Milk feeding quantity and feeding frequency: effects on growth performance, rumen fermentation and blood metabolites of Holstein dairy calves

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ABSTRACT

The aim of this study was to investigate interactions between milk feeding quantity (FL) and feeding frequency (FF) on intake, growth performance, rumen development and blood metabolites in dairy calves. A total of 48 Holstein calves (n = 12 calves per treatment: 6 males and 6 females) were randomly assigned to one of four treatments, including (1) two low plane of milk feeding (2 meals/d, LPM-2/C2; 3 meals/d, LPM-3/C2; total milk intake = 210 L) and two high plane of milk feeding (2 meals/d, HPM-2/C2; 3 meals/d, HPM-3/C2; total milk intake = 371 L). Calves fed HPM had higher BW, ADG, blood glucose and triglycerides concentration during the preweaning. Calves fed HPM received more ME, total DMI and ate less starter than LPM calves during preweaning. Regardless of the milk feeding quantity, with increasing FF, starter intake, total DMI and ME were reduced without a negative effect on ADG. Calves fed 3 times/d compared with 2 times/d had a lower final hip width. Calves fed HPM had higher ruminal pH and molar proportion of acetate and acetate to propionate ratio, but less molar proportion of propionate and butyrate and total VFA compared with calves fed LPM on d 35 of the study. Interaction between milk feeding level and feeding frequency was observed for plasma glucose concentration with HPM-3/C2 calves having the greatest value at d 35 of study. In conclusion, at both levels of milk feeding, calves did not benefit from the increased feeding frequency, but calves benefitted from HPM.

HIGHLIGHTS

- Calves did not benefit from increased milk feeding frequency at either low or high plane of milk feeding.
- The performance of calves on low plane of milk feeding was constrained by the low nutrient intake and not the feeding frequency.
- Regardless of plane of milk feeding, feeding 3 meals/d reduced total DM and starter intake with no detrimental effects on overall average daily gain.

Introduction

Many studies have explored the best milk feeding pattern and quantity in dairy calves that can improve their performance and health without negatively affecting solid feed consumption or rumen development (Silper et al. 2014; Daneshvar et al. 2015; Omidi-Mirzaei et al. 2015). In the traditional feeding method, the calf is usually fed a restricted amount (about 4 L/d or 10% of the birth weight) of milk or milk replacer (MR) allotted into two meals per day (Jasper and Weary 2002). Feeding a restricted amount of milk to increase solid dry matter intake, promote rumen
development, and reduce the age at weaning has been widely practiced on dairy calf enterprises (Davis and Drackley 1998; Khan et al. 2011). However, rearing calves on a low amount of milk or milk replacer (MR) leads to negative effects on calf welfare, for example, hunger and vocalisation (Vieira et al. 2008; Rosenberger et al. 2017) and compromises their pre-weaning growth compared with calves receiving a higher milk allowance (20% of the initial BW) (Khan et al. 2011). Recent studies have shown that feeding more milk could improve growth and feed intake, reduce non-nutritional behaviours and illnesses (Khan et al. 2011; Silper et al. 2014; Orellana Rivas et al. 2020), and potentially improve milk production in the first lactation (Soberon et al. 2012; Gelsinger et al. 2016; Chester-Jones et al. 2017). Feeding high planes of milk (≥ 8 L/d, about 20% of the birth weight) might have some disadvantages such as reduced calf starter intake, delayed rumen development and increased age at weaning (Vi et al. 2004; Terre et al. 2009; De Paula et al. 2017). Besides, some of the growth advantages realised with the high plane of milk feeding may be lost after weaning, because calves are not prepared to maintain their growth solely from solid feed (Bach et al. 2013). For this reason, a gradual reduction in the volume of liquid feed in the last weeks before weaning has been proposed as a good strategy for stimulating concentrate intake, facilitating weaning, and preventing reduced growth performance (De Paula et al. 2017).

A high level of milk feeding (for instance 6 L/d) has the potential to sufficiently support calves’ performance during the pre-weaning period under farm practical conditions. An incremental step-up/step-down pattern of milk supply to calves has been proposed (Khan et al. 2007). In this method, milk feeding is gradually increased to reach a peak in the middle of the milk feeding period before it is gradually decreased to the original level towards the end of the period (Omidi-Mirzae et al. 2015). In the step-up phase, the calf receives more nutrients from milk while in the step-down phase, lesser amount of milk is supplied to stimulate solid feed intake. Omidi-Mirzae et al. (2015) showed starter intake, total dry matter intake, and average daily gain were greater in calves fed using the step-up/step-down procedure compared to those in the conventional procedure during the preweaning period.

Apart from the quantity of milk offered to calves, the milk feeding frequency (FF) can also be manipulated to alter the nutrient intake throughout the day. In nature, a calf left with its dam can on average suckle 7 to 10 times per day resulting in a well distributed nutrient supply (Albright and Arave 1997). Modern farms try to imitate the natural conditions by using automatic milk feeders that allow the calf to consume their daily ration in several meals per day. But, some dairy farms might favour feeding whole milk and waste milk as a result of changes in milk replacer prices (Saldana et al. 2019). Several studies have indicated that feeding milk or milk replacer once daily reduced labour-costs without any effect on health, weight gain and solid feed consumption (Willett et al. 1969; Stanley et al. 2002; Saldana et al. 2019), yet careful management is vital for its success. It has been suggested that feeding high plane of milk in calves can lead to problems such as milk backflow into the reticulorumen (Van den Borne et al. 2006). Besides, rapid intake of a large quantity of milk over the abomasal capacity may lead to clotting protein source and thus slow release and absorption of AA during the day (Van den Borne et al. 2006). Clotting of milk in the abomasum, due to enzymes (chymosin and pepsin) and hydrochloric acid acting on casein and fat in the milk has for long been thought to be important in the milk digestion process of preruminant calves (Longenbach and Heinrichs 1998). Clotting binds much of the casein and fat into a clump, or curd, which is digested slowly by stomach enzymes over a period of 12 to 18 h, allowing calves to absorb and assimilate the nutrients more slowly and efficiently (Cruywagen and Horn-Quass 1991). In a study by Okada et al. (2010), frequent feeding of milk and milk replacer in Holstein calves (6×/d) led to formation of looser curds compared to 2×/d (Okada et al. 2010). This might have consequences on nutrient supply in pre-weaning calves, hence the need for further investigation. Providing large amounts of milk twice daily have linked to reduced insulin sensitivity which may have consequences for future metabolic responses (Bach et al. 2013; MacPherson et al. 2019). Alternatively, calves could be fed higher volumes in several meals to mitigate the challenges stated. An increased FF was shown to enhance the efficiency of energy and protein utilisation in heavy veal calves (Van den Borne et al. 2006). However, recently, it has been shown that calves fed milk replacer containing different sources of protein (whey protein vs. a combination of whey protein, bovine plasma protein, and modified wheat protein) did not benefit from increased FF (Grice et al. 2020).

We hypothesised that increasing FF at high milk feeding level (FL) would positively affect the health, solid feed consumption and rumen development of
the calf hence improve overall calf performance. Therefore, the purpose of this study was to investigate the interaction between the milk feeding quantity (low vs. high) and milk feeding frequency (2 times vs. 3 times) on growth performance, health and rumen development of calves.

Materials and methods

All the animal procedures were approved by the Animal Care and Use Committee of Isfahan University of Technology (IACUC #2018005) as outlined in the Iranian Council of Animal Care (1995).

