Iranian Society of Animal Science

Journal of Livestock Science and Technologies

Shahid Bahonar University of Kerman

ISSN: 2322-3553 (Print) ISSN: 2322-374X (Online)

Paper type: Original Research

Productive and metabolic responses of Holstein dairy calves to different milk dry matter concentrations and two free water provision schedules

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Received: 07 Jul 2023,
Accepted: 20 Oct 2023,
Published online: 20 Oct 2023,

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Abstract This study was aimed to assess the effects of reducing free water access time and feeding milk at various dry matter (DM) concentrations on water consumption, growth performance, DM intake (DMI), and blood metabolites of Holstein dairy calves. Sixty Holstein dairy calves [42 ± 3.0 kg of body weight (BW)] were used in a 3 × 2 factorial randomized block design with the main factors including milk DM (low, medium, and high DM; LDM, MDM, and HDM, 125, 140, and 155 g/kg, respectively) and water access time (short and long-time free water access, ST and LT respectively). Neither milk DM concentration nor water access time had any effect on the mean water intake (L/d). Increasing the concentration of liquid feed in both water provision schedules, improved the final BW, average daily gain (ADG), heart girth, and withers and hip heights. However, main blood metabolites were not affected by milk DM. The LT calves that consumed the highest milk DM (HDM) had the greatest ADG compared to other treatments ($P<0.05$). A trend was found for the interaction between milk DM and water access time as in the ST calves, the final BW was greater than LT at 125 and 140 g/kg milk concentrations ($P=0.08$) but at 155 g/kg milk DM, the highest final BW was found in LT calves. It was concluded that under the environmental condition similar to this study, water provision duration for neonatal calves could be reduced to once a day (just 2 h) however, we do not recommend increasing the DM content of milk by more than 140 g/kg when water is provided freely, 2 h once a day.

Keywords: dairy calves; milk DM; water provision schedule

Introduction

Water is an essential nutrient that must be supplied daily for the normal health and performance of dairy calves (Lowe et al., 2022). Beside milk water content, provision of drinking water is also essential for rumen development (Amaral-Phillips et al., 2015; Lowe et al., 2022). In neonatal dairy calves without free access to drinking water, starter intake and weight gain were reduced by 31 and 38%, respectively, compared to *ad libitum* water intake (Kertz et al., 1984).

However, there are still perceptions that water provision must be restricted during the early age of neonatal calves because of an experienced concern that it may cause diarrhea (Wickramasinghe et al., 2019). When young calves were reared outdoors and the ambient temperature was low, it was recommended to offer water within warmer hours of the day (Amaral-Phillips et al., 2015). Jensen and Vestergaard (2021) suggested that instead of permanent water access throughout 24 h, calves can have access to drinking water as much as they need but within short periods after milk consumption. By this practice, providing sufficient drinking water at desirable temperatures could be more warranted (Huuskonen et al., 2011). To our best of knowledge, research on reducing free access time to drinking water in suckling calves is lacking.

The critical period of a calf's lifetime is from birth up to weaning because enhanced growth in the pre-weaning period would result in lower age at first calving and increased performance during the first lactation (Meyer et al., 2006; Moallem et al., 2010). This could be achieved by maximizing solid feed intake through feed starter, liquid feed or both (von Keyserlingk et al., 2009; Omidi-Mirzaei et al., 2018). On industrial dairy farms, liquid feed intake of dairy calves is restricted to approximately 10% of birth BW or about 4 to 6 L of milk/d (Hill et al., 2016) mainly to prevent diarrhea and stimulate DMI. Nevertheless, feeding a fixed amount of milk may not meet the calves' requirements (Flower and Weary, 2001; Khan et al., 2011; Mahmoud et al., 2016). Niwińska et al. (2022) highlighted that although liquid feed restriction increased the starter intake, the compensatory increased starter intake was not sufficient for maximum body and organ growth.

Another strategy for feeding adequate nutrients to young calves is increasing the solid content of whole milk (Azevedo et al., 2016) or milk replacer (MR) (Quigley et al., 2006). It is now established that increasing DM of milk up to 150 g/kg can improve growth performance without negative effects on the calf health (Abbaslou et al., 2020; Norouzi et al., 2021). An improved ADG, weaning BW, growth, performance, and feed efficiency in suckling calves were observed when the DM content of milk or MR was increased above normal milk DM (125 g/kg) (Quigley et al., 2006; Azevedo et al., 2016; Norouzi et al., 2021). An increase in water consumption was also reported as a consequence of feeding high solid milk (more than 150 g/kg DM) probably because of the animal's attempts for regulating osmolality in the digestive tract (Azevedo et al., 2016).

We hypothesized that increasing the DM content of whole milk by MR powder (MRP) is detrimental for normal health and growth performance of young calves when free water access provision is limited to just 2 h in the morning. Therefore, the objective of the present study was to test the above hypothesis.

