Animal xxx (xxxx) xxx

Contents lists available at ScienceDirect

Animal
The international journal of animal biosciences

Extended transition milk feeding for 3 weeks improves growth performance and reduces the susceptibility to diarrhea in newborn female Holstein calves

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ARTICLE INFO

Article history:
Received 5 February 2020
Received in revised form 5 November 2020
Accepted 9 November 2020
Available online xxxx

Keywords:
Colostrum
Health
Liquid feed
Weaning
Weight gain

ABSTRACT

Dairy calves may benefit from extending the duration of feeding transition milk (**TM**; the subsequent two to six milkings after parturition) to enhance performance and health during early life. The objective of this study was to assess the effect of replacing pasteurized waste milk (non-saleable milk containing antibiotic and/or drug residues) with pasteurized TM for 3 weeks on the growth performance and health of dairy calves. A total of 84 healthy newborn female Holstein calves were blocked by birth order and assigned randomly to 4 treatment groups with partial replacement of pasteurized waste milk by TM (second milkings after parturition) at 0 (0 l/day TM + 6 l/day milk), 0.5 (0.5 l/day TM + 5.5 l/day milk), 1 (1 l/day TM + 5 l/day milk), or 2 l (2 l/day TM + 4 l/day milk) for a 21-day period. From day 22 onward, all calves were fed individually with 6 l/day pasteurized waste milk. Calves were weaned on day 60 and monitored until day 90 of the study. Liquid feed DM intake (**DMI**) was increased with increasing levels of TM ($P = 0.001$). Starter feed DMI and total DMI (liquid feed DMI + starter feed DMI) were not affected by the treatment effect. Calves were fed 2 l/day TM gained more BW compared with those in the control group during the postweaning and overall periods. The average daily gain tended ($P = 0.06$) to be higher in calves fed 2 l/day TM compared with calves fed 0 (+ 65 g/day), 0.5 (+ 53 g/day), or 1 (+ 76 g/day) l/day TM during the preweaning period. Daily weight gain was also higher in calves fed 2 l/day TM compared with calves in the control group during the postweaning (+ 137 g/day; $P = 0.04$) and overall (+89 g/day; $P < 0.01$) periods, respectively. Calves fed 2 l/day TM had a higher feed efficiency compared with calves in the control group during all studied periods. The calves fed TM2 had a lower chance of having diarrhea compared with other treatment groups. Duration but not the frequency of diarrhea was lower in calves fed TM2 vs TM0 (2.5 vs 4.2 days; $P = 0.03$). In general, partial replacement of waste milk with TM (2 l/day) may be recommended to feed dairy calves at an early stage of life to support a higher growth rate and health benefits.

Implications

In this study, transition milk was replaced with waste milk at graded levels (0, 0.5, 1, and 2 l/day) for 3 weeks postbirth in dairy calves. Extending transition milk feeding to dairy calves was associated with a lower risk of diarrhea during the early weeks of life. Feeding higher levels of transition milk (2 l/day) increased weight gain, withers height, and feed efficiency of calves. In general, partial replacing waste milk with transition milk (2 l/day) may be recommended to support higher growth and health benefits in dairy calves.

Introduction

Bovine colostrum contains not only immunoglobulin but also several bioactive compounds such as bioactive peptides (cytokines), lactoferrin, oligosaccharides, hormones (i.e., IGF-I and II, epidermal growth factor, insulin, and prolactin), and immune-related micro ribonucleic acids (Blattler et al., 2001) that are important for the health of the new-born calf. Furthermore, it was demonstrated that feeding colostrum to neonatal calves for 3 days increased binding capacities for IGFs and insulin in the small intestine and colon than those fed milk replacer, suggesting that

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https://doi.org/10.1016/j.animal.2020.100151
1751-7311/

colostrum feeding may alter intestinal growth through modification on growth factor functionality (Hammon and Blum, 1997). Schaff et al. (2014) observed improved glucose absorption in neonatal calves fed colostrum vs formula feeding, primarily by enhancing postnatal growth of the intestinal mucosa and by increasing absorptive capacity in the small intestine without stimulating intestinal glucose transporters. Following the first milking after parturition (defined as colostrum), the subsequent 2–6 milkings referred to as transition milk (TM) (Godden, 2008). Generally, the bovine colostrum composition (macro- and micro-elements) gradually changes to become mature milk on the fifth day postpartum (Abd El-Fattah et al., 2012). Similar to colostrum, TM contains elevated levels of IGF-I, nucleotides, growth hormone, and IGF-binding proteins (Blum and Hammon, 2000; Hammon et al., 2000; McGrath et al., 2016).

In a natural setting, a new-born calf experiences a gradual transition from colostrum to mature milk, which is not commonly practiced on most dairy farms. Transition milk is commonly not delivered to newborn calf on-farm and simply it is put into the bulk tank and diluted out, resulting in the calf being transitioned from colostrum directly onto other available liquid feeds (including whole milk, waste milk, or milk replacer) (Kargar et al., 2020). Therefore, based on recent research evidence, strategies to optimize gastrointestinal tract (**GIT**) development and health of calves, such as the timely feeding of colostrum to increase passive transfer (Fischer et al., 2018; Pyo et al., 2018) and feeding TM for an extended period (Conneely et al., 2014), are encouraged to reduce the risk of mortality and morbidity related to abnormal GIT function. Recently, Ghaffari et al. (2020) concluded that the bioactive substances in colostrum are probably regulating the utilization of the branched-chain amino acids (leucine, isoleucine, and valine) through anabolic means in the small intestine of neonatal calves. Fischer-Tlustos et al. (2020) reported that bovine TM contains higher concentrations of certain oligosaccharides than mature milk, which may serve as carbon sources for beneficial bacteria in the intestine (Fischer et al., 2018), prevent the pathogen adhesion to the intestinal epithelium, and increase the immunoglobulin G (**IgG**) uptake (Gill et al., 1999). However, there is limited knowledge available evaluating the long-term effect of TM feeding on the growth performance and health of dairy calves.

