Effects of physical forms of starter and milk allowance on growth performance, ruminal fermentation, and blood metabolites of Holstein dairy calves

A. Jafari,¹ A. Azarfar,^{1*} G. R. Ghorbani,² M. Mirzaei,³ M. A. Khan,⁴ H. Omid-Mirzaei,⁵ A. Pakdel,⁶ and M. H. Ghaffari^{7*}

¹Department of Animal Science, Faculty of Agriculture, Lorestan University, PO Box 465, Khorramabad, 68151-44316, Iran

²Department of Animal Science, College of Agriculture, Isfahan University of Technology, Isfahan 84156-83111, Iran

³Department of Animal Science, Faculty of Agriculture and Natural Resources, Arak University, Arak 38156-88349, Iran

⁴AgResearch Ltd., Grasslands Research Centre, Private Bag 11008, Palmerston North 4474, New Zealand

⁵Animal Science Research Department, Isfahan Agricultural and Natural Resources Research and Education Center, AREEO, Isfahan, Iran

⁶Department of Animal Science, Isfahan Agricultural and Natural Resources Research and Education Center, AREEO, Isfahan, Iran

⁷Institute of Animal Science, University of Bonn, 53111 Bonn, Germany

ABSTRACT

A 2×2 factorial study was conducted to evaluate the effects of milk allowance and physical forms of starter on growth performance, ruminal fermentation, and blood metabolites of Holstein dairy calves. A total of 48 calves [40.4 ± 1.55 kg of body weight (BW), $n = 12$ per treatment: 6 males and 6 females] were randomly assigned to 1 of the 4 treatments: (1) calves fed low milk allowance and finely ground (FG) starter feed [low-FG; 1.47 ± 2.12 -mm geometric mean particle size (GMLP)], (2) calves fed low milk allowance and textured (TS) starter feed [low-TS, includes steam-flaked grains (corn and barley) with a pelleted supplement, GMLP 4.15 ± 1.77 mm], (3) calves fed high milk allowance and FG starter feed (high-FG); and (4) calves fed high milk allowance and TS starter feed (high-TS). The starter diets were blended with 7% of chopped alfalfa hay as a proportion of diet dry matter (DM). No milk refusal was observed in any treatments, and calves on both treatments were weaned from milk by wk 8 of the study using a gradual weaning procedure. We observed no interaction between milk allowance and physical forms of starter on feed intake, average daily gain, feed efficiency, BW, and structural growth. Calves fed high milk allowance had lower starter feed intake but greater feed efficiency and overall BW compared with those fed low allowance. Total DM intake and average daily gain were not different among treatments. Regardless of the

physical form of starter feed, hip height, heart girth, the molar proportion of ruminal acetate, acetate to propionate ratio, plasma cholesterol, and high-density lipoprotein were greater, but ruminal total volatile fatty acids, the molar proportion of propionate, and plasma β -hydroxybutyrate were lower in calves fed high milk allowance compared with those fed low allowance. Regardless of the milk allowance, calves fed the FG starter feeds had greater blood urea nitrogen concentrations compared with calves fed the TS starter diets. In conclusion, both forms of the starter feeds can be used when calves are fed high milk allowance with no negative effect on their performance.

Key words: calf, milk feeding level, starter physical form

INTRODUCTION

Rearing calves on a low quantity of milk or milk replacer (MR) leads to negative effects on calf welfare (e.g., hunger and vocalization; de Paula Vieira et al., 2008; Rosenberger et al., 2017) and compromises their preweaning growth compared with calves receiving a higher milk allowance (20% of the initial BW; Khan et al., 2011). Alternatively, feeding calves with greater milk or MR allowance promotes preweaning growth rate (Jasper and Weary, 2002; Khan et al., 2007), feed efficiency (FE; Mirzaei, et al., 2018), and first-lactation milk yield (Soberon et al., 2012). Also, calves fed an intensified MR feeding regimen with CP concentrations greater than 20% (i.e., 28% CP and 20% fat or 26% CP and 18% fat) had greater preweaning ADG compared with conventional MR (20% CP:20% fat) feeding regimen (Cowles et al., 2006; Guindon et al., 2015; Chapman et al., 2017). However, feeding a low amount of milk or MR (approximately 4–5 L/d) is still commonly

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*Corresponding authors: arash.azarfar@gmail.com and morteza1@uni-bonn.de

practiced for stimulating ruminal development to allow for early weaning (Suarez-Mena et al., 2011; Kertz and Loften, 2013; Daneshvar et al., 2015), thereby offsetting rearing cost, which makes feeding low level of milk or MR to dairy calves popular among dairy farmers.