Materials and methods

Animals, housing, and treatments

This experiment was conducted at a commercial dairy farm (Dasht dairy farm) in Neyshabour, Iran. Sixty Holstein dairy calves (30 males and 30 females), were selected and separated from their dams at birth, weighed (42 ± 3.0 kg BW), and housed outdoor in individual pens which were bedded with straw (2.5 × 2.2 × 1.6 m; length × width × height). Daily temperature in the nursery was measured twice daily in the morning (0630 h) and afternoon (1430 h). During the experiment, the temperature in the morning and afternoon ranged from 3-18 and 8-26 °C, respectively. Calves were fed 3 L colostrum during the first 8 hours of life (1.5 L at 1 h after birth and 1.5 L at 8 h after the first feeding) followed by feeding transition milk (10% of the initial BW) thereafter. Calves (7 d old) enrolled in the study were fed 6 L/d of pasteurized whole milk (34.8 ± 0.1, 31.5 ± 0.1, 53.3 ± 2.6, and 125.1 ± 0.9 g/kg DM fat, crude protein (CP) and lactose, respectively) in buckets twice daily at 0600 and 1400 h from d 7 to 50, followed by morning feeding (2 L/d) from d 51 to 56 of age. Calves were used in a 3 × 2 factorial randomized block design with the main factors including milk DM (low, medium, and high DM; LDM, MDM, and HDM; 125, 140, and 155 g/kg, respectively) and water access time (short and long-time free water access, ST and LT respectively).

Calves were blocked by sex and randomly allotted to 1 of 6 treatments (each treatment having five males and females) from 7 to 56 days as follows: 1) control treatment containing whole milk (LDM, 125 g/kg) with LT (24 h) (LDMLT), 2) whole milk (LDM, 125 g/kg) with ST (0800 to 1000 h) (LDMST), 3) whole milk plus 15 g/kg added MRP (MDM, 140 g/kg) with LT (MDMLT), 4) whole milk and 15 g/kg added MRP (MDM, 140 g/kg) with ST (MDMST), 5) whole milk containing 30 g/kg MRP (HDM, 155 g/kg) with LT (HDMLT) and 6) whole milk including 30 g/kg MRP (HDM, 155 g/kg) with ST (HDMST). Water was freely provided with buckets whenever the calves had access to water.

The amount of MRP (JOSERA Brillant, Germany) to be added to the pasteurized whole milk (w/v) was adjusted to achieve the desired DM content for each treatment. Analytical constituents of MRP were: 220, 180, 82, 0.4, 19 ,9, 7.5, 7.5 g/kg CP, ether extract (EE), ash, crude fiber (CF), lysine, calcium, sodium, and phosphorus, respectively, with basic composition of 250 g/kg skim milk powder, refined vegetable fat, highly digestible whey protein, hydrolyzed soluble wheat protein, immunoglobulins and prebiotics). One hundred mL of tank milk for each meal was analyzed using Milko-Scan 605 analyzer (Foss Electric, Hillerod, Denmark) for adjusting DM to 125 g/kg. The MRP was added to the whole milk immediately before being fed to the calves.

Starter feed

The mash starter was offered *ad libitum* throughout the experiment. The total amount of the estimated starter was prepared once and subsamples were taken for chemical analysis (Table 1). Analyzes included DM: method 930.15; (AOAC, 2000), CP: Kjeldahl method 988.05; (AOAC, 2000), EE: alkaline treatment with Roese-Gottlieb method 932.06 for MR, diethyl ether extraction method 2003.05 for starters; (AOAC, 2000), neutral detergent fiber (NDF) expressed inclusive of residual ash by the procedure of (Van Soest et al., 1991) without sodium sulfite and α-amylase, acid detergent fiber (ADF) expressed inclusive of residual ash (Robertson and Soest, 1981), and ash: oven method 942.05; (AOAC, 2000).

Table 1. Dietary ingredients and nutrient composition of the starter

Items	g/kg
Ingredients	
Alfalfa hay	100
Ground barley grain	252
Ground corn grain	135
Soybean meal	180
Wheat bran	108
Cotton seed meal	54
Flaxseed	81
Corn germ	36
Whole cotton seed	36
Salt	2
Dicalcium phosphate	2
Mineral and vitamin premix[1]	14
Nutrients composition (DM basis unless otherwise indicated)	
Dry matter	922
Ether extract	61
Neutral detergent fiber	258
Acid detergent fiber	121
Non-fibrous carbohydrate	423
Starch[2]	266.2
Ash	43
Metabolizable Energy (Mcal/kg)[3]	2.5
CP	203.3

[1] The premix contained: vitamin A 500000 IU/kg, vitamin D3 60000 IU/kg, vitamin E 3000 IU/kg, vitamin K 100 IU/kg, vitamin B1 350 IU/kg, vitamin B2 325 IU/kg, vitamin B3 1000 IU/kg, vitamin B5 1550 IU/kg, vitamin B6 325 IU/kg, vitamin B9 25 IU/kg, vitamin B12 4 IU/kg, vitamin Biotin 10 IU/kg, vitamin Choline 50000 IU/kg, vitamin C 30000 IU/kg, Ca 80000 mg/kg, P 40000 mg/kg, Mg 80000 mg/kg, Na 120000 mg/kg, Fe 5000 mg/kg, Mn 2000 mg/kg, Zn 2000 mg/kg, Cu 500 mg/kg, I 20 mg/kg, Se 15 mg/kg, Co 10 mg/kg, Antioxidant 1000 mg/kg and Carrier up to 1000 g.
[2] Starch, calculated based on NASEM values.
[3] ME, calculated using NASEM (NASEM, 2021) equations.