Neonatal calves are at high risk of illness in the first few weeks of life. Diarrhea is the most common cause of morbidity and mortality among dairy calves in the first few weeks of life, causing substantial economic losses due to growth depression and medication (Larson et al., 2005). Overall, the bioactive components in colostrum contribute to the calf's ability to cope with infection (Langel et al., 2015) and may promote the growth performance of dairy calves (Kargar et al., 2020). Previous studies have shown that feeding calves TM after the initial colostrum meal have reduced their odds of being assigned a worse eye/ear score, which is associated with a lower incidence of infection/disease (Conneely et al., 2014), and have greater small intestinal surface area and intestinal villi height (Pyo et al., 2018) compared with calves fed whole milk. However, recommendations on feeding TM have not been well established yet, and further research on the effects of extending the duration of TM feeding on calf performance during the pre- and postweaning periods is needed. As the bioactive substances and hormones in TM (Blum and Hammon, 2000; McGrath et al., 2016) are probably stimulating post-natal development of the neonatal calf GIT (Blum and Hammon, 2000), we test a hypothesis that feeding TM for an extended period of 3 weeks may improve the growth performance and health of dairy calves. Therefore, the objective of this study was to assess the effects of replacing pasteurized waste milk with pasteurized TM for 3 weeks on the growth performance and health of dairy calves during the pre- and postweaning periods.

Material and methods

Calves, treatments, management, and climatic conditions

This study was performed from November 19, 2018, to March 10, 2019, at the Ghiam Agriculture and Animal Husbandry, Isfahan, Iran. All the animal procedures were approved by the Animal Care and Use Committee of Shiraz University (IACUC # 201809) by the Iranian Council of Animal Care (1995). During the study period, air temperature (°C) and relative humidity (%) in the calf barn were recorded daily (Hobo Pro Series Temp probes; Onset Computer Corporation, Pocasset, MA, USA) and temperature-humidity index was computed according to National Research Council (NRC) (2001). The average meteorological values were 19.5 ± 3.5 (maximum ± SD), 9.9 ± 2.7 (mean ± SD), and 0.2 ± 3.0 °C (minimum ± SD), respectively, for temperature, 45.2 ± 8.4, 36.3 ± 4.9, and 27.5 ± 4.0 for relative humidity, and 63 ± 3.7, 53 ± 2.9, and 43 ± 3.3 for temperature-humidity index during the overall study period.

Several selection criteria were used to enroll calves in the study. Calves with low or high birth BW were not enrolled in the study emphasizing select calves with more than 30 and less than 40 kg at birth which were close to herd average. Calves with 24-h blood total protein less than 5.5 mg/dl were not enrolled in the study even with fitted BW in the range mentioned. Calves with diarrhea, fever, physical disabilities, failure of suckling, and other health-related issues were not enrolled in the study, in turn, healthy calves with a good general appearance were used. Calves were enrolled in the study in chronological order according to their birth date. During 21 consecutive days, calves were enrolled in the individual pens (4 calves daily; one calf per treatment per day) progressively as born. A total of 84 healthy newborn female Holstein calves (3 days of age; BW = 35.8 ± 0.56 kg; dam parity = 2.3 ± 0.30; mean ± standard error) were weighed (3 days after birth) and housed in a naturally ventilated barn with individual pens (3.0 m × 1.2 m × 1.8 m; length × width × height). A fresh blend of sand and sawdust (7:1) was used as bedding and refreshed every day and manure was removed daily to keep the pens visibly clean and dry.

Daily yielded colostrum was pooled (colostrum from cows with Johne's disease or clinical mastitis history was discarded), pasteurized (60 °C for 30 min; Model SM-392510; Shirmack Pasteurizer, Shirmack Livestock Engineering Group, Isfahan, Iran), and fed to all calves at 12% of birth BW (6% of birth BW at each feeding time) within 1 h of life using a nipple bottle and again 12 h postbirth. The average Brix refractometer reading was 25.7% ± 2.55 (mean ± SD) which was measured using a Reichert AR 200 Digital Handheld Refractometer (Reichert Inc., Depew, NY, USA). On day 2 of life, the calves received pooled pasteurized waste milk 2.5 l in the morning (at 0800 h) by steel bucket and 2.5 l in the evening (at 1700 h). To train a calf to drink from a bucket, two fingers were inserted in its mouth and allowed the calf to start suckling and slowly guided its head down toward the milk in the bucket. Then helped the calf realize there is milk in the bucket and that it can drink it by putting a hand down into the bucket while the calf is still suckling on fingers. The hand was put into the milk, so the calf started to suck it up as it suckled on fingers. Fingers were then left in the calf's mouth as it started to suck the milk out of the bucket. Fingers were removed gradually and allowed the calf to drink from the bucket on its own. If the calf stopped drinking, fingers were replaced and helped it again until the content of the bucket was gone. Most calves caught on very quickly and no animal refused the training.

Calves were in a maternity hospital during the first 2 days of life and then transported to individual pens on day 3 of age to be enrolled in different treatment groups. The healthy animals were blocked by birth order and assigned randomly to one of the following four groups: (1) TM0 [daily pooled pasteurized waste milk (non-saleable milk containing antibiotic and/or drug residues) at 6 l without TM; $n = 21$], (2) TM0.5 (a daily mixture of 5.5 l pooled pasteurized waste milk with 0.5 l pooled pasteurized TM from second milking; $n = 21$), (3) TM1 (a daily mixture of pooled pasteurized waste milk with 1 l pooled pasteurized TM from second milking; $n = 21$), and (4) TM2 (a daily mixture of 4 l pooled pasteurized waste milk with 2 l pooled pasteurized TM from second milking; $n = 21$). Treatments were applied for a 21-day period and all calves were then fed individually with 6 l/day pooled pasteurized waste milk (temperature set at 39 ± 0.5 °C using a water bath) from day 22 to 55, 4 l/day milk from day 56 to 57 of the study followed by a single morning feeding 2 l/day milk from day 58 to 59 of the study. Calves were weaned on day 60 and the study was terminated on day 90.

The study was conducted in a commercial dairy farm where waste milk was the first option of feeding liquid feed to calves (due to its high availability and impact on lowering raising costs). Although feeding milk replacer as an alternative liquid feed for waste milk could potentially avoid day-to-day variations in nutrient offered to calves, we supposed that day-to-day variations in nutrient composition of waste milk may have a similar impact on the performance of calves in different treatment groups due to daily feeding of waste milk from single pooled pasteurized source. Daily yielded waste milk in the farm was transported to a refrigerator and blender containing bulk tank and after pasteurizing (64 °C for 90 min; Model MS-3000 Pasteurizer; Sanaye Boroodati Nasr Inc., Isfahan, Iran) was offered. The pooled pasteurized waste milk (TM from cows with Johne's disease or clinical mastitis history was discarded) was then mixed with pooled pasteurized TM (60 °C for 30 min; Model SM-392510; Shirmack Pasteurizer, Shirmack Livestock Engineering Group, Isfahan, Iran) in separated bulk tanks in ratios of 11:1 (TM0.5), 5:1 (TM1), and 2:1 (TM2) before being fed to calves in different treatment groups. Pasteurization is an option to use to decrease pathogen load and utilize liquid feeds with high bacteria count. This process kills bacteria that can cause diseases; however, pasteurization is not sterilization and pasteurized colostrum/TM still may contain measurable amounts of pathogenic bacteria to survive the pasteurization process. For this reason and as farm protocol, colostrum and TM coming from cows with Johne's disease and clinical mastitis history were excluded to minimize the potential transmission of organisms that cause disease, such as Johne's or infectious microorganisms that cause mastitis. From day 3 onwards, the calves were individually fed liquid feed in steel buckets in two equal meals (at 0830 and 1730 h). From day 1 to 90 of the study, all calves had free access to fresh and clean drinking water and a starter feed diet (fed in steel buckets) formulated according to National Research Council guidelines, allowing at least 10% refusals. The starter feed diet was made on-farm in ground form and blended by 4.6% of DM wheat straw. The ingredients and nutrient compositions of the starter feed diet are given in Table 1.