In addition to milk allowance, physiochemical properties of calf starter could also influence the initiation of solid feed, ruminal development, and growth performance of calves by weaning (Pazoki et al., 2017; ZeidAli-Nejad et al., 2018). Processing of cereal grains (e.g., grinding, flaking, rolling) along with methods used in feed manufacturing also influences the ruminal and intestinal digestibility of feed and starch (Firkins et al., 2001; Hu et al., 2018) and performance (Khan et al., 2008; Gressley et al., 2011) of both young and adult cattle. Available studies in the literature have reported conflicting results regarding the effects of starter physical form on performance and ruminal development of dairy calves. Our previous study showed that the starter feed intake, ADG, and FE were greater for calves fed textured starter (TS) feeds compared with those fed coarse ground starter feeds (Omid-Mirzaei et al., 2018). Similarly, Bateman et al. (2009) reported that calves fed TS consumed more starter and gained more weight than those fed finely ground (FG) starter. Data reported by Porter et al. (2007) suggest that parakeratosis of the rumen and its dysfunction are minimized when 75% of the particles in a starter exceed 1.19 mm in diameter. In contrast, other studies showed no differences in the intake and growth performance of calves fed ground starter feed compared with the TS starter feed (Mirzaei et al., 2016; Pazoki et al., 2017). Recently, van Niekerk et al. (2020) reported that the combination of MR allowance and corn processing in calf starter had no effect on weight gain and nutrient digestibility, and the intake of starter feed was similar between the whole versus steam-flaked corn. However, there are a few studies where the influence of milk allowance that affects the solid feed intake is evaluated in combination with starter feeds varying in their physical forms (van Niekerk et al., 2020) or nutrient content (Araujo et al., 2014) on calf performance.

In the present study, we tested the hypothesis that feeding TS starter in combination with high milk allowance would promote nutrient intake, thereby enhancing the growth of calves compared with those fed high milk allowance and FG starter diet, and with low milk-fed calves offered either TS or FG starter feed. The objective of this study was to evaluate the interaction effects of milk allowance (high vs. low) and the physical form of starter feeds (TS vs. FG) on starter intake, growth performance, ruminal fermentation, and blood metabolites in dairy calves.

MATERIALS AND METHODS

Animals, Management, and Treatments

The present study was carried out from April to July 2018 at Fazil Agri. Industrial Co., Isfahan, Iran. All the animal procedures were approved by the Animal Care and Use Committee of Isfahan University of Technology (IACUC #2018005) outlined by the Iranian Council of Animal Care (1995). The average ambient temperature during the experimental period was 24°C (range: 19 to 30°C). A total of 48 Holstein calves (40.40 ± 1.55 kg of BW, $n = 12$ calves per treatment: 6 males and 6 females) were enrolled in the experiment. Calves were separated from their dams immediately after birth, weighed, and moved to a naturally ventilated barn with individual pens (1.5×2.8 m). A fresh blend of sand and sawdust (7:1) was used as bedding and refreshed every day, and manure was removed daily to keep the pens visibly clean and dry. The calves were fed 2.5 L of fresh high-quality colostrum (Brix value > 22) via nipple bottles within 3 h of birth and another 2.5 L 12 h after the first feeding. From the second feeding time until d 3 of life, all calves received colostrum and transition milk twice daily at 0830 and 1630 h. The quality of colostrum was measured with a digital Brix refractometer (PAL-1, Atago Co. Ltd., Bellevue, WA) and was discarded if it measured less than 22 on the Brix scale (Bielmann et al., 2010). Blood samples were collected by venipuncture from the jugular vein, using Clot Activator Vacutainers (BD Vacutainer, Franklin Lakes, NJ), 24 h after the first feeding of colostrum, and serum total protein was determined using a commercially available handheld refractometer (VET 360; Reichert Inc., Depew, NY). Only calves with a serum protein level > 5.5 mg/dL were included in the study. From 3 d of age (d 0 of the study), whole pasteurized milk was provided in steel buckets twice daily at 0830 and 1630 h. Whole milk used to feed calves was pasteurized at 63°C for 35 min.

In a 2×2 factorial arrangement of treatments calves were randomly assigned to 1 of 4 feeding programs: (1) calves fed low milk allowance (4 L/d of milk from wk 1 to 7, and 2 L/d of milk for the last week) and FG starter feed [low-FG; geometric mean particle size (GMLP) 1.47 ± 2.12 mm in diameter], (2) calves fed low milk allowance and TS starter feed [low-TS; includes steam-flaked grains (corn and barley) with a pelleted supplement, GMLP 4.15 ± 1.77 mm in diameter], (3) calves fed high milk allowance (4 L/d of milk during wk 1, 6 L/d of milk during wk 2, 8 L/d of milk during wk 3, 10 L/d of milk during wk 4 and 5, 6 L/d of milk during wk 6 and 7, followed by 3 L/d of milk for the last week) and

Figure 1. The schematic diagram represents the amounts of milk consumed (L/d) by calves fed milk by different procedures and weaned at 56 d of the study. Calves were assigned to 1 of 4 dietary treatments: (1) calves fed low milk allowance (4 L/d of milk from d 0–48, and 2 L/d of milk from d 49–55 of the study) and finely ground starter feed (Low-FG; includes grinding of corn and barley in a hammer mill); (2) calves fed low milk allowance and textured starter feed [Low-TS, includes steam-flaked grains (corn and barley) with a pelleted supplement, mean particle size 4.15 mm diameter]; (3) calves fed high milk allowance (4 L/d of milk from d 0–6, 6 L/d from d 7–13, 8 L/d from d 14–20, 10 L/d from d 21–34, 6 L/d from d 35–48, and 3 L/d from d 49–55 of the study) and finely ground starter feed (High-FG); and (4) calves fed high milk allowance and textured starter feed (High-TS). The starter diets were blended with 7% of chopped alfalfa hay as a proportion of diet DM.