Sample collection and analysis

Body weight, body frame development, starter, and water intake were monitored between 7 and 56 days of age. The average daily intake of starter and water were calculated by subtracting the refusals from the amount of provided and starter further adjusted for DM content to determine DMI from the starter feed. Calves were weighed at 0, 7, 21, 35, 49, and 56 days of age. Body measurements, including the heart girth (circumference behind the shoulders), body length (distance between the point of the shoulder and the rump), withers height (distance from the base of the front feet to the withers), hip height (distance from the base of the rear feet to the hook bones), and hip width (distance between the points of hook bones) of the calves, were recorded on d 7, 37, and 56 of age according to the method described by Fouladgar et al. (2016).

Health status and fecal scores were monitored daily. Calves that required medication were treated per veterinary recommendation and treatments were recorded daily. A 5-point scale of 1 = normal, 2 = soft to loose, 3 = loose to watery, 4 = watery, mucous, and 5 = watery, mucous, and bloody (Heinrichs et al., 2003) was used for scoring feces and average fecal score (AFS) was calculated. The fecal scores of 4 and 5 were considered to be diarrhea. Accordingly, the number of animals affected by diarrhea and days with diarrhea were recorded. The rectal body temperature (RBT) was measured at 7 and 37 days and weaning.

Jugular blood was sampled via venipuncture at 7, 37, and 56 days, 4 h after the morning liquid feeding. Blood was collected into evacuated tubes and maintained until coagulated and then centrifuged at 3000 x g for 10 min. Serum was obtained and frozen at –20 ˚C. Serum metabolites including glucose, urea, triglycerides (TG), alanine transaminase (ALT), aspartate transferase (AST), alkaline phosphatase (ALP), beta-hydroxybutyric acid (BHBA) and total protein (TP), were analyzed by commercial kits (Pars Azmoon Kit; Pars Azmoon, Karaj, Iran) using an automated analyzer (Vital Scientific N.V, Netherlands).

Statistical analysis

Before analyzes, all data were screened for normality using the UNIVARIATE procedure of SAS. Data analysis was performed for a 3 × 2 factorial randomized block design using the following statistical model:

$$Y_{igjkl} = \mu + C_i + S_g + M_j + W_k + A_l + (M_j \times A_l) + (W_k \times A_l) + (M_j \times W_k) + (M_j \times W_k \times A_l) + e_{igjkl}$$

where, Y_{igjkl} is the dependent variable; μ is the overall population mean; C_i is the random effect of the calf; S_g is the effect of the block (sex); M_j is the fixed effect of milk DM; W_k is the fixed effect of water access time; A_l is the fixed effect of the age; ($M_j \times A_l$) is the interaction between milk DM and age; ($W_k \times A_l$) is the interaction between water access time and age; ($M_j \times W_k$) is the interaction between the milk DM and water access time; ($M_j \times W_k \times A_l$) is the tripartite effect of milk DM, water access time, and age, and e_{igjkl} is the random residual error term (assumed to be random and independently distributed). The data were analyzed using a repeated measures MIXED procedure by SAS software, version 9.4 (SAS Institute Inc., Cary, NC).

Results

Feed and water consumption and growth of calves

Table 2 shows the effect of milk DM levels, two free water access time scenarios (W), sex (S), age (A) and their interactions on feed intake, growth performance and body measurements of neonatal calves. The HDM group showed greater final BW (P<0.05) than LDM; however, water provision time had no significant effect on BW. Calves that received HDM had significantly greater ADG (P<0.05) than LDM, while an interaction between milk DM and age was found for ADG (P<0.05). The male calves had greater final BW and ADG than the females (P<0.05).

Increasing the milk DM had no significant effect on the starter intake (Table 2) however, the ST calves tended to have higher (P=0.08) starter intake than LT group (708.2 vs 634.0 g/d, Table 2) and there was an interaction between water access time and age (P<0.05) on starter intake (Figure 1, a). Neither milk DM concentration nor water access time had an effect on average water intake. An interaction between milk DM and × age (P<0.01) was found on average water intake. The differences between male and female calves for water intake were not significant. Water and starter intakes and growth performance parameters were affected by the calf age (P<0.01, Table 2).