Table 1
Ingredients and chemical composition (% of DM unless otherwise noted) of the basal starter feed diet of Holstein dairy calves.

Ingredient composition	Diet
Wheat straw	4.6
Corn grain, ground	44.6
Barley grain, ground	7.7
Soybean meal	32.5
Soybean, full fat	1.9
Fish meal	2.9
Vitamin supplement[1]	1.0
Mineral supplement[2]	1.0
Calcium carbonate	1.0
Sodium bicarbonate	1.5
Calcium phosphate (Di-)	0.2
Magnesium oxide	0.3
Salt	0.5
Bentonite	0.3
Chemical composition	
DM	89.8
CP	22.4
Non-fibrous carbohydrate (NFC)[3]	46.1
NDF	16.6
Ether extract (EE)	5.4
Ash	9.5
Calcium[4]	0.76
Phosphorous[4]	0.52
Metabolizable energy,[4] Mcal/kg of DM	3.25
Net energy for maintenance,[4] Mcal/kg of DM	2.43
Net energy for growth,[4] Mcal/kg of DM	1.85

[1] Contained per kilogram of the supplement: 16 g of Zn, 12 g of Mn, 3.5 g of Cu, 120 mg of I, 120 mg of Co, and 80 mg of Se.
[2] Contained per kilogram of the supplement: 1 500 000 IU of vitamin A, 250 000 IU of vitamin D, and 10 000 IU of vitamin E.
[3] NFC = 100 − (CP + NDF + EE + Ash); according to National Research Council guidelines.
[4] Calculated according to National Research Council guidelines.

Sampling and analyses

Throughout the study, refusals were removed daily immediately before the provision of fresh starter feed (at 0900 h). Individual feed intake was determined daily by weighing the amounts of starter feed offered and the amounts refused. Samples of liquid feeds (waste milk and TM) and their blends from daily pooled pasteurized source (two samples daily; one sample at each feeding time) were preserved with potassium dichromate, stored at 4 °C, and submitted to the Central Milk Testing Laboratory of the farm to determine DM as well as fat and protein concentrations using an IR analyzer (MilkoScan 134 BN; Foss Electric, Hillerød, Denmark; Table 2). The values on nutrient composition were used to calculate daily nutrient intake.

Calves consumed all milk offered and no milk refusal was measured indicating successful transitioning from bottle to bucket feeding and no possible variation between calves by milk delivery method. Twenty-four hour after the first feeding of colostrum, jugular blood samples were taken into Vacutainer tubes (BD Vacutainer, Franklin Lakes, NJ, USA) containing spray-coated silica to determine serum total protein using a commercially available handheld clinical refractometer (Model ATA-2771; Atago Co. Ltd., Tokyo, Japan). The bottom threshold (>5.5 mg/dl) for passive transfer of Igs was equal in all treatment groups and only calves with a serum total protein level > 5.5 mg/dl were included in the study. The average serum total protein was 6.1 ± 0.40 mg/dl (mean ± SD), 6.2 ± 0.45, 6.0 ± 0.48, and 6.3 ± 0.44 in TM0, TM0.5, TM1, and TM2, respectively.

To measure DM and chemical analyses, representative samples of wheat straw ($n = 10$; pooled by forage every 10 days over the study period), basal starter feed diet ($n = 10$; pooled by diet every 10 days over the study period), and individual refusals ($n = 9$; pooled by calf every 10 days over the study period) were gathered immediately before the morning feeding. The DM concentration of samples was measured by drying at 100 °C in a forced-air oven for 24 h (AOAC International, 2002; method 925.40). After mixing, samples were ground to pass a 1-mm screen in a Wiley mill (Ogawa Seiki Co. Ltd., Tokyo, Japan) and analyzed in triplicate for CP using the Kjeldahl method (Kjeltec 1030 Auto Analyzer, Tecator, Höganäs, Sweden; AOAC International, 2002; method 955.04), ether extract (AOAC International, 2002; method 920.39), ash (AOAC International, 2002; method 942.05), and NDF using a heat-stable α-amylase (100 μl/0.5 g of sample) and sodium sulfite (Van Soest et al., 1991).

Body weight was measured on the first day of the trial and every 10 days thereafter before the morning feeding [using 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], and average daily gain (**ADG**; kg of BW/d) was calculated as the difference between BW taken every 10 days apart divided by 10. Values from BW were also used to compute weight gains during the preweaning (day 1–60), postweaning (day 61–90), and overall (day 1–90) periods. Feed efficiency (**FE**) was computed as kg of ADG/total DM intake (**DMI**; liquid feed DMI + starter feed DMI). Body size measurements including heart girth (circumference of the chest) and withers height (distance from the base of the front feet to the withers) of the calves were measured manually on the first day of study and every 10 days thereafter as described by Pazoki et al. (2017). Heart girth and withers height were measured using an ordinary measuring tape and measuring stick, respectively. A single observer measured body frame measurements over the study period after establishing high inter-

Table 2
Average daily nutrient composition of transition milk (TM) and the experimental liquid feeds for Holstein dairy calves.