Animals, management, and treatments

The experiment was performed on a dairy cattle farm (Fazil Agri. Industrial Co., Isfahan, Iran) from the beginning of spring 2018 for a 3-mo period. The average ambient temperature during the experimental period was 24°C (range: 19 to 30°C). A total of 48 Holstein calves (40.4 ± 1.5 kg of BW, n = 12) per treatment (6 males and 6 females) were enrolled in the experiment. Calves were separated from their dams immediately after birth, weighed, and moved in a naturally ventilated barn with individual pens (from birth until d 3 in 1.2 × 2.4 m pens and thereafter in 1.8 × 2.8 m pens). A fresh blend of sand and sawdust (7:1) was used as bedding and refreshed every day. Manure was removed daily to keep the pens visibly clean and dry. The calves were fed 2.5 L of colostrum within 1 h of birth and 2.5 L 6 h later. From the second feeding time until d 3 of life, all calves received colostrum and transition milk via bottles with nipple twice daily. 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. Furthermore, six representative samples (starter only) were obtained after mixing them and used by the Penn State Particle Separator (PSPS; Table 1) to evaluate particle size distribution of the feeds provided (starter and forage) (ASAE, 1996; method S424.1).

This study used a randomised complete block design with a 2 × 2 factorial arrangements, with different milk feeding rates and frequency. Treatments included: (1) calves fed low plane of milk (LPM) fed twice daily (LPM-2 ×), 4 L/d of milk from wk 1 to 7, and 2 L/d of milk for the last week; total milk intake = 210 L); (2) calves fed LPM intake and three times daily (LPM-3 ×); total milk intake = 210 L); (3) calves fed high plane of milk (HPM) and twice daily (HPM-2 ×), 4 L/d of milk during wk 1, 6 L/d of milk during wk 2, and 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 total milk intake = 371 L); (4) calves fed HPM intake and three times daily (HPM-3 ×), total milk intake = 371 L). Calves were weaned on d 56 and remained in the study until d 70 of the study. All calves were fed pasteurised waste milk containing 3.65 ± 0.07% fat, 2.85 ± 0.02% CP, 4.34 ± 0.03% lactose, and 11.50 ± 0.18% total solids. Milk pasteurisation was performed for 30 min at 60°C and milk was fed at a temperature of 39 ± 0.5°C by the bucket. Calves on LPM-2 × and HPM-2 × treatments were fed milk at 08:30 and 16:30 h from wk 1 to 7 and half in each meal during wk 8. Calves on LPM-3 × and HPM-3 × treatments received 3 meals/d at 08:30, 16:30 and 23:00 h from wk 1 to 7 and half in each meal during wk 8. From the third day until the end of the study, calves were allowed free access to a texturised calf starter and water. The starter diet was blended with 7% of chopped alfalfa hay as a proportion of diet DM. The starter feed contained steam-flaked corn and barley with a 3 mm pellet of other components. The ingredients and nutrient composition of the starter feed and hay is given in Table 1. The starter feed offered was 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 that fell sick were treated by a veterinarian based on the standard operating procedures at the Fazil Agri. Industrial Co. (Isfahan, Iran).

Six representative samples (for calf starter only) were used for fractional distribution of particle size. The particular size distribution of experimental starters was determined by dry sieving using an automated sieve shaker (model 120; Techno Khak, Khavaranz, Tehran, Iran) with screens of 4, 75-, 2, 36-, 1, 18-, 0.6-, 0.3-, and 0.15 mm diameter (Table 1). Approximately 200 g of sample was put on the top screen and the sieve stack was shaken until there was no change in material distribution (about 10 min). Geometric mean particle size was calculated as described by the American Society of Agricultural Engineers (ASAE, 1983). Furthermore, six representative samples (starter mixed with chopped alfalfa hay at 93:7 ratio) were obtained after mixing them and used by the Penn State Particle Separator (PSPS; Table 1) to evaluate particle size distribution of the feeds provided (starter and forage) (ASAE, 1996; method S424.1).
Chemical analyses. Subsamples of feed and refusals were collected weekly throughout the trial and stored at −20°C before chemical analyses. Subsamples of feed 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 starter feed and refusals were analysed for DM (AOAC, 2000; method 925.40), CP (Kjeltec 1030 Auto Analyser, Tocater, Höganas, Sweden; AOAC International, 2002; method 955.04), ether extract (EE; AOAC, 2000; method 920.39), ash (AOAC, 2000; method 942.05), NDF using a heat-stable x-amylase (100 μL/0.5 g of sample) with sodium sulphite (Van Soest et al. 1991). Non-fibrous carbohydrate (NFC) component was computed as 100 – (CP + NDF + EE + ash) (NRC 2001). Calves were weighed on d 1 of the study and thereafter, each week by an electronic scale (Model EES-500; Ettehad Inc., Isfahan, Iran), which was calibrated by the manufacturer’s agent before initiation of the study and every month thereafter. Individual starter intake and milk intake were computed daily to determine total DMI. Values from BW measurements were used to compute weight gains. Feed efficiency (FE) was calculated as kg of ADG/total DMI (liquid feed DMI + starter feed DMI). Body measurements including body length (BL, distance between the points of shoulder and rump), withers height (WH, distance from base of the front feet to the withers), heart girth (HG, circumference of the chest), body depth (BD, circumference of the belly before feeding), hip-height (HH, distance from base of rear feet to hook bones), and hip-width (HW, distance between points of hook bones) were also recorded at first day of study (d 0), at weaning (d 56) and the end of the study (d 70) according to (Lesmeister and Heinrichs 2004).

Rumen fluid samples were collected with a stomach tube fitted to a vacuum pump 3 h after morning feeding on d 35 and 70. The first 50 mL was discarded to avoid saliva contamination. Samples were subsequently squeezed through 4 layers of cheesecloth to collect rumen fluid, and 8 mL of the rumen fluid was immediately acidified with 2 mL of 25% metaphosphoric acid and stored at −20°C pending VFA analysis. Rumen VFA concentrations were measured by GC (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;}

### Table 1. Ingredients, chemical composition, and particle size distribution of the experimental diet.

| Ingredient                        | % of dry matter (DM) | Texturised starter |
|-----------------------------------|----------------------|--------------------|
| Chopped alfalfa hay               | 7.0                  |                    |
| Steam-flaked corn                 | 37.2                 |                    |
| Steam-rolled barley               | 18.6                 |                    |
| Soybean meal (45% crude protein (CP)) | 28.8               |                    |
| Full fat soybean                  | 4.7                  |                    |
| Calcium carbonate                 | 0.7                  |                    |
| Vitamin and mineral mix           | 1.8                  |                    |
| Sodium bicarbonate                | 0.7                  |                    |
| Salt                              | 0.5                  |                    |
| DM                               |                      |                    |
| Metabolizable energy (ME) (Mcal/kg DM) | 3.13                |                    |
| Crude protein (CP)                | 21.7                 |                    |
| Ether extract (EE)                | 3.6                  |                    |
| Neutral detergent fiber (NDF)     | 17.4                 |                    |
| Non-fiber carbohydrates (NFC)     | 48.8                 |                    |
| Calcium (Ca)                      | 0.9                  |                    |
| Phosphorous (P)                   | 0.5                  |                    |
| Particle size distribution of starter feed, % retained on sieve |                     |                    |
| 4.75 mm                           | 30.2 ± 2.9           |                    |
| 2.36 mm                           | 65.3 ± 4.2           |                    |
| 1.18 mm                           | 1.8 ± 0.3            |                    |
| 0.6 mm                            | 1.4 ± 0.3            |                    |
| 0.3 mm                            | 1.1 ± 0.2            |                    |
| 0.15 mm                           | 0.3 ± 0.1            |                    |
| Pan                               | 0.3 ± 0.5            |                    |
| GMPLf, mm                         | 2.74 ± 0.01          |                    |
| Particle size distribution of mixed feed (starter feed with alfalfa hay), % retained on sieve |                     |                    |
| 19                                | 0.3 ± 0.1            |                    |
| 18                                | 15.5 ± 2.2           |                    |
| 1.18                              | 79.5 ± 3.2           |                    |
| Pan                               | 6.4 ± 2.1            |                    |
| GMPLe, mm                         | 4.15 ± 1.77          |                    |