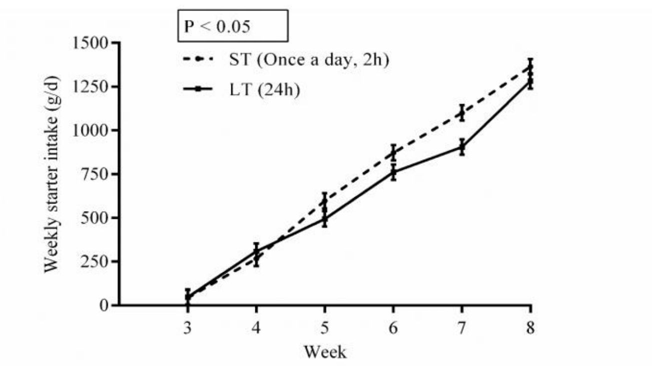

Figure 1a. Weekly starter intake in ST and LT calves; ST = short time free water access (once, 2 hours); LT = long time free water access (24 hours).

There were no significant differences between different milk DM concentrations and two water provision times on hip width and body length (Table 2). While the differences between ST and LT calves for body measurements were not significant, the heart girth (P<0.05), withers height (P<0.01), and hip height (P<0.01) were affected by milk DM (Table 2). Interactions between milk DM and age were found for hip width, heart girth (P<0.05) and body length (P<0.05); however, there were no interactions between water access time and age on any of the body frame measurements (Table 2). The male calves had greater hip width (P<0.05), body length (P<0.01), heart girth (P<0.01), withers height (P<0.05) and hip height (P<0.01) than the female calves.

The effects of water provision schedule and milk DM

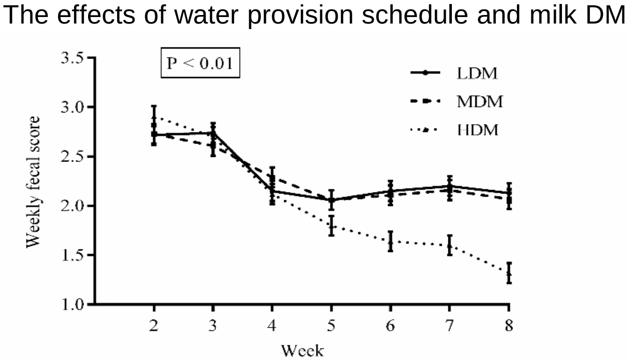

Figure 1b. Weekly fecal score in the LDM, MDM and HDM groups. LDM = low dry matter (125 g/kg); MDM = medium dry matter (140 g/kg); HDM = high dry matter (155 g/kg).

enhancement on water intake and body measurements are presented in Table 3. There were no significant interactions between milk DM and water access time on water intake and body frame measurements but a significant interaction between milk DM, water access time, and age was found for withers height (P<0.05). Figures 2 and 3 (a, b) also show the interactions of main factors (milk DM and water access time) on average starter intake (g/d), ADG (Figure 3, a) and final BW (Figure 3, b) during 56 days of the pre-weaning period, respectively. When DM concentration of milk was 125 or 140 g/kg, the ST calves consumed more (P<0.05) starter than the other groups (Figure 2). The LT calves that consumed HDM had the greatest ADG (P<0.05, Figure 3, a). A trend was found for the interaction between milk DM and water access time; as in the ST calves, final BW was greater than LT at 140 g/kg milk DM concentration (P=0.08) but at 155 g/kg milk DM, the highest final BW was found in LT calves (Figure 3, b).

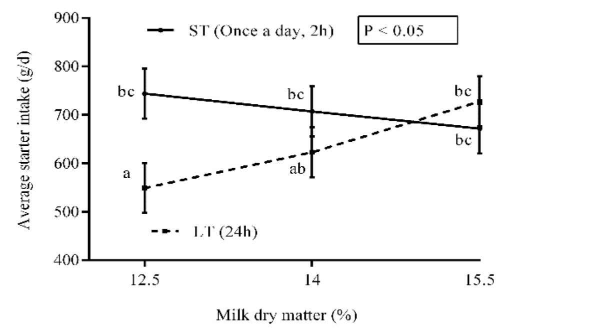

Figure 2. Significant effect of water provision schedule and milk dry matter enhancement on average starter intake (g/d) of neonatal calves during 56 days pre-weaning period. ST = short time free water access (once, 2 hours); LT = long time free water access (24 hours).

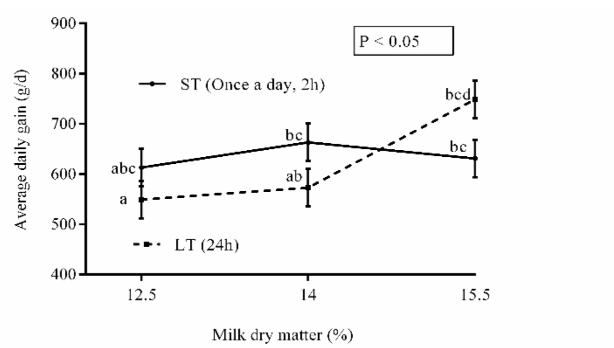

Figure 3a. Effect of water provision schedule and milk dry matter enhancement on average daily gain; ST = short time free water access (once, 2 hours); LT = long time free water access (24 hours).