FG starter feed (high-FG), and (4) calves fed high milk allowance and TS starter feed (high-TS). The starter diets were blended with 7% of chopped alfalfa hay as a proportion of diet DM. Calves were weaned by reducing whole milk offered by 50% during wk 8 and remained on the study until wk 10 (Figure 1). No milk refusal was observed in any treatment. All calves had free access to water and starter diets throughout the study. The ground and TS diets had the same ingredients and nutrient compositions (Table 1) and differed only in their physical aspects such as particle size, uniformity, and hardness. The offered diets were adjusted daily to achieve 5 to 10% orts (i.e., the portion of the starter not consumed over a 24-h period); orts were collected and weighed daily at 0800 h. Calves were treated according to the recommendations and protocols established by the Fazil Agri. Industrial Co., and sick calves were treated by a veterinarian accordingly. Calves were checked daily for incidences of health disorders before morning and afternoon milk feeding by the farm veterinarian and a member of the research team. Inappetence, breathing difficulties, feces with blood, and attitude scores were recorded for individual calves. If calves had

a fecal score of >2 or had signs such as fever with temperatures $\geq 39.5^{\circ}\text{C}$, inappetence, droopy ears, nasal discharge, and labored breathing, they were treated by the veterinarian. During the entire experiment, 2 calves in high-FG, 2 calves in high-TS, and 1 calf in low-FG treatments were treated for diarrhea.

Sampling and Laboratory Analyses

Milk intake was measured daily. Ten representative samples of milk were collected throughout the study and submitted to the Central Milk Testing Laboratory of the farm to determine DM, ether extract (**EE**), and CP concentrations using an infrared analyzer (MilkoScan 134 BN; Foss Electric, Hillerød, Denmark). The average DM, EE, CP, and lactose content of whole milk are shown in Table 2. Throughout the study, feed refusals were removed daily immediately before the provision of the fresh diets in the morning. Individual feed intake was determined daily by weighing the amounts of diets offered and the amounts refused. Body weight was recorded weekly using a digital scale before the morning feeding [using an electronic scale (Model EES-

Table 1. Ingredients, chemical compositions, and particle size distribution of the experimental diets

Item ¹	Diet	
	Ground	Textured
Ingredient, % of DM basis		
Chopped alfalfa hay	7.0	7.0
Corn grain, ground	37.2	—
Barley grain, ground	18.6	—
Steam-flaked corn	—	37.2
Steam-rolled barley	—	18.6
Soybean meal (45% CP)	28.8	28.8
Full fat soybean	4.7	4.7
Calcium carbonate	0.7	0.7
Vitamin and mineral mix	1.8	1.8
Sodium bicarbonate	0.7	0.7
Salt	0.5	0.5
Chemical composition, % of DM		
DM	90.8	90.1
ME (Mcal/kg of DM)	3.09	3.13
CP	21.6	21.7
Ether extract (EE)	3.5	3.6
NDF	17.0	17.4
NFC	49.20	48.8
Ca	0.9	0.9
P	0.5	0.5
Particle size distribution, % retained on sieve		
19	0.04 ± 0.02	0.3 ± 0.1
18	1.2 ± 0.7	15.5 ± 2.2
1.18	36.2 ± 3.5	79.5 ± 3.2
Pan	62.5 ± 4.1	6.4 ± 2.1
GMPL, mm	1.47 ± 2.12	4.15 ± 1.77

¹Vitamin and mineral mix (per kg) contained: vitamin A = 750,000 IU, vitamin D₃ = 25,000 IU, vitamin E = 500 IU, Ca = 100 g, P = 10 g, Mg = 20 g, Zn = 1,000 mg, Cu = 250 mg, I = 50 mg, Co = 60 mg, Mn = 500 mg, Se = 50 mg; ME was calculated from NRC (2001); NFC = 100 - (% NDF + % CP + % EE + % ash) (NRC, 2001); Ca was estimated using the NRC (2001) model; GMPL = geometric mean particle size calculated as described by the American Society of Agricultural Engineers (ASAE, 1996; method S424.1).

500; Ettehad Inc., Isfahan, Iran), which was calibrated by the manufacturer's agent before initiation of the study and every month thereafter] throughout the experiment. Feed samples were collected weekly throughout the trial and stored at -20°C pending chemical analyses. Subsamples of feeds and refusals were mixed thoroughly, dried at 55°C for 48 h, and ground to pass through a 1-mm screen in a Wiley mill (Ogaw Seiki Co. Ltd., Tokyo, Japan). Triplicate samples of diets and refusals were analyzed for DM (AOAC International, 2002; method 925.40), CP (Kjeltec 1030 Auto Analyzer, Tecator, Höganäs, Sweden; AOAC International, 2002; method 955.04), EE (AOAC International, 2002; method 920.39), ash (AOAC International, 2002; method

942.05), and NDF using a heat-stable α -amylase (100 μ L/0.5 g of sample) with sodium sulfite (Van Soest et al., 1991). The NFC component was computed as 100 - (CP + NDF + EE + ash) (NRC, 2001).