| Ingredient                        | % of dry matter (DM) | Texturised starter |
|-----------------------------------|----------------------|--------------------|
| Chopped alfalfa hay               | 7.0                  |                    |
| Steam-flaked corn                 | 37.2                 |                    |
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| Full fat soybean                  | 4.7                  |                    |
| Calcium carbonate                 | 0.7                  |                    |
| Vitamin and mineral mix           | 1.8                  |                    |
| Sodium bicarbonate                | 0.7                  |                    |
| Salt                              | 0.5                  |                    |
| DM                               |                      |                    |
| Metabolizable energy (ME) (Mcal/kg DM) | 3.13                |                    |
| Crude protein (CP)                | 21.7                 |                    |
| Ether extract (EE)                | 3.6                  |                    |
| Neutral detergent fiber (NDF)     | 17.4                 |                    |
| Non-fiber carbohydrates (NFC)     | 48.8                 |                    |
| Calcium (Ca)                      | 0.9                  |                    |
| Phosphorous (P)                   | 0.5                  |                    |
| Particle size distribution of starter feed, % retained on sieve |                     |                    |
| 4.75 mm                           | 30.2 ± 2.9           |                    |
| 2.36 mm                           | 65.3 ± 4.2           |                    |
| 1.18 mm                           | 1.8 ± 0.3            |                    |
| 0.6 mm                            | 1.4 ± 0.3            |                    |
| 0.3 mm                            | 1.1 ± 0.2            |                    |
| 0.15 mm                           | 0.3 ± 0.1            |                    |
| Pan                               | 0.3 ± 0.5            |                    |
| GMPLf, mm                         | 2.74 ± 0.01          |                    |
| Particle size distribution of mixed feed (starter feed with alfalfa hay), % retained on sieve |                     |                    |
| 19                                | 0.3 ± 0.1            |                    |
| 18                                | 15.5 ± 2.2           |                    |
| 1.18                              | 79.5 ± 3.2           |                    |
| Pan                               | 6.4 ± 2.1            |                    |
| GMPLe, mm                         | 4.15 ± 1.77          |                    |

*a*All these ingredients were pelleted.

*Contained per kg of supplement: Vitamin (Vit) A (IU) = 750000, Vit D3 (IU) = 25000, Vit E (IU) = 500, Ca (g) = 100, P (g) = 10, Magnesium (g) = 20, Zinc (Vellena et al.) = 1000, Copper (Vellena et al.) = 250, Iodine (Vellena et al.) = 50, Cobalt (Vellena et al.) = 60, Manganese (Vellena et al.) = 500, Selenium (Vellena et al.) = 50.

*NFC = 100 − (% NDF + % CP + % ether extract + % ash) (NRC 2001).*

*Estimated using the NRC (2001) model.*

*GMPL: geometric mean particle size; calculated as described by the American Society of Agricultural Engineers (1983).*

*GMPLe: geometric mean particle size calculated as described by the American Society of Agricultural Engineers (1996; method 5424.1).*

### Table 2. Mean chemical composition of corn, barley, soybean meal (g/kg of DM), and whole milk (%).

| Item               | Corn grain | Barley grain | Soybean meal | Whole milk |
|--------------------|------------|--------------|--------------|------------|
| DM                 | 88.1       | 91.0         | 89.1         | 11.50 ± 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         | ND           | ND           | 4.34 ± 0.03 |

ND: not determined.

**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, fat, protein and lactose concentrations using an infra-red analyser (MilkoScan 134 BN; Foss Electric, Hillerød, Denmark; Table 2). Fresh starter and hay were fed every morning at 8.00 a.m. Individual feed intake was determined daily by weighing the amounts of feed offered and refused to determine DMI. Feed samples were collected weekly throughout the trial and stored at −20°C before chemical analyses. Subsamples of feed 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).
Table 3. Effects of milk feeding level [(High (HPM) (up to 10 L/d) vs. Low (LPM) (4 L/d)] and milk feeding frequency (2× vs. 3×) on Dry matter intake (DMI), Starter intake (SI), Feed intake (FI), Metabolizable energy (MEI), Average daily gain (ADG), ADG/ME intake, and feed efficiency of dairy calves.

| Item                                    | HPM-2× | HPM-3× | LPM-2× | LPM-3× | SEM | FL | FF | W | FL × FF | FL × W | FL × W | FL × FF × W |
|-----------------------------------------|--------|--------|--------|--------|-----|----|----|---|--------|--------|--------|------------|
| Total DMI (g/d)                         | 997.24 | 903.42 | 873.59 | 779.62 | 18.26 | <0.01 | 0.007 | <0.01 | 0.95 | <0.01 | 0.004 | 0.12 |
| Postweaning                             | 1569.95 | 1315.29 | 1741.59 | 1554.08 | 42.96 | 0.01 | 0.007 | <0.01 | 0.66 | 0.08 | 0.25 | 0.45 |
| Overall                                 | 1115.52 | 985.18 | 1047.45 | 934.77 | 21.98 | 0.07 | <0.01 | <0.01 | 0.84 | <0.01 | 0.04 | 0.74 |
| Preweaning,% of BW                      | 1.69 | 1.53 | 1.58 | 1.43 | 0.02 | 0.005 | <0.01 | <0.01 | 0.92 | 0.002 | 0.14 | 0.06 |
| Postweaning, % of BW                    | 1.80 | 1.57 | 2.12 | 1.94 | 0.05 | <0.01 | 0.009 | <0.01 | 0.81 | 0.04 | 0.14 | 0.38 |
| Overall, % of BW                        | 1.71 | 1.54 | 1.69 | 1.53 | 0.02 | 0.58 | <0.01 | <0.01 | 0.84 | <0.01 | 0.02 | 0.46 |
| Starter DM and forage intake (g/d)      | 235.36 | 141.54 | 442.34 | 348.37 | 19.55 | <0.01 | 0.009 | <0.01 | 0.98 | <0.01 | 0.001 | 0.31 |
| Preweaning                              | 1569.95 | 1315.29 | 1741.59 | 1554.08 | 42.96 | 0.01 | 0.007 | <0.01 | 0.66 | 0.08 | 0.25 | 0.45 |
| Overall                                 | 502.00 | 386.58 | 702.47 | 589.80 | 32.77 | <0.01 | <0.01 | <0.01 | 0.84 | <0.01 | 0.04 | 0.74 |
| Preweaning, % of BW                     | 0.34 | 0.21 | 0.73 | 0.58 | 0.03 | <0.01 | 0.001 | <0.01 | 0.83 | <0.01 | 0.005 | 0.14 |
| Postweaning, % of BW                    | 1.80 | 1.57 | 2.12 | 1.94 | 0.05 | <0.01 | 0.009 | <0.01 | 0.81 | 0.04 | 0.14 | 0.38 |
| Overall, % of BW                        | 0.63 | 0.48 | 1.01 | 0.85 | 0.04 | <0.01 | <0.01 | <0.01 | 0.92 | <0.01 | 0.03 | 0.44 |
| ME intake, Mcal/d                       | 4.82 | 4.53 | 3.68 | 3.39 | 0.07 | <0.01 | 0.009 | <0.01 | 0.98 | <0.01 | 0.001 | 0.31 |
| Preweaning                              | 4.85 | 4.06 | 5.38 | 4.80 | 0.14 | 0.01 | 0.007 | <0.01 | 0.66 | 0.08 | 0.25 | 0.45 |
| Overall                                 | 4.82 | 4.44 | 4.02 | 3.68 | 0.07 | <0.01 | <0.01 | <0.01 | 0.84 | <0.01 | 0.04 | 0.74 |
| ADG, kg/d                               | 0.615 | 0.605 | 0.520 | 0.518 | 0.02 | <0.01 | 0.80 | <0.01 | 0.85 | <0.01 | 0.28 | 0.14 |
| Preweaning                              | 1.064 | 0.863 | 1.126 | 1.043 | 0.039 | 0.14 | 0.09 | 0.56 | 0.46 | 0.01 | 0.007 | 0.97 |
| Overall                                 | 0.705 | 0.657 | 0.641 | 0.624 | 0.018 | 0.07 | 0.22 | <0.01 | 0.57 | <0.01 | 0.006 | 0.46 |
| ADG/ME intake, kg/Mcal                  | 0.12 | 0.13 | 0.13 | 0.15 | 0.003 | 0.04 | 0.05 | <0.01 | 0.26 | <0.01 | 0.70 | 0.14 |
| Preweaning                              | 0.22 | 0.21 | 0.21 | 0.22 | 0.007 | 0.98 | 0.93 | <0.01 | 0.60 | 0.09 | 0.01 | 0.70 |
| Overall                                 | 0.15 | 0.15 | 0.15 | 0.16 | 0.003 | 0.11 | 0.12 | <0.01 | 0.25 | <0.01 | 0.33 | 0.34 |
| Feed efficiencyb                        | 0.62 | 0.66 | 0.57 | 0.65 | 0.01 | 0.17 | 0.009 | <0.01 | 0.38 | <0.01 | 0.68 | 0.27 |
| Preweaning                              | 0.69 | 0.66 | 0.66 | 0.69 | 0.02 | 0.98 | 0.92 | <0.01 | 0.60 | 0.09 | 0.01 | 0.70 |
| Overall                                 | 0.63 | 0.66 | 0.58 | 0.65 | 0.01 | 0.25 | 0.02 | <0.01 | 0.31 | <0.01 | 0.45 | 0.38 |
| Faecal score                            | 1.74 | 1.76 | 1.80 | 1.69 | 0.03 | 0.89 | 0.52 | – | 0.25 | – | – | – |
| Preweaning                              | 1.48 | 1.58 | 1.37 | 1.48 | 0.07 | 0.45 | 0.44 | – | 0.96 | – | – | – |
| Overall                                 | 1.68 | 1.72 | 1.70 | 1.65 | 0.03 | 0.64 | 0.93 | – | 0.43 | – | – | – |