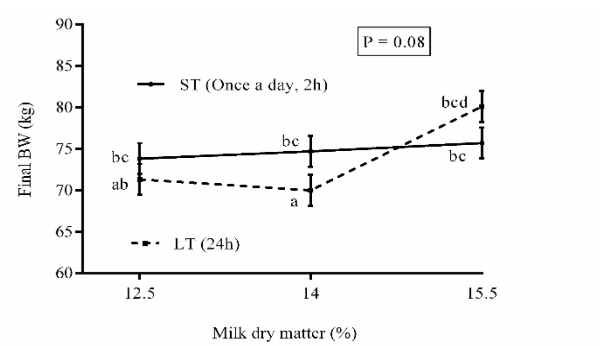

Figure 3b. Effect of water provision schedule and milk dry matter enhancement on final BW of neonatal calves during 56 days pre-weaning period. ST = short time free water access (once, 2 hours); LT = long time free water access (24 hours).

Table 2. Effects of the milk dry matter and water provision schedule, and their interactions with age, and sex on water and starter intake and growth parameters in neonatal calves during 56 days pre-weaning period

Items	Main factors													
	M [1]				W [5]		S[8]		P-Value[11]					
	LDM[2]	MDM[3]	HDM[4]	SEM	LT[6]	ST[7]	Male	Female	M	W	S	A[9]	M×A	W×A
Final BW (kg)	72.59[a]	72.36[a]	77.93[b]	1.32	73.84	74.75	76.99	71.60	< 0.05	0.59	< 0.05	-	-	-
ADG[10] (g/d)	581.4[a]	618.5[a]	690.3[b]	26.5	624.2	635.9	668.4	591.7	< 0.05	0.70	< 0.05	< 0.01	< 0.05	0.92
Average starter intake (g/d)	647.2	665.7	700.4	36.5	634.0	708.2	688.7	653.5	0.58	0.08	0.41	< 0.01	0.35	< 0.05
Average water intake (L/d)	1.04	1.02	1.07	0.15	0.96	1.12	1.07	1.02	0.97	0.36	0.76	< 0.01	< 0.01	0.94
Hip width (cm)	13.52	13.68	13.93	0.16	13.60	13.82	13.90	13.52	0.20	0.24	< 0.05	< 0.01	< 0.05	0.64
Body length (cm)	47.12	46.38	47.35	0.40	46.94	46.95	47.71	46.19	0.20	0.98	< 0.01	< 0.01	< 0.05	0.35
Heart girth (cm)	84.25[a]	83.67[a]	85.76[b]	0.52	84.41	84.69	85.54	83.56	< 0.05	0.64	< 0.01	< 0.01	0.15	0.71
Withers height (cm)	80.07[a]	78.13[b]	80.13[a]	0.46	79.62	79.27	80.07	78.82	< 0.01	0.50	< 0.05	< 0.01	0.86	0.72
Hip height (cm)	85.83[a]	83.75[b]	85.68[a]	0.52	85.19	85.00	85.78	84.40	< 0.01	0.74	< 0.05	< 0.01	0.10	0.39

[1]M = Milk dry matter [2]LDM = low dry matter (125 g/kg); [3]MDM = medium dry matter (140 g/kg); [4]HDM = high dry matter (155 g/kg); [5]W = Water provision schedule; [6]LT = long time free water access (24 hours); [7]ST = short time free water access (once, 2 hours).
[8]S = sex; [9]A = age. For initial BW, P-value obtained by analyzing data as complete random design (six treatments and each ten replicate, five male and five female) as there was no age effect at the beginning of experiment. For other parameters please see statistical analysis in the materials and methods section.
[10]ADG = Average daily gain.
[11] Within rows, mean values with common letter (s) do not differ (P>0.05).

Table 3. Effect of water provision schedule and milk dry matter enhancement on performance and intake of neonatal calves during 56 days pre-weaning period.

Items	LDM[1]		MDM[2]		HDM[3]		SEM	P-value	
	LT[4]	ST[5]	LT	ST	LT	ST		M×W[6]	M×W×A[7]
Initial BW (kg)	42.3	41.7	40.7	42.3	43.1	41.9	0.74	0.14	-
Average water intake (L/d)	0.81	1.28	0.91	1.12	1.17	0.97	0.21	0.28	0.76
Hip width (cm)	13.2	13.8	13.5	13.8	14.0	13.8	0.23	0.15	0.17
Body length (cm)	47.0	47.2	46.3	46.5	47.5	47.2	0.56	0.84	0.30
Heart girth (cm)	84.0	84.6	82.9	84.4	86.4	85.1	0.73	0.14	0.38
Withers height (cm)	80.0	80.2	78.2	78.1	80.8	79.5	0.65	0.47	< 0.05
Hip height (cm)	85.6	86.0	83.8	83.7	86.2	85.2	0.73	0.64	0.41

[1]LDM = low dry matter (125 g/kg); [2]MDM = medium dry matter (140 g/kg); [3]HDM = high dry matter (155 g/kg); [4]LT = long time free water access (24 hours). [5]ST = short time free water access (once, 2 hours); [6]M = Milk dry matter; W = Water provision schedule; [7]A = age. For initial BW, P-value obtained by analyzing data as complete random design (six treatments and each ten replicate, five male and five female) as there was no age effect at the beginning of experiment. For other parameters please see statistical analysis in the materials and methods section.