Item	TM	Treatment[1]			
		TM0	TM0.5	TM1	TM2
DM	17.93 ± 2.45	11.63 ± 1.36	12.16 ± 1.36	12.68 ± 1.24	13.73 ± 1.15
CP, % of DM	29.20 ± 2.06	28.54 ± 1.39	28.59 ± 0.91	28.65 ± 0.90	28.76 ± 0.98
Fat, % of DM	22.57 ± 5.41	24.15 ± 3.81	24.02 ± 2.42	23.89 ± 2.37	23.62 ± 2.55
Metabolizable energy,[2] Mcal/kg of DM	5.03 ± 0.23	5.10 ± 0.16	5.09 ± 0.10	5.09 ± 0.10	5.08 ± 0.11

[1] Treatments were: TM0 = calves received daily pooled pasteurized waste milk at 6 l from day 1 to 21 of the study; TM0.5 = calves received a daily mixture of 0.5 l pooled pasteurized TM from second milking and 5.5 l pooled pasteurized waste milk from day 1 to 21 of the study; TM1 = calves received a daily mixture of 1 l pooled pasteurized TM and 5 l pooled pasteurized waste milk from day 1 to 21 of the study, and TM2 = calves received a daily mixture of 2 l pooled pasteurized TM and 4 l pooled pasteurized waste milk from day 1 to 21 of the study. From day 22 onward, all calves were fed individually with 6 l/day pasteurized waste milk, weaned on day 60, and monitored until day 90 of the study.
[2] Calculated according to National Research Council guidelines.

observer reliability [Cohen's kappa coefficient (κ_W) > 0.83)] for each measurement. These values were used to calculate skeletal growth at three discrete periods as pre-weaning, post-weaning, and overall periods.

Health

Calves were checked daily for health status based on their appetite to consume milk and starter as well as their general appearance by a veterinarian, blinded to the treatments, during the experimental period (day 1–90). Feces were scored daily based on a 1–5 system (1 = normal; 2 = soft to loose; 3 = loose to watery; 4 = watery, mucous, and slightly bloody; and 5 = watery, mucous, and bloody) while calves were in individual pens (Pazoki et al., 2017). Fecal score (1–5 scale) was categorized as a number of days with fecal score ≥ 3 and donated as days with the abnormal fecal score. In the case of having fecal score ≥ 3, the calves were examined by the veterinarian to confirm diarrhea diagnoses. Calves with diarrhea were medicated with standard procedures prescribed by the veterinarian. Calves with diarrhea received water-based oral rehydration salt solution [4 l/day per calf in two equal meals (at 0300 and 1500 h) for two consecutive days; Damyaran-e-Arak Co., Arak, Iran] and sulfadimidine syrup (sulfadimidine sodium 33.3%; 15 ml/day per calf for two consecutive days; Fan-Avari Zisti Kimia Co., Arak, Iran). Nonresponding individuals were treated for five more days with enrofloxacin (10%; 2 ml per calf for five consecutive days; Rooyan Darou Co., Semnan, Iran) and Norodine 24 [sulfadiazine (200 mg/ml) + trimethoprim (40 mg/ml); 5 ml per calf for five consecutive days; Norbrook Laboratories Ltd., Newry, UK). Calves not experienced pneumonia and no animal died during the experimental period.

Statistical analysis

Prestudy power analysis for sample size estimation was performed for the primary response variables, including feed intake, BW, and BW gain based on previously published values (Pazoki et al., 2017; Kargar et al., 2020). From the power test analysis, using $\alpha = 0.05$ and power = 0.85, the projected sample size was 12 calves per treatment for starter intake, DMI, ADG, and BW. Therefore, 84 animals were enough to get a significant result with adequate probability (power) in performance among the treatments according to the power analysis performed by POWER PROC of SAS (version 9.4, SAS Institute Inc., Cary, NC, USA). All data were screened for normality using PROC UNIVARIATE and normalized as required using a log10 transformation (skeletal measurements) and a square root transformation (feed intake, ADG, and FE). Means shown in Table 3 for these variables are back-transformed. Repeated-measures data (feed intake and growth performance) were analyzed for preweaning, postweaning, and overall periods using the PROC MIXED of SAS. The model consisted of the order of introduction to the individual pens (i.e., birth order), treatment (Treat; TM0, TM0.5, TM1 or TM2), period (P; 1- or 10-day period), and Treat × P as the fixed effects and calf within period and THI as the random effects. The lowest Bayesian information criterion (fit statistic) level was used to select the covariance structure, and the compound symmetry structure was accordingly modeled. Initial skeletal size and starter feed intake were used as covariates in the skeletal measurements and ADG models, respectively. Data on initial BW and body size and those gains were analyzed using the same model without the P effect. Models for the occurrence of diarrhea (≥3) and the need for medication were examined by logistic regression, using a binomial distribution in PROC GLIMMIX. The odds ratio was used to compare the probability for calves on each treatment to experience any event. Frequency and duration of diarrhea, administration of medication, and the number of days with diarrhea (≥3) were examined with a Poisson distribution using PROC GENMOD. Data were reported as least square means and the Tukey–Kramer adjustment was applied to account for multiple comparisons. The threshold of significance was set at $P \le 0.05$; trends were declared at $0.05 < P \le 0.10$.

Cliff's delta (effect size) of five traits was also calculated for comparisons of two independent groups (TM2 vs TM0) according to the original formulation by Norman Cliff (Cliff, 1993) using the scikits bootstrap package: https://github.com/cgevans/scikits-bootstrap. The Cliff's delta estimator was obtained with the below equation:

$$d = \frac{\#(X1 > X2) - \#(X1 < X2)}{n1 \times n2}$$

In this expression, X_1 and X_2 were scored within group 1 (e.g., TM2) and group 2 (e.g., TM0), and n_1 and n_2 were the sizes of the sample groups. The cardinality symbol # indicates the number of times value from the test sample (e.g., TM2) exceeds (or is lesser than) values in the control sample (e.g., TM0). This statistic estimates the probability that the value selected from one of the groups is greater than a value selected from the other group, minus the reverse probability. Cliff's delta ranges from −1 to 1. An effect size of +1 or −1 indicates the absence of overlap between the two groups, whereas a 0.0 indicates that group distributions overlap completely. Cliff's delta was as per standard practice referred to delta as either 'negligible' ($d < 0.15$), 'small' ($d < 0.33$), 'moderate' ($d < 0.47$), or 'large' ($d > 0.47$, Kargar et al., 2020). Two-sided P-values using a non-parametric Brunner–Munzel test were used for comparisons of two independent groups (TM2 vs TM0).

Results

Replacing waste milk with TM increased liquid feed DM concentration but not the CP, fat, and metabolizable energy contents (Fig. 1A). Replacing waste milk with TM did not affect the starter feed DMI or total DMI during the preweaning, postweaning, and overall periods (Table 3, Fig. 1B); however, liquid feed DMI was increased with the increasing replacement of waste milk with TM ($P = 0.001$). Initial BW (Fig. 1C) and BW changes during the preweaning were not affected by treatment (Table 3). Calves fed the higher levels of TM (TM2) gained more BW compared with the calves fed TM0 during the postweaning

Table 3
Effects of replacing pasteurized waste milk with pasteurized transition milk (TM) for 3 weeks on intake, growth performance, and changes in skeletal growth of Holstein dairy calves (n = 21 per group). DMI - dry matter intake.