*Statistical comparisons: FL = milk feeding level; FF = milk feeding frequency, W = period (Week), FL × FF = milk feeding level × milk feeding frequency, FL × W = milk feeding level × period, FF × W = milk feeding frequency × period, and FL × FF × W = milk feeding level × milk feeding frequency × period, respectively.

*Feed efficiency = kg of BW gain/kg of total DMI.

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. Detector and injector temperatures were set at 251°C.

Blood samples were collected before the morning meal at d 35, 56, and 70 of study. Briefly, blood was withdrawn from the jugular vein into evacuated tubes containing K2EDTA (Becton Dickinson Vacutainer Systems, Franklin Lakes, NJ). Blood samples were kept on ice after collection and centrifuged at 3,000 × g for 20 min at 4°C. Plasma was separated and stored at −20°C freezer until subsequent analysis. Blood metabolites concentrations were spectrophotometrically (UNICCO, 2100; Zistchemi Co., Tehran, Iran) determined using commercially available kits (Pars Azmoon Company, Tehran, Iran), glucose, cholesterol, triglyceride, albumin, total protein, high-density lipoprotein (HDL), and urea nitrogen according to the manufacturer’s instructions. Plasma BHB was determined with an auto-analyser using a laboratory kit (Randox Laboratories Ltd., Ardmore, UK).

**Faecal score**

Faecal score was established as 1 = normal, thick in consistency; 2 = normal, but less thick; 3 = abnormally thin, but not watery; 4 = watery; 5 = watery with abnormal colouring (Kertz and Chester-Jones 2004). No animal died during the experimental period.

**Statistical analyses**

Data were analysed separately for the preweaning (wk 1–8 of the study), postweaning (wk 9–10 of the study), and the overall (wk 1–10 of the study) periods. Data were analysed using the MIXED procedure of SAS (SAS...
Table 4. Effects of milk feeding level ([High (HPM) (up to 10 L/d) vs. Low (LPM) (4 L/d)] and milk feeding frequency (2× vs. 3×) on Body weight (BW) and body measurements of dairy calves.

| Item               | Treatment | p Valuea |
|--------------------|-----------|----------|
|                    | HPM-2×    | HPM-3×   | LPM-2×   | LPM-3×   | SEM   | FL    | FF    | FL × FF |
| BW, kg             |           |          |          |          |       |       |       |         |
| Initial (d 0)      | 40.7      | 40.6     | 40.5     | 40.6     | 0.24  | 0.72  | 0.96  | 0.85     |
| Weaning (d 56)     | 75.0      | 74.5     | 69.8     | 69.1     | 0.97  | 0.02  | 0.70  | 0.95     |
| Final (d 70)       | 89.2      | 86.6     | 85.5     | 83.7     | 1.16  | 0.09  | 0.20  | 0.72     |
| Body length, cm    |           |          |          |          |       |       |       |         |
| d 0                | 46.48     | 46.63    | 46.19    | 47.18    | 0.31  | 0.34  | 0.37  | 0.51     |
| d 56               | 55.45     | 56.21    | 56.32    | 56.54    | 0.31  | 0.36  | 0.46  | 0.68     |
| d 70               | 59.06     | 58.39    | 58.83    | 58.61    | 0.39  | 0.98  | 0.59  | 0.78     |
| Wither height, cm  |           |          |          |          |       |       |       |         |
| d 0                | 80.31     | 79.24    | 79.46    | 80.24    | 0.33  | 0.92  | 0.83  | 0.19     |
| d 56               | 89.75     | 88.70    | 89.36    | 88.36    | 0.34  | 0.60  | 0.14  | 0.97     |
| d 70               | 92.75     | 92.14    | 92.25    | 92.03    | 0.43  | 0.74  | 0.65  | 0.83     |
| Heart girth, cm    |           |          |          |          |       |       |       |         |
| d 0                | 80.56     | 80.99    | 80.33    | 80.55    | 0.28  | 0.57  | 0.59  | 0.86     |
| d 56               | 102.21    | 101.46   | 101.79   | 98.79    | 0.54  | 0.14  | 0.08  | 0.28     |
| d 70               | 107.62    | 109.60   | 106.04   | 106.71   | 0.66  | 0.09  | 0.32  | 0.62     |
| Body depth, cm     |           |          |          |          |       |       |       |         |
| d 0                | 80.54     | 80.24    | 80.57    | 80.24    | 0.28  | 0.98  | 0.60  | 0.98     |
| d 56               | 109.05    | 107.39   | 114.50   | 108.28   | 0.95  | 0.08  | 0.03  | 0.20     |
| d 70               | 122.21    | 122.23   | 124.90   | 121.68   | 0.93  | 0.58  | 0.41  | 0.41     |
| Hip height, cm     |           |          |          |          |       |       |       |         |
| d 0                | 82.06     | 81.82    | 81.49    | 82.22    | 0.29  | 0.91  | 0.64  | 0.03     |
| d 56               | 92.52     | 91.37    | 91.81    | 91.04    | 0.36  | 0.48  | 0.20  | 0.80     |
| d 70               | 95.54     | 95.24    | 95.02    | 94.91    | 0.45  | 0.65  | 0.83  | 0.92     |
| Hip width, cm      |           |          |          |          |       |       |       |         |
| d 0                | 15.44     | 15.22    | 15.56    | 15.34    | 0.12  | 0.66  | 0.29  | 0.99     |
| d 56               | 18.32     | 18.34    | 18.67    | 17.79    | 0.14  | 0.71  | 0.12  | 0.11     |
| d 70               | 20.40     | 19.90    | 20.50    | 19.70    | 0.22  | 0.88  | 0.05  | 0.65     |

Statistical comparisons: FL = milk feeding level; FF = milk feeding frequency, FL × FF = milk feeding level by feeding frequency interaction, respectively.