Health and blood parameters

The main effects of milk DM, water access time and their interactions with age (A), and the effects of sex (S) and age (A) on blood parameters and health indicators such as RBT, fecal score, and diarrhea duration are presented in Table 4. A trend was found for higher alkaline phosphatase (ALP) concentration by increasing the milk DM (P=0.06, Table 4). The LT calves tended (P=0.08) to have higher AST than ST group (Table 4). However, the effect of milk DM concentration or water provision schedule on the other measured blood parameters was not significant (Table 4). The HDM calves had the lowest (P<0.01) AFS (Table 4) and an interaction between milk DM and age (P<0.01) was found on AFS (Figure 1, b). The effects of milk DM concentration and water provision schedule on RBT and diarrhea cases and days were not significant. The male calves showed lower diarrhea cases and days (P<0.01) than the female calves. Except blood urea and ALT, the calf age significantly influenced other blood parameters and health indicators. No interaction was found between water access time and calf age (Table 4).

Table 5 displays the interactions of main factors on the blood and health parameters. An interaction between milk DM and water access time was observed for blood TG (P<0.05) and ALP (P<0.05) concentrations (Table 5). The ST calves exhibited greater ALP level in the blood serum at milk DM of 155 g/kg. Interactions between milk DM, water access time and age were not significant for any parameter listed in the Table 5.

Discussion

Milk concentration increment

The calves fed a fixed amount of liquid feed (10% of initial BW) during the experiment always consumed all their daily liquid feed allowance. Interestingly, increasing the milk DM concentration enhanced nutrients intake via liquid feed without a negative effect on the starter intake. Therefore, supplying nutrients by using the high solid-liquid feed, as shown in this study (at an average temperature of 9˚C), could increase the overall nutrient intake. This is very important during the cold periods which is often difficult to ensure nutritional sufficiency in dairy calves through solid feed (Drackley, 2005). As shown in figures 3 (a) and (b), the main response to increased DMI via solid and liquid feeds was observed on the growth performance. Similar to our findings, Norouzi et al. (2021) and Azevedo et al. (2016) also observed that enhancing the liquid feed concentration of suckling calves resulted in an increase in final BW and ADG without influencing the starter intake.

Beside BW improvement, skeletal measures such as hearth girth and withers and hip heights were also enhanced in calves fed concentrated whole milk. Norouzi et al. (2021) also found that increasing the solids in liquid feed caused greater body length at weaning. Furthermore, Azevedo et al. (2016) observed an increase in the heart girth and wither height by increasing the concentration of solids in the liquid feed.

In the present study, diarrhea days and cases, and RBT were similar in different liquid feed concentrations (Table 4) which is in line with previous reports (Azevedo et al., 2016; Norouzi et al., 2021) when MRP was added to the whole milk for getting targeted milk DM. Even so, the HDM calves showed the lowest AFS (Figure 1, b). In contrast, Glosson et al. (2015) observed that increasing the milk DM by 175 g/kg, using MRP enhanced the fecal scores. Therefore, it seems that increasing the milk DM concentration by 155 g/kg is a tolerable level in Holstein calves that could increase the nutrient supply without affecting the calf health.

It was found that increasing the quantity of liquid feed in dairy calves reduced the starter intake (Hill et al., 2010, 2013; Gelsinger et al., 2016) and led to undesirable effects such as weight loss at weaning and difficulties in transiting from liquid to solid feed post-weaning. Therefore, increasing the concentration of liquid feed may provide advantages over the high level of liquid with usual DM content (Rosenberger et al., 2017). Calves of indigenous cattle breeds had more milk total solids than the current high-producing breeds (Sharma et al., 2018). Based on the earliest practice aimed to increase milk concentration (Pettyjohn et al., 1963), the latest relevant publication (Norouzi et al., 2021) and the present work that liquid feed concentration was increased, it can be established that feeding milk with a DM concentration of about 150 g/kg was a successful strategy for increasing DMI of neonatal calves.

Water provision schedule

One of the worries in restricting water provision for neonatal calves might be a reduction in water intake and subsequent influence on the starter intake and animal health. As presented in Tables 2-5, there were no adverse effect of reducing free water provision time from LT to ST on water intake, starter intake, and growth and health parameters. In line with our observations, it was found that restriction of water access time did not negatively impact on ADG and weaning BW in dairy calves (Broucek, 2019; Wickramasinghe et al., 2019). Jensen and Vestergaard (2021) reported that when access to water is limited, an increase in the motivation to drink may occur that could be associated with a negative subjective experience of thirst. For such an adaptation to short time free water access, calves in the ST groups consumed significantly greater amount of water per unit of time compared to those that on LT schedule.