Means for ADG and FE [kg of BW gain/kg of total DMI (liquid feed DMI + diets DMI)] were calculated. Body size measurements including body length (the distance between the points of shoulder and rump), withers height (the distance from the base of the front feet to the withers), heart girth (the circumference of the chest), body depth (the circumference of the belly before feeding), hip height (the distance from the base of the rear feet to hook bones), and hip width (the distance between the points of hook bones) of the calves

Table 2. Mean chemical composition of corn, barley, soybean meal (g/kg of DM), and whole milk (g/kg; $\pm$ SD)

Item	Corn grain	Barley grain	Soybean meal	Whole milk
DM	88.1	91.0	89.1	11.5 ± 0.18
CP	8.2	11.5	45.6	2.85 ± 0.02
Ether extract	4.2	2.3	1.2	3.65 ± 0.07
Lactose	ND ¹	ND	ND	4.34 ± 0.03

¹ND = not determined.

were measured manually on d 0, 56, and 70 of the study as described by Pazoki et al. (2017).

Ruminal fluid was collected on d 35 and 70 by a stomach tube fitted to a vacuum pump at 3 h after feeding; the first 50 mL was discarded to avoid saliva contamination. Ruminal contents were squeezed through 4 layers of cheesecloth, and 8 mL of the ruminal fluid was immediately acidified with 2 mL of 25% metaphosphoric acid and stored at -20°C pending VFA analysis. Ruminal concentrations of VFA were measured by gas chromatography (model CP-9002; Chrompack, Middelburg, the Netherlands) fitted with a 50-m (0.32-mm i.d.) silica-fused column (CP-Wax Chrompack Capillary Column; Varian, Palo Alto, CA), with crotonic acid (1:7, vol/vol) as an internal standard, as described by Bal et al. (2000). Helium was used as the carrier gas, and initial and final oven temperatures were 55 and 196°C , respectively. The detector and injector temperatures were set at 251°C .

Blood samples were collected before the morning meal on d 35, 56, and 70 of the study. Briefly, blood was withdrawn from the jugular vein into evacuated tubes containing K2 EDTA (Becton Dickinson Vacutainer Systems, Franklin Lakes, NJ), immediately placed on ice, and centrifuged at $3,000 \times g$ for 20 min at 4°C . Plasma subsamples stored at -20°C subsequently were analyzed to determine concentrations of glucose, cholesterol, triglycerides, albumin, total protein, high-density lipoprotein (HDL), and BUN using commercial kits in accordance with the manufacturer's instructions (Pars Azmoon Co., Tehran, Iran). Plasma BHB was determined with an autoanalyzer using a laboratory kit (Randox Laboratories Ltd., Ardmore, UK).

Statistical Analyses

Data were analyzed as a completely randomized design with a 2×2 factorial arrangement of treatments with the factors of starter physical form (FG vs. TS) and milk allowance (low vs. high). Data were subjected to ANOVA using the MIXED procedure of SAS (SAS 9.4, SAS Institute Inc., Cary, NC) with time as repeated measures for starter feed intake, total DMI, BW, ADG, FE, structural growth, ruminal fermentation characteristics, and blood metabolites with the individual calf as the experimental unit. The model included fixed effects of the physical forms of starter, the milk allowance, period (d or wk), and their interactions. Calf and sex of calf were considered as random effects. Before analyses, all data were screened for normality using the UNIVARIATE procedure of SAS. Three variance-covariance structures (autoregressive type 1, compound symmetry, and Toeplitz) were tested, and the covariance structure that minimized the Schwarz's

Bayesian information criterion was chosen. Initial BW was considered as a covariate for overall BW. The main effects of milk allowance and starter physical form and interactions were tested using ANOVA. The threshold of significance was set at $P \leq 0.05$; trends were declared at $0.05 < P \leq 0.10$.

RESULTS

Total DMI, starter DMI, ADG, FE, and BW data are presented in Table 3. We observed no interaction between the milk allowance and the physical forms of starter for total DMI, starter feed intake, ADG, FE, BW, and structural growth. Calves fed high and low milk allowance had no milk refusals. A milk allowance $\times$ week interaction was observed for starter feed intake (from wk 4–10), total DMI (from wk 2–10), ADG (from wk 2–4 and wk 7–9), and FE (from wk 3–5), although there was no 3-way interaction (milk allowance $\times$ physical forms of starter $\times$ week). Intake of starter feed was lower ($P < 0.01$) in calves fed high milk allowance compared with those fed low allowance (Figure 2A). Total DMI (Figure 2B) was greater ($P < 0.01$) from wk 2 to 5 and was lower from wk 7 to 10 in calves fed high milk allowance compared with those fed low allowance due to the step-up/step-down protocol followed for calves fed high milk allowance. The ADG (Figure 2C) was greater ($P < 0.01$) from wk 2 to 5 and lower from wk 7 to 9 in calves fed high milk allowance compared with those fed low allowance. The overall FE ($P = 0.02$) and BW ($P < 0.01$) were greater in calves fed high milk allowance compared with those fed low allowance. The BW was also affected by a milk allowance $\times$ week interaction ($P < 0.01$; Figure 2D), with calves fed high milk allowance had greater BW from wk 3 to 7 compared with those fed low allowance. No difference was observed on total DMI, ADG, and BW for calves fed the TS starter diets compared with calves fed the FG starter diets. Calves fed the FG starter diets tended to have greater starter DMI ($P = 0.07$) but lower FE ($P < 0.01$) compared with those fed the TS starter diets.

Skeletal growth data are presented in Table 4. We observed no interaction between the main effects and time on the skeletal growth measurements. Regardless of the physical form of starter feed, hip height ($P = 0.03$), heart girth ($P < 0.05$), and wither height (tendency, $P = 0.07$) were greater in calves fed high milk allowance compared with those fed low allowance.