9.4, SAS Institute Inc., Cary, NC) with period (week) as repeated measures for starter feed intake, DMI, ME intake, BW, ADG, FE, ruminal fermentation characteristics, blood metabolites, and structural growth variables with the individual calf as the experimental unit. The model included fixed effects of milk feeding level, milk feeding frequency, their interactions and random effect of block. Before analyses, all data were screened for normality using the UNIVARIATE procedure of SAS. Three variance-covariance structures (auto-regressive type 1, compound symmetry, and Toeplitz) were tested and the covariance structure that minimised the Schwarz’s Bayesian information criterion was chosen. Body weight and structural growth variables were analysed using a similar model, but without the effect of time. The SLICE option of the LSMEANS statement from the MIXED procedure of SAS was used to determine difference among dietary treatments [milk feeding level (FL) × milk feeding frequency (FF)] at every time point. Initial BW was considered as a covariate for the weaning BW and final BW, but excluded from the model as it was not significant. The effect of the milk feeding quantity, the milk feeding frequency, and their interactions on faecal score were tested using the GLIMMIX procedure of SAS version 9.4, with Poisson distribution; the model included fixed effects of milk feeding level, milk feeding frequency, and their interactions. Least squares means for treatment effects were separated by the use of PDIF statement when the overall F-test was $P \leq 0.05$.

Results

Effects of milk feeding quantity

Intake and growth performance data are presented in Tables 3 and 4. Calves fed HPM had greater (Figure 1; $p < 0.01$) milk intake between d 10 and 59 compared with calves fed LPM, which led to 88.5% increase in total milk intake for calves fed HPM (343 L) compared with calves fed LPM (182 L). Calves fed HPM and LPM had no milk refusals. HPM-calves had greater ($p < 0.01$) BW during the preweaning and overall periods compared to LPM calves. Calf starter intake (as g/d and % of BW) was higher (Table 3; $p < 0.01$) during both pre- and postweaning periods in calves fed LPM versus HPM, which resulted in greater overall starter intake in calves fed LPM versus HPM ($p < 0.01$). Total DMI intake (g/d, Table 3) was higher ($p < 0.01$), while it was lower ($p = .01$) postweaning in calves fed HPM versus LPM due to the step-up/step-down protocol used for the
HPM calves. Total DM intake as % of BW during the preweaning was greater (Table 3; \(p = .005\)) and lower \((p < .01)\) during the postweaning period for calves fed HPM compared with those fed LPM. Calves fed HPM were heavier \((p < .01; \text{Table } 3)\) compared with calves fed LPM in preweaning and overall. The ADG was greater during the preweaning \((p < .01)\) and overall (tendency, \(p = .07\)) in HPM calves than LMP calves. The ADG/ME intake ratio was greater for calves fed LPM compared with calves fed HPM during the preweaning \((p = .04)\). Feed efficiency was unaffected by the milk feeding quantity. Calves on HPM had greater BW compared with LPM calves at d 56 \((p = .002)\) and 70 (tendency, \(p = .09\)) of study. At 70 d of the study, HG had a tendency \((p = .09)\) to be greater for calves fed HPM compared with LPM calves. Final BW and other body measurements were unaffected by the milk feeding rate, regardless of the milk feeding frequency (Table 4).

Data on rumen fermentation parameters are presented in Table 5. At d 35 of the study, the molar proportion of acetate, and acetate to propionate ratio were greater \((p < .01)\) for calves fed HPM compared with those fed LPM. The molar proportion of butyrate \((p = .05)\) and propionate and total VFA in the rumen were greater \((p < .01)\) for calves fed LPM compared with calves fed HPM on d 35, but were unaffected by milk feeding quantity at d 70 of the study.

Blood metabolites data are presented in Table 6. Plasma concentration of glucose was greater \((p < .01)\) in calves fed HPM compared with calves fed LPM at d 35, but at d 56 HPM calves had lower \((p = .02)\) glucose levels compared with calves fed LPM. Cholesterol was greater \((p = .004)\) in calves fed HPM compared with those fed LPM at d 56, but was unaffected by milk quantity at d 35 and 70. Plasma triglycerides were greater \((p = .02)\) at d 35 and lower \((p = .05)\) at d 56 for calves fed HPM versus LPM. Plasma HDL concentration was greater for calves fed HPM compared with calves fed LPM at d 56 \((p = .005)\) and d 70 \((p = .04)\). Plasma BHB concentrations was greater \((p < .01)\) for calves fed LPM compared with calves fed HPM at d 56. Calves fed HPM had lower \((p = .008)\) plasma urea concentration at d 35, but tended to be greater \((p = .07)\) at d 70 compared with calves fed LPM. Calves fed HPM had greater \((p = .02)\) plasma albumin concentration at d 70, but similar at d 35 and d 56 compared with calves fed LPM. Plasma concentration of albumin and total protein were unaffected by the milk feeding rate, regardless of the milk feeding frequency.

**Effects of milk feeding frequency**

Calves fed 2×/d had greater \((p < .01)\) starter feed intake (as g/d and % of BW, Table 3) during the preweaning, postweaning and overall periods compared with calves fed 3×/d. Calves fed 2×/d had greater total DM intake (as g/d and % of BW, Figure 2(B)) and ME intake (Figure 2(C)) compared with 3×/d during the preweaning, postweaning and overall periods. There was a tendency to greater ADG for calves fed 2×/d compared with those fed 3×/d during the
postweaning ($p = .09$) period, but preweaning and overall ADG of calves were not affected (Table 3). ADG/ME intake was greater for calves fed 3×/d compared with 2×/d in the preweaning ($p = .05$) and unaffected by the milk feeding frequency in the postweaning and overall periods. Feed efficiency was also greater in the 3×/d compared with 2×/d calves in the preweaning ($p = .009$) and overall ($p = .02$) periods. Calves on 2×/d had greater HG (tendency, $p = .08$) and BD ($p = .03$) at weaning, and HW ($p = .05$) at d 70 compared with calves fed 3×/d. No effect was observed for BW, BL, WH and HH of calves fed 2 times/d versus 3 times/d, regardless of the milk FF.

The rumen fermentation profile was altered by milk FF. Rumen total VFA ($p = .02$) and molar proportion of butyrate (tendency, $p = .06$) were greater in 2×-fed calves than in 3× calves on d 35 of the study, respectively (Table 5). However, the molar proportion of acetate and propionate did not differ between calves fed 2× versus 3×/d (Table 5).

Feeding frequency had no effect on plasma glucose concentration and HDL during the study. Increased FF from 2 to 3 times/d resulted in higher ($p = .02$) concentrations of plasma cholesterol and lower (tendency, $p = .06$) concentration of triglycerides on d 56. Calves fed 2 times/d had a greater ($p < .01$) blood BHB concentration than those fed 3 times/d on d 56, but similar at d 35 and 70. The FF had no significant effect on plasma urea concentration of calves. Calves fed 3×/d had a greater concentration of plasma albumin and total protein at d 35 (tendency, $p = .06$) and 70, but their levels were similar at d 56 compared with calves fed 2×/d.

**Milk feeding quantity × milk feeding frequency interaction**

No significant interactions were observed between the milk feeding quantity and frequency for total DMI (g/d or percentage of BW), starter feed intake (g/d or percentage of BW), ME intake, ADG, ADG/ME intake, and FE during the preweaning, postweaning and overall periods. Similarly, an interaction trend ($p = .06$) was observed for rumen concentration of acetate with HPM-3× calves having the greatest concentration. The interaction of milk feeding quantity and frequency ($p = .001$) was significant for plasma concentration of glucose, with the highest plasma concentration reported for HPM-3× on d 35.

**Discussion**

The goal of this study was to determine the effect of the milk feeding quantity and feeding frequency on growth performance, rumen fermentation, and blood metabolites of dairy calves during the pre- and postweaning periods.