When milk was fed at low DM (LDM), increasing the duration of free water access was not advantageous to the ST scenario for any of the performance parameters. Therefore, although water is an essential nutrient in livestock nutrition that must be supplied daily for normal health and performance, it does not mean that water needs to be provided throughout 24 h especially for young calves that are able to obtain most of their water needs through liquid feed (Jensen and Vestergaard, 2021). Dracy and Kurtenbach (1968) showed that following consumption of liquid feed at 7.78 °C temperature, a sudden drop of 10 °C in the rumen temperature occurred and it took about an hour to return to the recover to normal values. Presumably, during the cold season, shortening the water provision time reduced rumen temperature fluctuation in the ST than LT groups that was overall for the health benefit of the calves. Figures 2 and 3 show that starter intake and growth performance were numerically greater for ST groups than LT at low and medium milk DM levels.

Effects of time and interaction of milk DM and water provision schedule and main factors with time

The present study was conducted during the period of rumen development in growing calves. This event increased the solid feed intake and thus, significant impact of the age on many parameters particularly intake and growth performance is a usual event. It was also found that the AFS decreased in growing calves (Figure 1, b) which could be due to the reduction in the incidence of diarrhea, which was more notable in the HDM calves. The ST calves in the LDM and HDM had greater serum TG than LT but not in the MDM group for un-known reasons. The ST calves in the HDM group had the highest blood ALP concentration (Table 5). This might be attributed to the body's effort in response to ST scenario at HDM regime. Beside this, Figure 2 indicates that there was a negative slope in the linear response of the starter intake to milk concentration when the 2 h free water provision schedule was used. Probably by increasing the milk DM to 155 g/kg, calves required more water and they could not obtain it within 2 h (Table 3). The growth indicators presented in Figure 3 also showed falling ADG and final BW in the ST calves below that of LT calves when milk DM was 155 g/kg; therefore, when water provision is 2 h once a day, increasing the solid content of milk by 140 g/kg might be advisable.

Conclusions

With provision of free water throughout the day, condensation of the whole milk up to 155 g/kg could be recommended as a practical strategy for increasing the DM intake and performance of dairy calves. Water provision duration for neonatal calves could be reduced to once a day (just 2 h) however; we do not recommend increasing the solid content of milk by more than 140 g/kg when water provision is 2 h once a day.

Institutional Review Board Statement

The animal study protocol was approved by the Animal Care and Use Committee of Ferdowsi University of Mashhad (FUM) outlined by the Iranian Council of Animal Care (Iranian Council of Animal Care, 1995). Furthermore, the Ethics Committee of FUM accepted and supervised all experimental processes (130/21/06/2018).

Acknowledgments

The authors are grateful to Mr. Mohammad Ali Dabiri, Head and Mr. Mohsen Mahroughi, the nursery expert of the Dasht dairy farm, Neyshabour, Iran, for providing the required experimental facilities and supporting this study. The research of the corresponding authors is supported by a grant from Ferdowsi University of Mashhad (N. 3/ 45939).

Conflicts of Interest

The authors declare no conflict of interest.

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Table 4. Effects of main factors (the milk dry matter and water provision schedule), their interactions with age and sex on blood and health parameters of neonatal calves during 56 days pre-weaning period.

	Main factors													
	M[1]				W[5]		S[8]		P-Value					
Items	LDM[2]	MDM[3]	HDM[4]	SEM	LT[6]	ST[7]	Male	Female	M	W	S	A[9]	M×T	W×T
Glucose (mg/dL)	101.8	99.67	91.92	7.91	97.89	97.72	91.33	104.28	0.65	0.98	0.16	< 0.01	0.12	0.89
Urea (mg/dL)	25.79	32.58	29.33	4.56	30.94	27.53	31.53	26.95	0.58	0.52	0.39	0.32	0.34	0.46
Creatinine (mg/dL)	1.08	1.04	1.12	0.03	1.06	1.10	1.11	1.05	0.29	0.34	0.14	< 0.01	0.55	0.42
Cholesterol (mg/dL)	83.12	89.50	94.21	5.73	86.22	91.77	88.08	89.91	0.41	0.41	0.78	< 0.01	0.20	0.97
Triglyceride (mg/dL)	38.21	41.08	41.37	2.83	40.14	40.31	37.81	42.64	0.68	0.96	0.15	< 0.01	0.81	0.94
Total protein (g/dL)	6.00	5.92	5.93	0.10	5.92	5.99	6.10	5.80	0.85	0.55	0.02	< 0.01	0.14	0.25
AST[10] (IU/L)	43.42	48.67	44.71	2.59	48.25	42.94	44.78	46.42	0.34	0.08	0.59	< 0.01	0.02	0.14
ALT[11] (IU/L)	16.58	15.75	14.21	2.60	15.61	15.42	14.5	16.53	0.81	0.95	0.50	0.09	0.98	0.94
ALP[12] (IU/L)	469.7	464.2	637.7	55.89	483.0	564.7	471.7	576.1	0.06	0.21	0.11	< 0.01	0.38	0.70
BHBA[13] (mmol/L)	1.40	1.12	1.36	0.11	1.28	1.31	1.28	1.30	0.16	0.84	0.88	0.05	0.97	0.31
RBT[14] (˚C)	38.67	38.72	38.62	0.07	38.73	38.61	38.64	38.70	0.66	0.15	0.54	0.03	0.53	0.13
Average diarrhea cases[15]	0.90	1.00	0.60	0.15	0.93	0.73	0.50	1.17	0.14	0.24	< 0.01	-	-	-
Average diarrhea days[16]	1.75	2.00	1.30	0.35	1.87	1.50	1.03	2.33	0.34	0.35	< 0.01	< 0.01	0.84	0.86
AFS[17]	2.31[a]	2.29[a]	2.01[b]	0.06	2.22	2.19	0.15	2.25	< 0.01	0.58	0.14	< 0.01	< 0.01	0.12

