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Effects of plane of milk-replacer nutrition on the health, behavior, and performance of high-risk Holstein bull calves from a commercial calf ranch

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Objective: This research study aimed to determine whether preweaning plane of milk-replacer nutrition influences health, standing and oral behaviors, and performance of high-risk calves.

Materials and Methods: Thirty-six Holstein bull calves (1 d of age) from a commercial calf ranch were assigned to either a high plane of milk-replacer nutrition (HPN; n = 18; 20 and 28% DM lipid and protein, respectively, Cow’s Match, Land O’Lakes Animal Milk Protein Co., Shoreview, MN) or a low plane of milk-replacer nutrition (LPN; n = 18; 20% DM lipid and protein, Herd Maker, Land O’Lakes Animal Milk Protein Co.) through weaning. Total serum protein concentrations confirmed that 72 and 76% of calves had failure of passive transfer (<5.2 g/dL) in HPN and LPN, respectively. All calves were bottle fed twice daily. The LPN were fed 455 g of DM/d of milk replacer (MR) until weaning, and the HPN calves were fed 830 g of DM/d of milk replacer during the first 10 d and 1,080 g from 11 d until weaning. Calf starter and water were offered ad libitum. Calves were step-down weaned beginning at 51 d and completely weaned after 58 d when consuming 1 kg of calf starter (as-fed basis). Data are reported as HPN versus LPN throughout, respectively.

Results and Discussion: Risk for bloat and scouring were greater for calves fed the HPN, but there was no difference in antibiotic treatment or mortality. The HPN calves had greater performance over the preweaning period including final BW, ADG, and feed:gain (total kg of milk replacer + calf starter, kg of DM/kg of BW gain), but weaning was more stressful for HPN calves.

Implications and Applications: In conclusion, the high-risk calves fed a HPN had increased preweaning ADG, but there was also an increased incidence of scours. There may be differences in the health status of calves that may affect the ability to consume and use high MR levels. When feeding high-risk calves there may be a need for alternate strategies when determining the quality and quantity of MR being fed when compared with low-risk calves. Differences in gut integrity may influence the ability of high-risk calves to handle high levels of MR early in life. Potential issues with high-risk calves being fed a HPN might be reduced with increased feeding times per day, a gradual step-up method, or other nutritional strategies that improve gastrointestinal development and function.

Key words: dairy, gastrointestinal health, weaning

INTRODUCTION

Determining the effects of an increased plane of nutrition (PON) on the health, behavior, and performance of high-risk dairy calves can help establish recommendations for feeding calves more milk solids to increase measures of gain and animal health without compromising normal calf behavior. Feeding calves more milk earlier in life has the potential to increase BW and age at first weaning and may have implications into the entire lifespan and productivity of the calf.

A common practice in the dairy industry is to restrict the quantity of milk solids fed to calves to accelerate starter consumption and decrease the age of weaning. However, an increasing number of operations are beginning to feed greater quantities of milk solids (NAHMS, 2014). A higher PON increases preweaning growth performance as well as future lactation performance (Davis Rincker et al., 2011; Ollivett et al., 2012; Soberon et al., 2012).

The influence that PON has on the risk for enteric disease during the preweaning period continues to be debated. A study completed by Quigley et al. (2006) reported that calves force fed greater quantities of milk solids had increased incidence of scours and antimicrobial treatments, whereas Ollivett et al. (2012) reported improved recovery and better hydration following a Cryptosporidium challenge in calves that were free fed. It is generally
accepted that when calves are fed greater quantities of milk solids, their fecal consistency appears looser or they tend to have more diarrhea. Liang et al. (2016) reported that fecal consistency may not be the best indicator of enteric health because even though the feces look more loose, there was no difference in the DM percentage of the feces from healthy calves fed lesser versus greater planes of milk-replacer nutrition. Another concern with feeding a reduced PON is that a lower PON does not satiate calves and possibly can cause prolonged expression of extra non-nutritive oral behaviors. High-risk calves experiencing failure of passive transfer (FPT) of colostrum require particular care and management, and increased rates of sickness may impede nonnutritive oral behavior development (Hulbert and Moisá, 2016). Increased health risks, including gastrointestinal ailments, are common in colostrum-deprived calves. High-risk calves are calves that may have FPT, increased exposure to pathogens, or increased stressors such as transport in the first few days of life. Thus, nutritional planes may especially influence these high-risk calves. Therefore, the objective of this study was to determine whether preweaning PON influences performance, behavior, and health in high-risk neonatal calves.

MATERIALS AND METHODS

Animals and Management

All experimental procedures were in compliance with the Guide for the Care and Use of Agricultural Animals in Research and Teaching and approved by the Institutional Animal Care and Use Committee of Texas Tech University.