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**Table 5. Effects of milk feeding level [(High (HPM) (up to 10 L/d) vs. Low (LPM) (4 L/d)] and milk feeding frequency (2× vs. 3×) on average ruminal pH, and volatile fatty acids (VFA) concentrations of dairy calves.**

| Item                  | HPM-2× | HPM-3× | LPM-2× | LPM-3× | SEM | FL   | FF  | D   | FL × FF | FF × D | FL × FF × D |
|-----------------------|--------|--------|--------|--------|-----|------|-----|-----|--------|--------|-------------|
| Total VFA, mmol/L     |        |        |        |        |     | <0.01| 0.02| 0.22| <0.01 | <0.01 | 0.03 0.52 |
| d 35                  | 63.24  | 45.50  | 116.60 | 108.39 | 7.32|      |     |     | <0.01 | <0.01 | <0.01 0.01 |
| d 70                  | 105.72 | 108.28 | 109.34 | 116.33 | 4.16| 0.38 | 0.58| 0.68| 0.38  0.58|       |             |
| Overall               | 84.64  | 72.65  | 112.37 | 111.16 | 4.46| <0.01| 0.21| <0.01| 0.30  0.01| 0.03  0.52|
| Individual VFA, mol/100 mol |        |        |        |        |     |      |     |     |        |        |             |
| Acetate               |        |        |        |        |     | <0.01| 0.27| 0.06| <0.01 | <0.01 | 0.03 0.14 |
| d 35                  | 63.35  | 69.78  | 52.19  | 49.61  | 2.34| <0.01| 0.27| 0.06| <0.01 | <0.01 | 0.03 0.14 |
| d 70                  | 49.22  | 48.05  | 50.35  | 49.45  | 0.67| 0.41 | 0.47| 0.94| <0.01 | <0.01 | 0.03 0.14 |
| Overall               | 57.04  | 60.42  | 51.24  | 49.43  | 1.43| <0.01| 0.57| <0.01| 0.07  0.01| 0.21  0.11|
| Propionate            |        |        |        |        |     | <0.01| 0.70| 0.15| <0.01 | <0.01 | 0.03 0.14 |
| d 35                  | 30.55  | 25.44  | 41.24  | 44.18  | 2.03| <0.01| 0.70| 0.15| <0.01 | <0.01 | 0.03 0.14 |
| d 70                  | 44.45  | 44.37  | 43.38  | 42.64  | 0.50| 0.21 | 0.69| 0.75| <0.01 | <0.01 | 0.03 0.14 |
| Overall               | 37.39  | 34.91  | 42.36  | 45.31  | 1.19| <0.01| 0.62| <0.01| 0.18  0.01| 0.21  0.17|
| Butyrate              |        |        |        |        |     | <0.01| 0.06| 0.10| <0.01 | <0.01 | 0.03 0.14 |
| d 35                  | 6.10   | 4.78   | 6.57   | 6.21   | 0.67| 0.05 | 0.06| 0.10| <0.01 | <0.01 | 0.03 0.14 |
| d 70                  | 6.33   | 7.58   | 6.27   | 7.91   | 0.60| 0.90 | 0.25| 0.86| <0.01 | <0.01 | 0.03 0.14 |
| Overall               | 6.88   | 4.67   | 6.40   | 7.06   | 0.48| 0.40 | 0.49| <0.01| 0.21  0.01| 0.21  0.17|
| Acetate:Propionate    |        |        |        |        |     | <0.01| 0.64| 0.40| <0.01 | <0.01 | 0.03 0.14 |
| d 35                  | 2.47   | 2.97   | 1.26   | 1.12   | 0.23| <0.01| 0.64| 0.40| <0.01 | <0.01 | 0.03 0.14 |
| d 70                  | 1.11   | 1.09   | 1.16   | 1.16   | 0.03| 0.26 | 0.84| 0.83| <0.01 | <0.01 | 0.03 0.14 |
| Overall               | 1.80   | 2.03   | 1.22   | 1.15   | 0.12| <0.01| 0.64| <0.01| 0.41  0.01| <0.01 0.59|

*Statistical comparisons: FL = milk feeding level, FF = milk feeding frequency, D = period (Day), FL × FF = milk feeding level × milk feeding frequency, FL × D = milk feeding level × period, FF × D = milk feeding frequency × period, and FL × FF × D = milk feeding level × milk feeding frequency × period, respectively.*
Effect of milk feeding quantity

It is well known that the effect of milk feeding on starter intake and growth performance in dairy calves is dependent on both the quality and quantity of milk (Silper et al. 2014). Usually, a negative correlation exists between the amount of milk fed and the amount of starter consumed by calves (Raeth-Knight et al. 2009; Terre et al. 2009; Gelsinger et al. 2016) and is exacerbated in calves on a greater plane of milk. In this study, we determined that calves performed differently when fed either HPM (371 L) or LPM (210 L). As shown in Figure 2, starter intake was low for all treatments during the first 2 wk. Calf starter intake increased greatly in calves fed LPM compared to the HPM consistent with previous studies (Khan et al. 2007, 2008). Starter intake of calves fed HPM was low until wk 5. A considerable increase was realised during wk 6 and 7, when the milk feeding quantity was reduced. This rapid increase in starter feed intake due to a reduction in milk for calves fed at higher levels was in line with previous studies (Quigley et al. 2018; Klopp et al. 2019; van Niekerk et al. 2020). Low starter intake is usually observed in trials that evaluate the effect of feeding high volumes versus low volumes of milk to calves (Hill et al. 2010; Silper et al. 2014). This decrease in feed intake can be attributed to an increase in the satiety levels as a result of elevated blood glucose and insulin, as well as continuous gut filling with curd formation (Khan et al. 2011).

### Table 6

| Item                  | Treatment | p Value* |
|-----------------------|-----------|----------|
| Glucose, mg/dL        | HPM-2 ×  | FL<0.01  |
| d 35                  | 101.61    | 0.15     |
| d 56                  | 94.87     | 0.75     |
| d 70                  | 99.54     | 0.003    |
| Overall               | 98.63     | 0.04     |
| Cholesterol, mg/dL    | HPM-2 ×  | D<0.01   |
| d 35                  | 122.77    | 0.27     |
| d 56                  | 127.90    | 0.27     |
| d 70                  | 68.31     | 0.001    |
| Overall               | 106.28    | 0.001    |
| Triglyceride, mg/dL   | HPM-2 ×  | FL<0.01  |
| d 35                  | 37.75     | 0.25     |
| d 56                  | 32.66     | 0.006    |
| d 70                  | 29.46     | 0.24     |
| Overall               | 32.78     | 0.23     |
| HDL, mg/dL            | HPM-2 ×  | D<0.01   |
| d 35                  | 68.60     | 0.001    |
| d 56                  | 76.97     | 0.001    |
| d 70                  | 61.74     | 0.001    |
| Overall               | 67.74     | 0.001    |
| Beta-hydroxy butyrate (BHBA), mmol/L | HPM-2 × | D<0.01   |
| d 35                  | 0.07      | 0.15     |
| d 56                  | 0.16      | 0.01     |
| d 70                  | 0.27      | 0.15     |
| Overall               | 0.16      | 0.001    |
| Urea, mg/dL           | HPM-2 ×  | D<0.01   |
| d 35                  | 14.26     | 0.001    |
| d 56                  | 22.91     | 0.01     |
| d 70                  | 27.07     | 0.001    |
| Overall               | 21.26     | 0.001    |
| Albumin, g/dL         | HPM-2 ×  | D<0.01   |
| d 35                  | 3.23      | 0.36     |
| d 56                  | 3.44      | 0.36     |
| d 70                  | 3.47      | 0.001    |
| Overall               | 3.38      | 0.001    |
| Total Protein, g/dL   | HPM-2 ×  | D<0.01   |
| d 35                  | 6.25      | 0.12     |
| d 56                  | 6.58      | 0.09     |
| d 70                  | 6.43      | 0.09     |
| Overall               | 6.44      | 0.09     |