[1]M = Milk dry matter; [2]LDM = low dry matter (125 g/kg); [3]MDM = medium dry matter (140 g/kg); [4]HDM = high dry matter (155 g/kg); [5]W = Water provision schedule; [6]ST = short time free water access (once, 2 hours); [7]LT = long time free water access (24 hours).
[8] S = sex; [9]A = age; [10]AST = Aspartate transaminase; [11]ALT = Alanine transaminase; [12]ALP = alkaline phosphatase; [13]BHBA = beta-hydroxybutyric acid; [14] RBT= Rectal body temperature; [15] Average affected cases during eight weeks' experiment in each group; [16] Average days with fecal score of 4 and 5 in each group = total diarrhea days/number of calves in the group; [17]AFS = average fecal score; fecal consistency was scored according to 1 = normal; 2 = soft to loose; 3 = loose to watery; 4 = watery, mucous; 5 = watery, mucous, and bloody.
Within rows, mean values with common letter (s) do not differ (P>0.05).

Table 5. Effect of water provision schedule and milk dry matter enhancement on average blood metabolites measurements of neonatal calves during 56 days pre-weaning period.

Items	LDM[1]		MDM[2]		HDM[3]			P-value	
	LT[4]	ST[5]	LT	ST	LT	ST	SEM	M×W[6]	M×W×A[7]
Glucose (mg/dL)	105.8	97.8	102.0	97.3	85.8	98.0	11.18	0.63	0.41
Urea (mg/dL)	25.8	25.7	37.8	27.3	29.2	29.5	6.45	0.64	0.34
Creatinine (mg/dL)	1.07	1.09	1.01	1.07	1.11	1.13	0.05	0.89	0.32
Cholesterol (mg/dL)	82.1	84.5	87.0	92.0	89.6	98.8	8.11	0.91	0.29
Triglyceride (mg/dL)	33.4[a]	43.0[bc]	48.1[bc]	34.1[a]	38.9[ab]	43.8[bc]	4.00	0.01	0.26
Total protein (g/dL)	6.00	6.00	5.90	5.95	5.85	6.02	0.15	0.84	0.36
AST[8] (IU/L)	46.9	39.9	53.3	44.0	44.5	44.9	3.66	0.39	0.94
ALT[9] (IU/L)	15.2	18.0	17.7	13.8	14.0	14.4	3.68	0.66	0.86
ALP[10] (IU/L)	486[a]	453[a]	483[a]	444[a]	479[a]	796[b]	79.0	< 0.05	0.37
BHBA[11] (mmol/L)	1.31	1.48	1.14	1.10	1.39	1.33	0.15	0.70	0.44
RBT[12] (˚C)	38.7	38.7	38.9	38.5	38.6	38.6	0.10	0.18	0.54
Average diarrhea cases[13]	1.1	0.7	1.1	0.9	0.6	0.6	0.21	0.63	-
Average diarrhea days[14]	2.2	1.3	2.1	1.9	1.3	1.3	0.48	0.62	0.99
AFS[15]	2.28	2.33	2.40	2.18	1.98	2.05	0.08	0.15	0.14

[1]LDM = low dry matter (125 g/kg); [2]MDM = medium dry matter (140 g/kg); [3]HDM = high dry matter (155 g/kg); [4]LT = long time free water access (24 hours). [5]ST = short time free water access (once, 2 hours); [6]M = Milk dry matter; W = Water provision schedule; [7]A = age; [8]AST = aspartate transferase; [9]ALT = Alanine transaminase; [10]ALP = alkaline phosphatase; [11]BHBA = beta-hydroxybutyric acid;[12]RBT = Rectal body temperature; [13] Average affected cases during eight weeks' experiment in each group; [14] Average days with fecal score of 4 and 5 in each group = total diarrhea days/number of calves in the group; [15]AFS = average fecal score; fecal consistency was scored according to 1 = normal; 2 = soft to loose; 3 = loose to watery; 4 = watery, mucous; 5 = watery, mucous, and bloody.
Within rows, mean values with common letter (s) do not differ (P>0.05).