Item	Treatment (Treat)[1]				SEM	*P*-value		
	TM0	TM0.5	TM1	TM2		Treat	Period (P)	Treat × P
Starter feed DM intake (DMI), g/d								
Preweaning (day 1–60)	515	500	455	480	21.77	0.19	0.001	0.75
Postweaning (day 61–90)	2 951	2 871	2 975	3 092	80.45	0.25	0.001	0.61
Overall (day 1–90)	1 327	1 290	1 295	1 352	30.29	0.39	0.001	0.41
Pasteurized TM DMI (day 1–21), g/day	0^{d}	87^{c}	174^{b}	347^{a}	1.98	0.001	0.001	0.93
Pasteurized milk DMI (day 1–60), g/day	675^{a}	654^{b}	633^{c}	592^{d}	2.53	0.001	0.001	0.001
Liquid feed DMI (day 1–60),[2] g/day	675^{d}	684^{c}	694^{b}	713^{a}	2.44	0.001	0.001	0.001
Total DMI,[3] g/day								
Preweaning (day 1–60)	1 191	1 184	1 149	1 193	21.70	0.39	0.001	0.12
Postweaning (day 61–90)	2 951	2 871	2 975	3 092	80.45	0.25	0.001	0.61
Overall (day 1–90)	1 777	1 746	1 757	1 828	30.26	0.21	0.001	0.35
Initial BW (day 1), kg	36.1	35.8	35.7	35.6	0.56	0.93	–	–
BW changes, kg/period								
Preweaning (day 1–60)	44.0	44.7	43.3	47.9	2.09	0.13	–	–
Postweaning (day 61–90)	31.6^{b}	33.3^{ab}	33.4^{ab}	35.7^{a}	1.10	0.02	–	–
Overall (day 1–90)	75.6^{b}	78.0^{ab}	76.7^{ab}	83.6^{a}	2.98	0.03	–	–
Average daily gain, g/day								
Preweaning (day 1–60)	733	745	722	798	34.29	0.06	0.001	0.001
Postweaning (day 61–90)	1 053^{b}	1 110^{ab}	1 113^{ab}	1 190^{a}	36.02	0.04	0.22	0.76
Overall (day 1–90)	840^{b}	867^{ab}	852^{ab}	929^{a}	35.58	0.005	0.001	0.05
Feed efficiency[4]								
Preweaning (day 1–60)	0.62^{b}	0.63^{ab}	0.63^{ab}	0.67^{a}	0.02	0.03	0.001	0.31
Postweaning (day 61–90)	0.36^{b}	0.39^{ab}	0.37^{ab}	0.39^{a}	0.01	0.02	0.001	0.69
Overall (day 1–90)	0.47^{b}	0.50^{ab}	0.49^{ab}	0.51^{a}	0.01	0.01	0.001	0.21
Initial heart girth (day 1), cm	79.0	79.0	78.5	78.3	0.70	0.84	–	–
Heart girth changes, cm/period								
Preweaning (day 1–60)	25.0	25.0	25.0	26.2	0.80	0.62	–	–
Postweaning (day 61–90)	10.1	10.5	10.3	9.7	0.81	0.91	–	–
Overall (day 1–90)	35.1	35.5	35.3	35.9	0.73	0.86	–	–
Initial withers height (day 1), cm	77.0	76.7	77.1	77.0	0.55	0.94	–	–
Withers height changes, cm/period								
Preweaning (day 1–60)	12.1^{b}	12.4^{ab}	12.9^{ab}	14.3^{a}	0.62	0.03	–	–
Postweaning (day 61–90)	8.3	9.3	8.7	8.6	0.64	0.71	–	–
Overall (day 1–90)	20.4^{b}	21.7^{ab}	21.6^{ab}	22.9^{a}	0.68	0.05	–	–

[a–d] Means within a row with different superscripts are significantly different ($P \le 0.05$). Data on feed intake, average daily gain, and feed efficiency were square root-transformed, and back-transformed values are presented in the table.
[1] Treatments were: TM0 = calves received daily pooled pasteurized waste milk at 6 l from day 1 to 21 of the study; TM0.5 = calves received daily mixture of 0.5 l pooled pasteurized TM from second milking and 5.5 l pooled pasteurized waste milk from day 1 to 21 of the study; TM1 = calves received daily mixture of 1 l pooled pasteurized TM and 5 l pooled pasteurized waste milk from day 1 to 21 of the study; and TM2 = calves received daily mixture of 2 l pooled pasteurized TM and 4 l pooled pasteurized waste milk from day 1 to 21 of the study. From day 22 onward, all calves were fed individually with 6 l/day pasteurized waste milk, weaned on day 60, and monitored until day 90 of the study.
[2] Liquid feed DMI (g/d) = pasteurized TM DMI + pasteurized waste milk DMI.
[3] Total DMI (g/d) = liquid feed DMI + starter feed DMI.
[4] Feed efficiency was calculated by dividing average daily gain by average total DMI.