*Statistical comparisons: FL = milk feeding level, FF = milk feeding frequency, D = period (Day), FL × FF = milk feeding level × milk feeding frequency, FL ÷ D = milk feeding level ÷ period, FF ÷ D = milk feeding frequency ÷ period, and FL × FF ÷ D = milk feeding level × milk feeding frequency ÷ period, respectively.
in the LPM-fed calves due to an average of 879 vs. 460 g/d DM consumed in milk. Intake of DM from milk declined from wk 6 as milk allotment was reduced due to weaning. Therefore, calves fed the HPM gained more weight than calves fed LPM during the first month of life. The total DM intake as a proportion of BW for HPM calves in the second month was less than the first month. Preweaning starter intake was lower for calves fed HPM than calves offered 4 L/d, which could not have induced sufficient rumen development and establishment of ruminal fermentation in these calves (Khan et al. 2011). However, during the preweaning, total DM intake was higher for calves fed HPM with a concomitant increase in ME intake. The results obtained in this study regarding the quantity of milk feeding on preweaning starter feed intake are similar to the findings of studies that fed milk replacer (Hill et al. 2016; Dennis et al. 2018, 2019). In agreement with studies by Orellana Rivas et al. (2020) and van Niekerk et al. (2020), ADG in calves fed the HPM program was greater during the preweaning period likely due to greater supply of digestible nutrients from milk. Generally, milk or MR is considerably more digestible than calf starter feed, as shown by Dennis et al. (2018), partly justifying

Figure 2. The effect of level milk (low vs. high) and feeding frequency (2× vs. 3×) on (A) starter intake, (B) total dry matter intake, (C) metabolisable energy intake, and (D) average daily gain of dairy calves (n = 12 calves per treatment). Calves were assigned to 1 of 4 dietary treatments, including LPM-2×: calves fed low plane of milk (LPM) intake and twice per day (4 L/d of milk from wk 1 to 7, and 2 L/d of milk for the last week; total milk intake = 210 L); LPM-3×: calves fed LPM intake and three times per day (total milk intake = 210 L); HPM-2×: calves fed high plane of milk (HPM) intake and twice per day (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; total milk intake = 371 L); and HPM-3×: calves fed HPM intake and three times per day (total milk intake = 371 L). Error bars represent SEM. Asterisks indicate a difference \([**p < .01], (*)p < .05)\] among treatments at a given time point.
why a higher plane of milk feeding during the preweaning period led to improved growth rates in calves. Calves fed HPM experienced a decrease in the proportion of ADG to ME intake, but FE was not different between treatments. Levels of milk or MR intake tend to have inconsistent effects on FE with some studies reporting a greater FE for calves fed at high levels of milk (Quigley et al. 2018), while others, similar to our study did not report any improvement (Hill et al. 2016; Rosenberger et al. 2017; Dennis et al. 2019) during the preweaning period. The greater ME intake from the higher quantity of milk feeding also resulted in greater BW and tended to increase the heart girth compared with LPM calves, which is in line with previous reports (Stamey et al. 2012; Rosenberger et al. 2017). On the other hand, a tendency to increase body depth was observed in calves fed HPM compared with calves fed HPM, which may be linked to greater starter intake that induced early and greater rumen development in these calves (Khan et al. 2011).

Calves fed HPM had less total VFA concentration and molar proportions of butyrate at d 35 compared to calves fed LPM, this finding is most likely due to the lower starter feed intake in HPM calves. In accordance with our findings, (Khan et al. 2007) recorded lower molar proportions of ruminal total VFA, propionate, and butyrate at d 28 of age, but at weaning and postweaning calves fed high volumes of milk had greater concentration compared to the conventionally milk-fed calves. They suggested that higher intakes of both starter and hay in calves fed high milk volumes and subjected to a step-down weaning method resulted in early initiation of ruminal fermentation relative to conventionally milk-fed calves. The acetate to propionate ratio observed in the current study was greater in calves fed HPM compared to calves fed LPM, which could be due to the improved rumen buffering capacity (Mirzaei et al. 2016). Grant et al. (1990) showed that the decline in rumen pH impairs the growth of cellulolytic bacteria, leading to a decrease in the acetate to propionate ratio.

As expected, calf age affected blood glucose concentrations in response to increased consumption of solid feed. In dairy calves, glucose derived from intestinal absorption is the primary source of energy substrates...
et al. 2017), but with the initiation of solid feed intake, the energy source shifts to VFA acquired from the ruminal fermentation process, especially propionate that is converted into glucose (Vi et al. 2004). Lower plasma glucose concentrations at d 35 might have resulted in increased satiety signals (Khan et al. 2011), prompting greater starter feed intake in calves fed LPM, as it has been reported in previous studies (Khan et al. 2007; Omidi-Mirzaei et al. 2015; De Paula et al. 2017). On the other hand, higher concentrations of plasma glucose in calves fed HPM, was consistent with previous studies (Terre et al. 2009; MacPherson et al. 2016; Mirzaei et al. 2018). Higher levels of lactose intake are likely able to increase blood glucose concentration (Palmquist et al. 1992). Contrary to our results, Silper et al. (2014) and De Paula et al. (2017) observed lower concentrations of plasma glucose at preweaning in intensively fed calves compared to restricted milk feeding. They suggested that differences in MR lactose intake were not sufficient to elicit glycaemia. At d 56 of the study, a decrease in plasma glucose concentration in HPM-fed calves was observed in response to a reduction in the amount of milk fed and weaning which are associated with growth and development of the rumen (Khan et al. 2007).

As expected BHB plasma concentrations were influenced by age as a result of increased solid feed intake and consequently the development of the rumen (Nemati et al. 2015; De Paula et al. 2017). In line with previous studies (Omidi-Mirzaei et al. 2015; De Paula et al. 2017), higher concentrations of plasma BHB were found in calves fed low milk levels, which is an indicator of a better functioning rumen. According to Bach (2014), it may take more time for calves fed higher volumes of liquid diet to start consuming starter concentrates, resulting in delayed rumen development. As calves grew older, plasma BHB concentration increased in all groups. The increase was concomitant with a reduction in milk feed, greater solid feed intake and complete weaning from milk. The concentration of plasma BHB increased about 2-fold from d 56 to d 70 in HPM calves independent of the milk feeding frequency, so that at the end of the study, HPM-fed calves had BHB plasma concentrations similar to LPM calves.

Plasma triglycerides were affected by the age of the calf. At d 35, the greater triglycerides concentration in HPM-fed calves could be due to the higher consumption of milk and ME relative to the LPM-fed calves. Similar to our results, Khan et al. (2007) reported a higher plasma concentration of triglycerides in calves fed high relative to those fed conventional low milk volumes before the weaning process had been initiated (d 3 to 28 of age). At d 56 of the study, we observed a higher plasma triglycerides concentration in LPM-fed calves compared to HPM-fed calves, which could be due to the higher intake of calf starter and ME. In the current study, we found higher plasma cholesterol and HDL concentrations for calves fed HPM relative to LPM calves which could be due to increased intake of saturated fatty acids with increased intake of milk.

In this study, the higher plasma concentrations of urea for calves fed LPM compared to calves fed HPM were due to higher starter intake. Schäff et al. (2016) reported a decrease in plasma urea concentration for calves fed ad libitum MR compared with restricted fed calves. We observed an equal concentration of total plasma protein in HPM and LPM treatments, which was in agreement with previous studies (Khan et al. 2007; Mirzaei et al. 2018). In line with the Omidi-Mirzaei et al. (2015) and Mirzaei et al. (2018) results, we observed similar plasma albumin concentrations for these groups except at d 70, where HPM calves had higher concentration than LPM calves.

**Effects of milk feeding frequency**

In the current study, calves fed 2 times/d and 3 times/d had similar milk volume intakes, while those on LPM-3× and HPM-3× treatments received an extra meal at night. Total milk intake per calf in the HPM feeding method was about 2 times more than the LPM. We hypothesised that feeding milk 3 times a day would stimulate solid feed intake, because the smaller meals were insufficient to satisfy the energy requirements of the calf. In comparison, calves fed 2 meals may have a greater solid feed intake compared to 3 meals, because they experience an extended time between the milk feedings.