Ruminal fermentation characteristics data are presented in Table 5. We observed no interaction between the main effects on ruminal fermentation characteristics. Total VFA concentrations and the molar proportion of acetate and propionate, and the acetate to propionate ratio were affected by a milk allowance $\times$

Table 3. Effects of milk allowance (low vs. high) and physical form of starter (textured vs. fine ground) on total DMI, starter DMI, ADG, feed efficiency, and BW of dairy calves during the entire experiment (wk 1–10, $n = 12$ per treatment)¹

Item	Treatment					P-value						
	High-TS	High-FG	Low-TS	Low-FG	SEM	M	S	W	M × S	M × W	S × W	M × S × W
Total DMI, g/d	1,116	1,116	1,109	1,207	26.3	0.49	0.28	<0.01	0.20	<0.01	0.40	0.89
Starter DMI ² , g/d	507	506	764	862	36.4	<0.01	0.07	<0.01	0.89	<0.01	0.21	0.98
ADG, g/d	711	661	686	669	20.0	0.12	0.14	<0.01	0.30	<0.01	0.26	0.26
Feed efficiency	0.64	0.58	0.60	0.53	0.01	0.02	<0.01	<0.01	0.77	<0.01	0.37	0.21
BW, kg	64.1	63.1	59.9	59.4	0.79	<0.01	0.68	<0.01	0.86	<0.01	0.26	0.32

¹M = milk allowance, S = physical forms of starter, W = period (week).²Blended with 7% of chopped alfalfa hay as a proportion of diet DM.

day interaction ($P < 0.01$), with calves fed high milk allowance had greater acetate and acetate to propionate ratio compared with those fed low allowance and vice versa for total VFA concentrations and propionate in the rumen on d 35. Regardless of the physical form of starter feed, the molar proportion of ruminal acetate, acetate to propionate ratio were greater but total VFA concentrations and the molar proportion of propionate in the rumen were lower in calves fed high milk allowance compared with those fed low allowance during the overall period ($P < 0.01$).

Blood metabolites data are presented in Table 6. We observed no interaction between the main effects on plasma metabolite except for triglycerides, with the highest (tendency, $P = 0.06$) plasma concentration were observed in high-FG calves. A milk allowance × day interaction was observed for the plasma concentrations of glucose ($P < 0.01$), cholesterol ($P = 0.03$), triglycerides ($P < 0.01$), and albumin (tendency, $P = 0.06$) although there was no 3-way interaction (milk allowance × physical forms of starter × day). Calves fed high milk allowance had greater glucose (d 35), cholesterol (d 35 and 56), triglycerides (d 35), albumin (d 70) compared with those fed low allowance. Regardless of the physical form of starter feed, calves fed high milk allowance had greater plasma concentrations of cholesterol ($P < 0.01$), HDL ($P < 0.01$), albumin (tendency, $P = 0.07$) but lower BHB ($P < 0.01$) compared with those fed low milk allowance. Regardless of the milk allowance, the BUN concentrations were lower ($P < 0.01$) for calves fed the TS starter diets compared with calves fed the FG starter diets.

DISCUSSION

Effect of Milk Allowance

As expected, the starter DMI was greater for calves fed low milk allowance compared with high allowance as the calves fed high milk allowance consumed less than 100 g/d of DM diets until wk 5 of the study. The starter intake rapidly increased for calves fed high milk allowance between wk 5 and 8 of the study when the level of milk offered was reduced before the weaning. This rapid increase in the diet intake due to step-down weaning in calves fed high milk allowance was in line with the previous studies (Khan et al., 2007; Quigley et al., 2018; Klopp et al., 2019). In this study, the starter intake increased more than 3-fold for calves fed low milk allowance and approximately 5-fold for calves fed high milk allowance between wk 5 and 8 of the study regardless of the starter physical forms.

Previously, Khan et al. (2007) suggested that a hypophagic condition caused by the chemical stimuli (higher

blood glucose and insulin) depressed diet intake, which is responsible for lower feed intake in preweaning calves fed high milk allowance. In this study, the starter in-

take was consistently higher from wk 3 to 10 in calves fed low milk allowance compared with those fed high milk allowance, and gradually increased toward the

Figure 2. The effect of the level of milk and physical form of starter on (A) starter feed intake, (B) total DMI, (C) ADG, and (D) BW of dairy calves ($n = 12$ calves per treatment). Calves were assigned to 1 of 4 dietary treatments: (1) calves fed low milk allowance (4 L/d of milk from d 0–48, and 2 L/d of milk from d 49–55 of the study) and finely ground starter feed (Low-FG; includes grinding of corn and barley in a hammer mill); (2) calves fed low milk allowance and textured starter feed [Low-TS, includes steam-flaked grains (corn and barley) with a pelleted supplement, mean particle size 4.15 mm diameter]; (3) calves fed high milk allowance (4 L/d of milk from d 0–6, 6 L/d from d 7–13, 8 L/d from d 14–20, 10 L/d from d 21–34, 6 L/d from d 35–48, and 3 L/d from d 49–55 of the study) and finely ground starter feed (High-FG); and (4) calves fed high milk allowance and textured starter feed (High-TS). Error bars represent SEM.