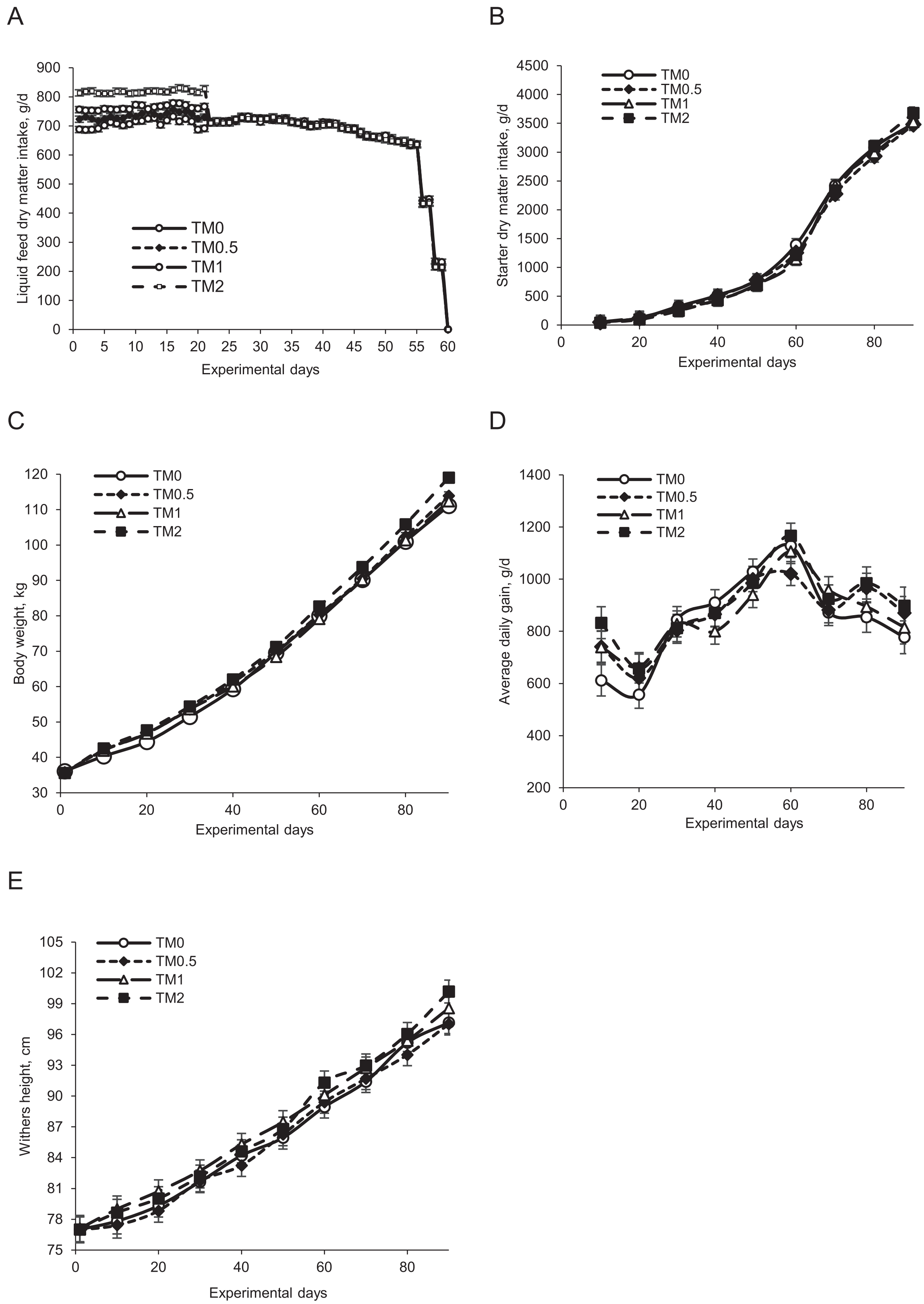
(+4.1 kg; $P = 0.02$) and overall (+8.0 kg; $P = 0.03$) periods. Calves fed TM2 were heavier than calves fed TM0 on day 80 ($P \le 0.10$) and 90 ($P \le 0.05$) of the study. The ADG was higher in calves fed TM2 vs TM0 on day 10 of the study ($P \le 0.05$; Fig. 1D) and also increased during the postweaning (1 190 vs 1 053 g/day for TM2 vs TM0; $P = 0.04$) and overall (929 vs 840 g/day for TM2 vs TM0; $P = 0.005$) periods. Feed efficiency increased in calves fed TM2 compared with calves fed TM0 during all studied periods. Replacing waste milk with TM did not affect the heart girth gain during the studied periods; however, calves fed the higher levels of TM (TM2) had greater withers height gain than calves fed TM0 during the preweaning and overall periods ($P = 0.05$; Table 3 and Fig. 1E).

In this study, we computed Cliff's delta for five traits including, FE, ADG, BW changes, withers height, and heart girth gain during the entire period (Fig. 2). Positive and statistically significant Cliff's delta (effect sizes) with large effect ($d > 0.47$) were observed for ADG ($d = 0.58$), and BW changes ($d = 0.53$) in calves fed 2 l TM compared to those fed only waste milk (TM0). Positive and statistically significant Cliff's delta with moderate effect ($d = 0.35$) was observed for FE and withers height gain. The effect size of heart girth gain was small ($d > 0.33$). No negative Cliff's delta was indicated for the measured traits.

Table 4 presents the logistic models for the occurrence of diarrhea (score ≥ 3) or needing to medication during the overall (day 1–90) period. The occurrence of diarrhea was lower in TM2 compared with TM0 (odds ratio = 0.59; $P = 0.003$), TM0.5 (odds ratio = 0.72; $P = 0.07$), and TM1 (odds ratio = 0.70; $P = 0.05$) without changing in the chance of medication among treatments. Table 5 presents the Poisson regression for frequency and duration of diarrhea (score ≥ 3) or medication. The frequency of diarrhea and medication was not affected by treatment; however, calves in TM2 experienced shorter days with diarrhea compared with those in TM0 (1.7 days; $P = 0.03$), TM0.5 (1.0 days; $P = 0.09$), or TM1 (1.1 days; $P = 0.08$).

Discussion

Following the colostrum, the subsequent 2–6 milkings referred to as TM (Godden, 2008). Transition milk similar to colostrum is rich sources of bioactive components (Blum and Hammon, 2000) that are beneficial for calf GIT development (McGrath et al., 2016). Though TM, similar to colostrum, contains an abundance of bioactive compounds that not present in whole milk (Blum and Hammon, 2000; McGrath et al., 2016), we conducted a trial to evaluate the effects of replacing pasteurized waste milk with pasteurized TM for 3 weeks on the growth


A
Liquid feed dry matter intake, g/d
Experimental days
TM0
TM0.5
TM1
TM2
B
Starter dry matter intake, g/d
Experimental days
TM0
TM0.5
TM1
TM2
C
Body weight, kg
Experimental days
TM0
TM0.5
TM1
TM2
D
Average daily gain, g/d
Experimental days
TM0
TM0.5
TM1
TM2
E
Withers height, cm
Experimental days
TM0
TM0.5
TM1
TM2

