, and C. Pomar. 1983. Heavy veal production with Holstein calves raised by multiple suckling or milk replacers and fattened with whole corn, barley or oats. *Can. J. Anim. Sci.* 63:643–653.
- Mirzaei, M., M. Khorvash, G. R. Ghorbani, M. Kazemi-Bonchenari, A. Riasi, A. Nabipour, and J. J. G. C. van den Borne. 2015. Effects of supplementation level and particle size of alfalfa hay on growth characteristics and rumen development in dairy calves. *J. Anim. Physiol. Anim. Nutr. (Berl.)* 99:553–564.
- Mirzaei, M., M. Khorvash, G. R. Ghorbani, M. Kazemi-Bonchenari, A. Riasi, A. Soltani, B. Moshiri, and M. H. Ghaffari. 2016. Interactions between the physical form of starter (mashed versus textured) and corn silage provision on performance, rumen fermentation, and structural growth of Holstein calves. *J. Anim. Sci.* 94:678–686.
- Nemati, M., H. Amanlou, M. Khorvash, M. Mirzaei, B. Moshiri, and M. H. Ghaffari. 2016. Effect of different alfalfa hay levels on growth performance, rumen fermentation, and structural growth of Holstein dairy calves. *J. Anim. Sci.* 94:1141–1148.
- NRC. 2001. *Nutrient Requirement of Dairy Cattle*. 7th rev. ed. Natl. Acad. Sci., Washington, DC.
- Phillips, C. J. C. 2004. The effects of forage provision and group size on the behavior of calves. *J. Dairy Sci.* 87:1380–1388.
- Porter, J. C., R. G. Warner, and A. F. Kertz. 2007. Effect of fiber level and physical form of starter on growth and development of dairy calves fed no forage. *Prof. Anim. Sci.* 23:395–400.
- Redbo, I. 1990. Changes in duration and frequency of stereotypies and their adjoining behaviours in heifers, before, during and after the grazing period. *Appl. Anim. Behav. Sci.* 26:57–67.
- Redbo, I., and A. Nordblad. 1997. Stereotypies in heifers are affected by feeding regime. *Appl. Anim. Behav. Sci.* 53:193–202.
- Suárez, B. J., C. G. V. Reenen, N. Stockhofe, J. Dijkstra, and W. J. J. Gerrits. 2007. Effect of roughage source and roughage to concentrate ratio on animal performance and rumen development in veal calves. *J. Dairy Sci.* 90:2390–2403.
- Terré, M., E. Pedrals, A. Dalmau, and A. Bach. 2013. What do pre-weaned and weaned calves need in the diet: A high fiber content or a forage source? *J. Dairy Sci.* 96:5217–5225.
- Van Soest, P. J., J. B. Robertson, and B. A. Lewis. 1991. Methods for dietary fiber, neutral detergent fiber nonstarch polysaccharide in relation to animal nutrition. *J. Dairy Sci.* 74:3583–3597.
- Žitnan, R., J. Voigt, U. Schonhuse, J. Wegner, M. Kokardova, H. Hagemeister, M. Levkut, S. Kuhla, and A. Sommer. 1998. Influence of dietary concentrate to forage ratio on the development of rumen mucosa in calves. *Arch. Anim. Nutr.* 51:279–291.