Table 4. Effects of milk allowance (low vs. high) and physical form of starter (textured vs. fine ground) on structural growth of dairy calves (n = 12 per treatment)¹

Item	Treatment				SEM	P-value						
	High-TS	High-FG	Low-TS	Low-FG		M	S	D	M × S	M × D	S × D	M × S × D
Body length, cm												
d 0	46.0	45.7	46.2	45.6	0.21							
d 56	55.3	55.9	56.1	54.2	0.32							
d 70	59.1	59.2	58.8	58.3	0.34							
Overall	53.5	53.6	53.7	52.7	0.52	0.48	0.36	<0.01	0.23	0.64	0.76	0.11
Heart girth, cm												
d 0	80.6	80.3	80.2	80.0	0.21							
d 56	102	101	101	99	0.53							
d 70	107	106	106	104	0.62							
Overall	96.5	95.8	95.5	94.2	1.04	0.05	0.14	<0.01	0.63	0.21	0.41	0.71
Body depth, cm												
d 0	81.0	81.3	80.4	80.0	0.30							
d 56	109	109	113	111	0.91							
d 70	122	120	124	123	0.90							
Overall	104	103	106	105	1.73	0.20	0.39	<0.01	0.89	0.11	0.71	0.93
Wither height, cm												
d 0	79.4	79.6	79.2	79.5	0.32							
d 56	89.7	89.7	89.1	87.5	0.33							
d 70	92.5	92.0	92.0	90.8	0.35							
Overall	87.2	87.1	86.8	86.0	0.52	0.07	0.29	<0.01	0.41	0.12	0.21	0.36
Hip height, cm												
d 0	82.0	81.1	81.3	81.0	0.32							
d 56	92.5	92.5	91.6	90.2	0.41							
d 70	95.3	94.9	94.8	93.6	0.31							
Overall	89.9	89.5	89.2	88.3	0.52	0.03	0.12	<0.01	0.57	0.18	0.98	0.34
Hip width, cm												
d 0	15.4	15.5	15.6	15.3	0.14							
d 56	18.6	18.5	18.4	18.2	0.11							
d 70	20.7	20.5	20.6	20.2	0.24							
Overall	18.2	18.2	18.2	17.9	0.24	0.47	0.38	<0.01	0.57	0.78	0.87	0.12

¹M = milk allowance, S = physical forms of starter, D = period (day).

Table 5. Effects milk allowance (low vs. high) and physical form of starter (textured vs. fine ground) on ruminal fermentation characteristics of dairy calves (n = 12 per treatment)¹

Item	Treatment				P-value				
	High-TS	High-FG	Low-TS	Low-FG	SEM	M	S	D	M × S M × D S × D M × S × D
Total VFA, mmol/L									
d 35	65	72	121	113	6.0				
d 70	108	100	108	93	3.2				
Overall	86	86	114	103	3.5	<0.01	0.23	0.02	<0.01 0.19 0.66
Individual VFA, mol/100 mol									
Acetate									
d 35	61.3	61.7	50.0	51.8	1.86				
d 70	48.3	49.3	49.1	49.4	0.77				
Overall	54.8	55.5	49.5	51.6	1.17	<0.01	0.63	<0.01	0.91 0.84
Propionate									
d 35	29.9	30.2	40.1	40.5	1.55				
d 70	43.6	40.6	42.4	42.9	0.64				
Overall	36.7	35.4	41.3	41.7	0.97	<0.01	0.75	<0.01	0.57 0.61
Butyrate									
d 35	5.9	6.0	6.3	6.1	0.53				
d 70	6.2	7.6	6.3	5.5	0.79				
Overall	6.1	6.8	6.3	5.8	0.48	0.58	0.92	0.44	0.51 0.72 0.40
Valerate									
d 35	2.9	2.1	3.6	1.6	0.52				
d 70	1.9	2.5	2.2	2.2	0.14				
Overall	2.4	2.3	2.9	1.9	0.25	0.99	0.21	0.79	0.18 0.08 0.95
Acetate to propionate ratio									
d 35	2.5	2.1	1.3	1.3	0.19				
d 70	1.1	1.2	1.2	1.2	0.03				
Overall	1.8	1.7	1.2	1.2	0.11	0.01	0.77	<0.01	0.67 0.01 0.56 0.52

¹M = milk allowance, S = physical forms of starter, D = period (day).

Table 6. Effects of milk allowance (low vs. high) and physical forms of starter (textured vs. fine ground) on average blood metabolites of dairy calves (n = 12 per treatment)¹