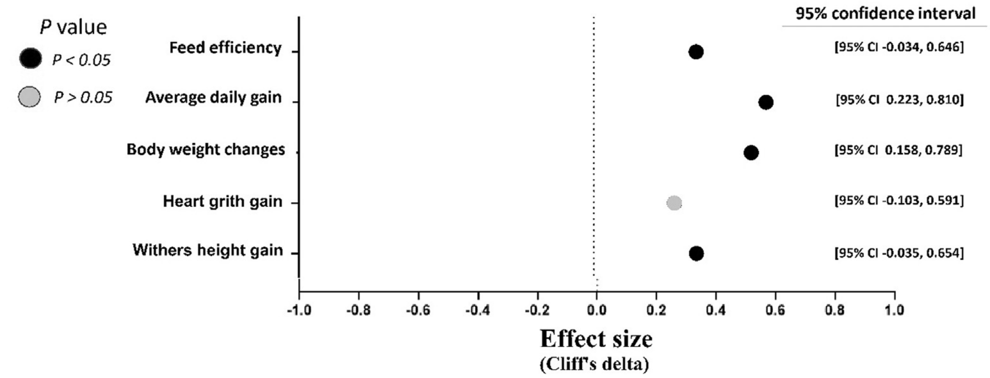


Fig. 2. The Cliff's delta indices of effect sizes (filled circles) of two independent groups (TM2 vs TM0) for five parameters, with 95% confidence intervals [CI width lower bound; upper bound]. Cliff's delta ranges from −1 to 1 and an effect size of +1 or −1 indicates the absence of overlap between the two groups, whereas a 0.0 indicates that group distributions overlap completely. Cliff's delta was as per standard practice referred to delta as either 'negligible' ($d < 0.15$), 'small' ($d < 0.33$), 'moderate' ($d < 0.47$), or 'large' ($d > 0.47$). Positive Cliff's delta for a given parameter indicates an increase in TM2 compared to respective control (TM0). Two-sided *P*-values using a non-parametric Brunner–Munzel test for comparisons of two independent groups (TM2 vs TM0). The significant ($P > 0.05$) *P*-value is shown in the above plot. TM0 = calves received daily pooled pasteurized waste milk at 6 l from day 1 to 21 of the study and TM2 = calves received a daily mixture of 2 l pooled pasteurized TM and 4 l pooled pasteurized waste milk from day 1 to 21 of the study. From day 22 onward, all calves were fed individually with 6 l/day pasteurized waste milk, weaned on day 60, and monitored until day 90 of the study.

performance and health of dairy calves. The extended TM feeding to calves for several days may be commonplace on some dairy farms; however, the benefits of this practice during the pre- and postweaning stages on the growth performance and health of calves remain unclear. Our findings showed that the inclusion of 2 l of TM in 6 kg of waste milk may be beneficial to the growth and health of dairy calves.

In the current study, liquid feed DMI was increased by increasing the concentration of TM in the liquid feed. Despite that TM DMI and total liquid feed DMI were substantially higher when waste milk was partially replaced with TM, extended TM feeding did not increase starter and total DM intakes compared with waste milk feeding. Our results imply that the partial replacement of waste milk with TM at 2 l/day during the first 3 weeks of life appears to have a carryover effect on the postweaning ADG, resulting in an improved weight gain. The partial replacement of waste milk with TM at 2 l/day improved the growth performance of calves in this study. In support of this observation, data from the previous study indicated that continuous feeding of smaller amounts of colostrum (10 g IgG) during the first 2 weeks of life improved the early weight gain (on day 28 of age) compared with control calves, but the ADG at 60 days was not significantly different from the control group (Berge et al., 2009). Another study showed that feeding enriched milk replacer with 150 g colostrum replacer powder (containing 14% IgG) during the first 14 days of life did not affect BW and ADG but reduced the occurrence of diseases and use of antibiotic therapy in dairy calves during the preweaning period (Chamorro et al., 2017). In the current study, the greater ADG in the TM2 calves compared with TM0 calves likely because of a combination of greater FE and lower duration of diarrhea (Table 5).

Calves fed TM2 used nutrients more efficiently during all studied periods than calves fed waste milk only. Jones et al. (2004) reported greater FE and a tendency to gain more BW in calves receiving 250 g of IgG via maternal colostrum than calves receiving the same dose via colostrum substitute during the first week of life. However, no difference was observed when the calves were weaned. Also, the improvement of FE in

Table 4
Logistic model for diarrhea (score ≥ 3) and medication occurrence during the overall (day 1–90) period as influenced by incremental replacement of pasteurized waste milk with transition milk (TM) in liquid feed for Holstein dairy calves ($n = 21$ per group).

Variable and comparison[1]	Coefficient	SEM	Odds ratio[2]	95% confidence interval	*P*-value
Diarrhea occurrence					
TM2 vs TM1	−0.3538	0.18	0.70	0.49, 1.00	0.05
TM2 vs TM0.5	−0.3260	0.18	0.72	0.50, 1.03	0.07
TM2 vs TM0	−0.5189	0.17	0.59	0.42, 0.84	0.003
TM1 vs TM0.5	0.0277	0.16	1.02	0.74, 1.42	0.86
TM1 vs TM0	−0.1651	0.15	0.84	0.62, 1.15	0.30
TM0.5 vs TM0	−0.1929	0.16	0.82	0.60, 1.13	0.23
Diarrhea medication occurrence					
TM2 vs TM1	−0.0868	0.18	0.91	0.63, 1.32	0.64
TM2 vs TM0.5	0.0489	0.18	1.05	0.73, 1.49	0.78
TM2 vs TM0	0.1694	0.17	1.18	0.83, 1.67	0.33
TM1 vs TM0.5	0.1357	0.18	1.14	0.79, 1.64	0.46
TM1 vs TM0	0.2562	0.17	1.29	0.90, 1.83	0.15
TM0.5 vs TM0	0.1205	0.17	1.12	0.80, 1.58	0.48

[1] Treatments were: TM0 = calves received daily pooled pasteurized waste milk at 6 l from day 1 to 21 of the study; TM0.5 = calves received daily mixture of 0.5 l pooled pasteurized TM from second milking and 5.5 l pooled pasteurized waste milk from day 1 to 21 of the study; TM1 = calves received daily mixture of 1 l pooled pasteurized TM and 5 l pooled pasteurized waste milk from day 1 to 21 of the study; and TM2 = calves received daily mixture of 2 l pooled pasteurized TM and 4 l pooled pasteurized waste milk from day 1 to 21 of the study. From day 22 onward, all calves were fed individually with 6 l/day pasteurized waste milk, weaned on day 60, and monitored until day 90 of the study.

[2] The odds ratio (OR) indicates the probability of having diarrhea (≥3) or needing medication in calves fed incremental levels of TM in the liquid feed. If the OR is >1, a given treatment in the comparison is more likely to have an occurrence of diarrhea or to be medicated than the other treatment by a factor of the difference above 1. If the OR is <1, a given treatment has a lower probability of occurrence than the other treatment.