In agreement with (Strzetelski et al. 2001), we found that an increase in FF reduced starter intake and total DMI in preweaned calves. Greater starter intake with decreased FF could be due to a longer interval between liquid feeding times, which leads the calves to seek for an alternative feed before weaning (Strzetelski et al. 2001). Our results were in contradiction with some reports. (Kmicikewycz et al. 2013) and MacPherson et al. (2019) did not find differences in feed intake and DMI by increasing FF from 2× to 4× per day. Saldana et al. (2019) reported that calf starter intake was 242.3 and 198.7 g/d for calves fed milk once and twice daily, respectively, even though the differences were not statistically significant. Similarly, (Thomas 2014), found no differences in starter consumption between calves fed two or three times daily. In their study, calves were given MR
(CP = 20%; fat = 20%) at 6:00 am and 5:00 pm and an additional meal at 12:00 noon for those fed three times/day. The differences in results could probably be due to the discrepancy in milk/MR feeding times. In our study, starter feed intake was continuously higher in calves fed 2 times compared with those fed 3 times/d and gradually increased until the end of the experiment. Calves fed 2×/d likely attempted to compensate for the lack of nutrients by consuming more starter to promote rumen development. However, increased frequency of feeding attenuated the level of DMI due to the reduced starter consumption in both milk feeding methods during the postweaning and overall periods. Reduced starter intake due to increased milk feeding frequency could be attributed to higher daily blood glucose levels, and hypophagia caused by the elevated blood glucose levels (Oba and Allen 2003) in calves on 3×/d vs. 2×/d during the day. Unfortunately, sampling for blood glucose measurement was done at a single time point which does not represent the dynamic changes of blood glucose as compared to more frequent sampling or use of a tolerance test. Therefore, further studies are warranted to prove this speculation. However, despite reduction in total DMI (mainly due to reduced starter intake), pre-weaning and overall ADG were not reduced by increased milk feeding frequency. In this experiment, according to other studies, increasing FF did not affect ADG (Strzetelski et al. 2001; Jensen 2004; Kmickewycz et al. 2013).

We observed a greater FE when calves were fed three times versus twice daily during the preweaning and overall period. However, in some of the previous studies no differences in FE were observed between varying milk feeding frequencies in the postweaning period (Kehoe et al. 2007; Kmickewycz et al. 2013). The FF had no significant effect on BW and skeletal traits of calves, but body depth and hip width were greater in calves fed 2 times versus 3 times/d on d 56 and 70, respectively, most likely due to a greater starter feed intake. Similarly, (Kmickewycz et al. 2013) did not find a correlation between MR feeding frequency and hip height, withers height, or hip-width gain, which was in contrast to their hypothesis that increased FF would result in increased starter and calf growth when calves were fed an accelerated MR program.

In the current study, higher total VFA concentration and molar butyrate in the rumen of calves fed 2 times on d 35, could be attributed to more starter intake and more rumen fermentable in 2×/d compared with 3×/d milk fed treatments.

In nature, suckling calves ingest colostrum or milk in several portions per day ad libitum (Vicari et al. 2008). Therefore, limit feeding milk or milk replacer to just one or two portions, which is mostly practiced on most dairy farms may not adequately support the calf physiologically (Abe et al. 1979; Hostettler-Allen et al. 1994; Jasper and Weary 2002). This becomes even more important when calves are fed with high volumes of milk or milk replacers (≥ 8 L/d, about 20% of the birth weight), and offered their milk allowances in one or two portions during the day. Under such conditions, glucose, fatty acids and amino acids might be absorbed and appear in blood more rapidly and in greater amounts than can be utilised by calves (Vicari et al. 2008). Therefore, one may expect that calves raised on a high plane of milk or milk replacer might benefit from increased milk FF. In a study by Kaufhold et al. (2000), increasing the FF at the same FL improved blood glucose homeostasis, reduced postprandial insulin levels and lowered plasma urea concentrations, suggesting that nitrogen (N) utilisation was enhanced. In our study however, increasing milk feeding frequency failed to exert any effect on plasma concentrations of glucose, triglycerides, and HDL, most likely because the amount and composition of milk were similar between 2× and 3×-fed calves (Senevirathne et al. 2017). Despite eating more starter, calves fed 2 times/d probably could not acquire sufficient propionate, which is the source of blood glucose in calves with a better developed rumen, to overcome the nutritional deficiencies during the night. In contrast, Vicari et al. (2008) reported that area under the curve of glucose concentration (AUC 12-18h) decreased with increasing FF in calves fed 1, 2 and 4 times/d. This could be attributed to whey protein as the only protein source in the milk replacer used. When skimmed milk protein is replaced by whey, milk clotting in the abomasum is reduced or absent, and protein and fat are more rapidly emptied from the abomasum into the absorptive sites in the small intestine (Beynen and Van Gils 1983) (Guilloteau et al. 1997). In this case amino acids, fatty acids and glucose might be absorbed and appear in blood faster and in greater amounts than if derived from casein, a situation that may exert metabolic stress on calves (Vicari et al. 2008). In such a condition, calves may benefit from increased FF, reflected as improved blood glucose homeostasis, reduced postprandial insulin levels, and lowered plasma urea concentration as an indicator of enhanced nitrogen (N) utilisation (Kaufhold et al. 2000). These benefits however, might be less prominent when calves are fed whole milk with casein as its main protein constituent. The higher plasma cholesterol concentration for 3×-fed calves was probably due to the intake of more fat in these calves at night. Compared to solid feed, milk nutrients are more digestible, containing 4 to 5 times more fat and is the source of blood lipids (Chapman
et al. 2016). In this study, the higher plasma BHB concentration for calves fed 2× compared to calves fed 3×/d would have also resulted from higher solid feed intake and better ruminal fermentation in these calves. Plasma urea nitrogen concentration, as the indicator of nitrogen utilisation in ruminants, was not affected by milk feeding frequency, which contradicts the findings of Kaufhold et al. (2000). Kehoe et al. (2007) did not find any differences in the concentration of plasma urea nitrogen when calves were fed once compared to twice daily. Likewise, similar plasma urea nitrogen between calves fed 1, 2, and 4 meals per day were observed in Vicari et al. (2008) study. However, plasma urea nitrogen concentration increased with age in all treatments as expected. This increase in plasma urea nitrogen demonstrated an increase in the amount of solid feed intake and protein digestibility by the calf (Thomas 2014). There was an effect on d 35 and 70, with albumin and total protein greater in calves fed milk 3 times compared to 2 times/d.

**Milk feeding quantity × milk feeding frequency interaction**

The molar proportion of acetate (tendency) was the greatest in HPM-3× calves at d 35 due to less starter intake. A significant interaction of milk feeding level and frequency for glucose at d 35, was mainly attributed to greater plasma concentration in HPM-3× calves probably as a result of more lactose intake from milk at night.

**Faecal score**

In this study, the occurrence of diarrhoea and general appearance score in calves were not affected by treatments (Table 3). We found no differences in faecal scores days with medical treatments in the current trial. Similar results were established in previous studies that calves can consume a large amount (16 to 24% of body mass) of liquid feeds (Drackley 2008; Chapman et al. 2016) and at different FF (Kmicikewycz et al. 2013; Saldana et al. 2019). The incidence of diarrhoea is usually due to calves’ health, management, and housing conditions rather than the amount of milk intake.

**Conclusions**

Increasing the amount of milk intake tended to increase ADG and final BW even though calves had less starter intake than calves fed LPM. Regardless of milk allowance, there were no differences in final BW and body measurements between calves fed milk 3×/d vs. 2×/d, but feeding 3 meals/d reduced total DM and starter intake with no detrimental effects on overall ADG. Contrary to our hypothesis, calves did not benefit from the increased milk feeding frequency at either amount of milk except for improved feed efficiency. The performance of calves fed LPM was constrained by the low nutrient intake.

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**Ethical approval**

All the animal procedures were approved by the Animal Care and Use Committee of Isfahan University of Technology (IACUC #2018005).

**Disclosure statement**

No potential conflict of interest was reported by the author(s).

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