Item	Treatment				SEM	P-value						
	High-TS	High-FG	Low-TS	Low-FG		M	S	D	M × S	M × D	S × D	M × S × D
Glucose, mg/dL												
d 35	102	105	99	87	2.50							
d 56	95	94	106	100	2.34							
d 70	100	93	110	101	2.41							
Overall	99	97	105	96	1.39	0.38	0.10	0.63	0.27	<0.01	0.76	0.52
Cholesterol, mg/dL												
d 35	123	128	97	106	5.94							
d 56	128	122	79	95	5.66							
d 70	70.9	69.7	61.9	58.9	2.97							
Overall	107	107	79	87	3.69	<0.01	0.58	<0.01	0.55	0.03	0.69	0.46
Triglycerides, mg/dL												
d 35	37.6	55.2	32.6	22.3	3.91							
d 56	23.3	23.8	30.1	28.1	1.18							
d 70	27.6	28.0	29.6	27.0	1.62							
Overall	29.4	35.6	30.7	25.7	1.52	0.12	0.82	<0.01	0.06	<0.01	0.71	0.08
HDL, mg/dL												
d 35	68.3	69.7	59.1	62.0	3.20							
d 56	76.6	76.6	46.0	55.6	4.06							
d 70	39.9	41.9	33.6	30.3	1.95							
Overall	61.6	62.8	46.3	49.3	2.32	<0.01	0.60	<0.01	0.81	0.21	0.73	0.54
BHB, mmol/L												
d 35	0.07	0.09	0.11	0.12	0.007							
d 56	0.16	0.17	0.23	0.19	0.01							
d 70	0.26	0.26	0.27	0.28	0.01							
Overall	0.16	0.18	0.21	0.20	0.01	<0.01	0.80	<0.01	0.30	0.45	0.63	0.58
Albumin, g/dL												
d 35	3.23	3.16	3.24	3.23	0.04							
d 56	3.44	3.43	3.43	3.31	0.04							
d 70	3.48	3.50	3.20	3.33	0.05							
Overall	3.38	3.36	3.29	3.29	0.03	0.07	0.80	<0.01	0.77	0.06	0.47	0.66
Total protein, g/dL												
d 35	6.25	6.15	6.55	5.95	0.10							
d 56	6.58	6.72	6.65	6.70	0.08							
d 70	6.47	6.73	6.61	6.51	0.07							
Overall	6.43	6.53	6.60	6.39	0.05	0.90	0.62	<0.01	0.17	0.92	0.05	0.51
BUN, mg/dL												
d 35	13.7	21.4	20.3	24.1	0.95							
d 56	22.9	27.9	23.0	30.6	0.91							
d 70	27.0	33.3	26.3	33.0	1.24							
Overall	21.2	27.5	23.2	29.2	0.75	0.11	<0.01	<0.01	0.90	0.11	0.95	0.32

¹M = milk allowance, S = physical forms of starter, D = period (day).

end of the experiment. Calves on a low milk allowance may attempt to compensate for the reduced nutrients from milk or MR by increasing their starter intake (Rosenberger et al., 2017). The total DMI was greater in calves fed high milk allowance compared with a low allowance from wk 2 to 5 because of the greater consumption of milk DM. However, the difference in milk DMI between calves fed high and low levels of milk was reduced with the initiation of weaning from wk 5 to 8 (step-down). Findings obtained in the present study regarding the influence of milk allowance on the preweaning starter intake are similar to those reported in the previous studies (Hill et al., 2016b; Dennis et al., 2018, 2019). Independent of the physical forms of the starter offered to calves, the greater milk allowance and an associated greater supply of nutrients and energy resulted in a greater preweaning ADG from wk 2 to 4 in calves fed high milk allowance compared with those fed low milk allowance.

Milk or MR allowance has been reported to have inconsistent effects on FE as some studies reported a greater FE for calves fed high milk allowance (Quigley et al., 2018), whereas the others did not report any improvement (Hill et al., 2016a, b; Rosenberger et al., 2017; Dennis et al., 2019) in FE during the preweaning period. Generally, for the first few weeks of life, milk or MR is considerably more digestible than calf solid feeds, as evident by Dennis et al. (2018), which may partly justify why a higher plane of milk feeding during the preweaning period led to a better FE of calves fed high versus low milk allowance.

Lower starter intake in calves fed high milk allowance compared with those fed low allowance has potentially resulted in lower concentrations of ruminal total VFA on d 35 of the study. In line with our findings, previously Khan et al. (2007) reported a lower concentration of ruminal total VFA, acetate, propionate, and butyrate on d 28 of age, but greater concentrations at weaning and postweaning for calves fed milk through a step-down method than in conventionally milk-fed calves. A greater solid feed intake has provided a greater amount of solid feed for ruminal fermentation to produce higher amounts of ruminal VFA in calves fed through step-down than conventional feeding methods. In our study, irrespective of the physical forms of starter, the milk allowance was observed to influence the feed intake and thereby lower concentrations of VFA in calves fed high milk allowance compared with low milk allowance. The acetate to propionate ratio in the rumen was greater in calves fed high milk allowance compared with those fed low allowance, which could be due to the higher ruminal pH associate with a lower build of the ruminal

VFA (Mirzaei et al., 2016). Previously, Grant et al. (1990) showed a reduction in ruminal pH with a higher buildup of VFA in the rumen impairs the growth of cellulolytic bacteria, therefore reducing acetate to propionate ratio.

In young calves, the primary source of energy is glucose derived from intestinal absorption (Khan et al., 2007), but with the initiation of solid feed consumption, ruminal fermentation proceeds, and VFA starts replacing glucose as the energy source (Baldwin et al., 2004). The higher blood glucose concentrations on d 35 of the study in calves fed high milk allowance compared with those fed low allowance could be attributed to a higher lactose intake that has been reported to increase blood glucose concentration (Terré et al., 2009; MacPherson et al., 2016; Mirzaei et al., 2018). On d 56 and 70 of the study, a decrease in plasma glucose concentrations in combination with increased solid feed intake and higher ruminal VFA concentrations in calves fed high milk allowance compared with those fed low allowance has reflected a shift in the energy precursor from glucose to VFA originating from ruminal fermentation (Khan et al., 2007).