Fig. 1. (A) Liquid feed (waste milk + transition milk) DM intake [DMI; g/day, the SEM = 0.82. Effects in model: treatment (Treat): $P = 0.001$; Period (P): $P = 0.001$; Treat×P: $P = 0.001$], (B) starter feed DMI [g/day; the SEM = 30.29. Effects in model: Treat: $P = 0.39$; Period (P): $P = 0.001$; Treat×P: $P = 0.41$], (C) Body weight [BW; kg, $P = 0.001$; Treat×P: $P = 0.01$], (D) average daily gain [ADG; g/day, the SEM = 16.58. Effects in model: Treat: $P = 0.005$; Period (P): $P = 0.001$; Treat×P: $P = 0.05$], and (E) withers height [cm, the SEM = 0.38. Effects in model: Treatment (Treat): $P = 0.01$; Period (P): $P = 0.001$; Treat×P: $P = 0.88$] as influenced by incremental replacement of pasteurized waste milk with pasteurized transition milk (TM) in liquid feed for newborn Holstein calves during the entire period. Treatments were: TM0 = calves received daily pooled pasteurized waste milk at 6 l from day 1 to 21 of the study; TM0.5 = calves received daily mixture of 0.5 l pooled pasteurized TM from second milking and 5.5 l pooled pasteurized waste milk from day 1 to 21 of the study; TM1 = calves received daily mixture of 1 l pooled pasteurized TM and 5 l pooled pasteurized waste milk from day 1 to 21 of the study; and TM2 = calves received daily mixture of 2 l pooled pasteurized TM and 4 l pooled pasteurized waste milk from day 1 to 21 of the study. From day 22 onward, all calves were fed individually with 6 l/day pasteurized waste milk, weaned on day 60, and monitored until day 90 of the study ($n = 21$ pre group).

Table 5
Poisson regression for frequency and duration of diarrhea (score ≥ 3) or medication during the overall (day 1–90) period as influenced by incremental replacement of pasteurized waste milk with transition milk (TM) in liquid feed for Holstein dairy calves (n = 21 per group).

Item	Treatment (Treat)[1]				SEM	P-value
	TM0	TM0.5	TM1	TM2		Treat
Diarrhea						
Frequency, no. of times diagnosed	1.7	1.2	1.6	1.1	0.18	0.30
Duration, days per diarrhea event	4.2[a]	3.5[ab]	3.6[ab]	2.5[b]	0.11	0.03
Diarrhea medicated						
Frequency, no. of times diagnosed	1.2	1.0	1.0	1.1	0.21	0.92
Duration, days per diarrhea event	3.5	3.1	2.7	2.9	0.12	0.50

[a,b]Means within a row with different superscripts are significantly different ($P \le 0.05$).
[1] Treatments were: TM0 = calves received daily pooled pasteurized waste milk at 6 l from day 1 to 21 of the study; TM0.5 = calves received daily mixture of 0.5 l pooled pasteurized TM from second milking and 5.5 l pooled pasteurized waste milk from day 1 to 21 of the study; TM1 = calves received daily mixture of 1 l pooled pasteurized TM and 5 l pooled pasteurized waste milk from day 1 to 21 of the study; and TM2 = calves received daily mixture of 2 l pooled pasteurized TM and 4 l pooled pasteurized waste milk from day 1 to 21 of the study. From day 22 onward, all calves were fed individually with 6 l/day pasteurized waste milk, weaned on day 60, and monitored until day 90 of the study.

replacement heifers is beneficial to the dairy industry, as feed is a major cost in raising dairy replacements. In the current study, Cliff's delta confirmed that the partial replacement of waste milk with TM at 2 l/day during the first 3 weeks of life improved efficiency in converting feed nutrients into increased body mass (weight gain).

In the present study, calves receiving further feedings of TM had a lower likelihood of diarrhea and the duration of diarrhea. However, partial replacement of milk with TM did not affect the likelihood of diarrhea medication throughout the study period. Perhaps the localized protective effects of colostral antibodies or other bioactive compounds (Godden, 2008) remaining in the lumen improved the gut immune system or protected calves against the colonization of pathogens. A recent study reported a tendency toward reducing the risk of diarrhea 14 days following diagnosis in dairy calves supplemented with lactoferrin (3 g of lactoferrin; an iron-binding protein found in colostrum) for three consecutive days (Pempek et al., 2019), although they concluded that lactoferrin as a treatment for calf diarrhea was not beneficial. In line with our findings, Conneely et al. (2014) reported that providing calves with further feedings of TM (2 or 4 subsequent feedings) following the initial feeding of colostrum reduced the likelihood of being assigned a worse eye/ear and nasal score. In another study, calves receiving 10 g of supplemental IgG in the milk replacer for 14 days had less diarrhea and received fewer antimicrobial treatments than control calves (Berge et al., 2009). Finally, our results indicated that partial replacement of milk with TM could help the calves improve the health status in early life. Our recent study showed that the partial replacement of pasteurized whole milk with pasteurized colostrum decreased the occurrence of diarrhea and resulted in fewer days with a rectal temperature ≥39.4 °C, general appearance ≥2, and pneumonia (Kargar et al., 2020). However, this is an area that warrants further research.

Conclusion

Our findings revealed that the partial replacement (2 l/day) of waste milk with TM for an extended period of 3 weeks improved the weight gain of dairy calves compared to the waste milk only treatment likely by improving FE and fewer days with diarrhea. Effect size (Cliff's delta) confirmed that feeding TM (2 l/day) improved efficiency in converting feed nutrients into increased body mass. Calves receiving the highest level of TM (2 l/day) had the lowest risk for diarrhea (odds ratio) and experienced shorter days with diarrhea during the trial. These findings extend our understanding of the effects of TM feeding on the growth performance and health of dairy calves. In general, the partial replacement of pasteurized waste milk with TM for newborn calves may be considered as a new model system that could be further used to improve the growth performance and health of dairy calves.

Ethics approval

All the animal procedures were approved by the Animal Care and Use Committee at Shiraz University (IACUC # 201809), Shiraz, Iran.

Data and model availability statement

None of the data were deposited in an official repository.

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Declaration of interest

None.

Acknowledgements

The authors are grateful to the Ghiam Agriculture and Animal Husbandry (Isfahan, Iran; grant number 12112018) and Shiraz University (Shiraz, Iran; grant number 9530751) for partial financial support of this study. The authors also express their kind appreciation to the farm staff for their help in conducting this research and diligent animal care. The authors also express their appreciation to several students from Shiraz University completing course requirements for their input to the research and laboratory analyses.

Financial support statement

This work was supported by the Ghiam Agriculture and Animal Husbandry (Isfahan, Iran; grant number 12112018) and Shiraz University (Shiraz, Iran; grant number 9530751).

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