On d 35 of the study, a higher triglycerides concentration in calves fed high milk allowance could be due to the higher milk-fat intake compared with those fed low milk allowance. Similar to our finding, Khan et al. (2007) reported a higher plasma concentration of triglycerides in calves receiving high versus low milk allowance. On d 35 of the study, the greater plasma concentrations of cholesterol and HDL in calves fed high milk allowance compared with those fed low allowance could be due to the increased intake of saturated fatty acids with increasing the milk intake. As expected, the plasma concentrations of BHB were affected by the age as a result of the increase in solid feed intake and the consequent ruminal development (Nemati et al., 2015; de Paula et al., 2017). Furthermore, the higher plasma concentrations of BHB in calves fed low milk allowance compared with high allowance may indicate a better development of the ruminal wall to metabolize VFA earlier in calves fed low versus high milk allowance (Baldwin et al., 2004). As calves age, the concentrations of plasma BHB increased in all groups, mainly after 5 wk of the study in calves fed high milk allowance, when starter intake increased due to milk weaning. The plasma concentrations of BHB increased more than 2-fold from d 35 to 56 and 3-fold from d 35 to 70 in calves fed high milk allowance independent of starter physical forms; so that calves fed high milk allowance had BHB plasma concentrations similar to calves fed low milk allowance at the end of the study. In agree-

ment with the findings of Omid-Mirzaei et al. (2015) and Mirzaei et al. (2018), milk feeding treatments did not affect the serum albumin content.

Effects of Starter Physical Forms

In the present study, starters were formulated to have the same ingredients and nutrient concentrations, but different particle sizes. Calf starter diets can be fed alone, blended with forages, or with free-choice provision of forages. In this study, the differences in the particle size of diets remained with the inclusion of 7% forage as a proportion of diet DM in both starters (TS diets had a GMLP of 4.15 mm, and FG starter diets had a GMLP of 1.47 mm). Feeding the FG starter diets to calves tended to increase starter intake compared with those fed the TS starter diets during preweaning and overall periods. Regardless of the milk allowance, we found no difference in postweaning starter feed intake, total DMI, ADG, BW, and skeletal growth when calves were fed either the FG or TS starter diets. In line with our findings, previous studies have reported no differences in BW or structural growth for calves fed ground and texturized starter diets (Pazoki et al., 2017; Mirzaei et al., 2016; Omid-Mirzaei et al., 2018). However, this trial was limited by too short a duration of the postweaning period.

Irrespective of the milk allowance offered, calves fed the TS starter diets had a greater FE compared with those fed the FG starter diets, which is in line with those reported by Moeini et al. (2017). In contrast, other studies have reported no difference in FE between calves reared on texturized and ground starters (Ghassemi-Nejad et al., 2012; Mirzaei et al., 2016; Pazoki et al., 2017). Recently, Quigley et al. (2018) reported that calves fed the TS starter diets had greater apparent total-tract digestibilities of DM, OM, and CP compared with calves fed a ground starter diet during postweaning (wk 1). In line with our results, Omid-Mirzaei et al. (2018) also reported a greater FE in calves fed TS starter diets (which includes steam-flaked corn and steam-rolled barley combined with a pelleted supplement) compared with those fed coarsely mashed diets (coarse ground grains combined with a mash supplement). In summary, our and other studies documenting the effects of starter physical forms on the ruminal development, ADG, and FE of calves have yielded inconsistent results owing to factors such as forage levels (Pazoki et al., 2017), processing methods of grains in the starter diet, milk volume offered, and the scours and health of the experimental calves (Bateman et al., 2009; Jarrah et al., 2013; Pezhveh et al., 2014). Therefore, FE results in our and other studies should be interpreted cautiously.

It is well established that the calf starter should be relatively rich in readily fermentable carbohydrates due to its role in ruminal propionate and butyrate production (Sander et al., 1959). Similar to our findings, previously Pazoki et al. (2017) also observed no differences in the molar proportion of ruminal acetate, butyrate, and acetate to propionate ratio between calves fed either FG or TS starter diets.

Blood urea is an indicator of N metabolism and disposal of excessive ruminal N because BUN value is positively correlated with ruminal $\text{NH}_3\text{-N}$ concentrations (DePeters and Ferguson, 1992). The high concentration of BUN is an indicator of low N efficiency (Broderick, 2006). In the current study, the lower BUN concentrations in calves fed the TS starter diets may reflect greater N utilization and higher N efficiency compared with those fed the FG starter diets. Additionally, no difference in the plasma concentration of BHB was found between the FG and TS diets, which is in line with the results of previous studies (Moeini et al., 2017; Omid-Mirzaei et al., 2018). This result was supported by the lack of dietary effects (TS vs. FG) on the molar proportion of ruminal butyrate in this study.

CONCLUSIONS

In summary, we found that the combination of milk allowance and physical forms of the starter did not influence the intake of starter, DM, ME, weight gain, or FE, which is contradictory to our initial hypothesis. Irrespective of the physical form of the starter, feeding a high milk allowance to calves resulted in a greater ADG, structural growth, and overall BW compared with calves offered a low milk allowance. However, this trial was limited by too short a duration of the postweaning period. In conclusion, both physical forms of starter diets (blended with 7% forage as a proportion of diet DM) can be used when calves are fed high milk allowance, with no negative effect on the performance of calves.

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