Thirty-six Holstein bull calves (1 d of age) were acquired from a local calf ranch and consisted of calves from 8 commercial dairies within a 160-km radius of Lubbock, Texas. Calves were randomly assigned to 1 of 2 dietary treatments (n = 18 calves per treatment): a low plane (LPN) of milk-replacer nutrition or a high plane (HPN) of milk-replacer nutrition. Calves fed the LPN were fed 455 g of DM/d from 1 d of age to 10 d of age and 1,080 g of DM/d from 11 d until weaning of a 28% CP, 20% lipid MR at 14.9 and 15.5% solids DM (Cow’s Match, Land O’Lakes Animal Milk Protein Co., Shoreview, MN; Table 1). Calves fed the HPN were fed 830 g of DM/d from 1 d of age to 10 d of age and 1,080 g of DM/d from 11 d until weaning of a 28% CP, 20% lipid MR at 14.9 and 15.5% solids DM (Cow’s Match, Land O’Lakes Animal Milk Protein Co., Table 1). Calves were fed MR from bottles twice daily at 0730 and 1630 h. All calves were offered ad libitum access to a texturized calf starter (Table 1) and water. Peripheral blood samples were collected from the jugular vein into evacuated tubes without any additive at enrollment and analyzed for total serum protein using a hand-held refractometer. A total serum protein concentration of 5.2 g/dL was equivalent to 1,000 mg/dL of serum IgG. Total serum protein concentrations confirmed that 72% of HPN and 76% of LPN calves had FPT (<5.2 g/dL). All calves were housed outdoors in commercially available calf hutches (2.13 × 1.09 m, Agri-Plastics, Cortland, NY) with an attached pen (1.83 × 1.09 m) bedded with sand, and bottle holder, water-bucket, and grain-bucket located inside the hutch. Calf starter and water intakes were measured daily. Calves were vaccinated with a 5-way (infectious bovine rhinotracheitis, bovine viral diarrhea virus I and II, bovine respiratory syncytial virus, parainfluenza-3; Triangle 5; Boehringer Ingelheim, Ridgefield, CT) vaccine at 28 d and at booster at 42 d. Weaning was initiated at 51 d by removal of the afternoon feeding. Calves were completely weaned from MR beginning after 58 d once they consumed 1 kg as fed/d of calf starter.

Experimental Design

Individual calf BW was recorded at enrollment and at 25, 49, and 68 d of the study period. Voluntary MR refusals were recorded 30 min after each feeding. Peripheral blood samples were collected into 6-mL heparin vacutainers (BD vacutainers, Fisher Scientific, Waltham, MA) at 0600 h on 0, 25, 38, 48, and 68 d relative to enrollment via jugular venipuncture. Blood samples were centrifuged at room temperature (1,500 × g for 20 min) within 25 min of collection, and plasma was stored at -40°C until subsequent analyses. Plasma glucose and urea nitrogen were analyzed using commercially available enzymatic kits (Stanbio Laboratory, Boerne, TX). All procedures were followed per the manufacturer’s instructions for the manual method; however, sample and reagent volumes were scaled for use in 96-well microplates. All colorimetric data were measured on a SpectraMax 340PC (Molecular Devices, Sunnyvale, CA). Control serum (Randox Laboratories, Oceanside, CA) was used to calculate the inter-assay CV of 4.1 and 5.0% for plasma glucose and urea nitrogen, respectively. The mean intra-assay CV for plasma glucose and urea nitrogen were 3.8 and 4.3%, respectively.

Fecal Scoring and Disposition Scoring

Fecal scores were classified twice daily around feeding according to the guidelines outlined by Larson et al. (1977), by 2 independent trained observers that were not blinded to treatment: 1 = firm, well-formed (not hard); 2 = soft, pudding like; 3 = runny, pancake batter; and 4 = liquid, splatters, pulpy orange juice. Data for each calf were averaged over 3-d periods before statistical analysis, and a scouring event was reported as 2 consecutive fecal scores of 4. The 2 independent evaluations were compared and any disagreement between the evaluations on a calf were reconciled by both observers looking at the calf together and agreeing on a fecal score. Bloat was assessed before and within 1 h after each feeding. Disposition scores for both the body and head were scored twice daily imme-
Immediately before each MR feeding. Calves were scored on a 1-to-3 scoring system. The body disposition scores were 1 = standing; 2 = lying sternal; and 3 = lying lateral. The head scores were 1 = alert, ears up; 2 = depressed, head distended or ears droopy; and 3 = head extended. Calves with either a body disposition score of 3 or head score of 2 or 3 had a rectal temperature taken before the a.m. feeding. Any calf with a rectal temperature greater than 39.5°C or less than 37.5°C was treated with Resflor Gold (Merck Animal Health, Kenilworth, NJ).

**Ex Vivo Leukocyte Function Measures**

Ex vivo leukocyte responses were performed as described by Hulbert et al. (2011) with minor modifications. Whole blood was stimulated with lipopolysaccharide from *Escherichia coli* O111:B4 to estimate the ability of whole blood cells to secrete tumor necrosis factor-α (TNF-α). For the cultures, 200 μL of whole heparinized blood was added to 800 μL of Roswell Park Memorial Institute (RPMI) medium 1640 (#11875-085, Invitrogen Life Technologies, Grand Island, NY) containing, at a final concentration, 1% antibiotic and antifungal (#15240-062, Invitrogen Life Technologies) and 1 μg/mL lipopolysaccharide from *E. coli* O111:B4, and incubated in a 39°C water-jacketed incubator at 5% CO₂ for 24 h. Following incubation, cultures were centrifuged at 1,200 × g for 15 min at room temperature, and resulting supernatant was collected and stored at −80°C until analysis for TNF-α concentration via commercially available bovine-specific ELISA kits (#DY2279 for TNF-α, RandD Systems, Minneapolis, MN) using 7-step serially diluted recombinant bovine TNF-α standard.

**Automated Behaviors**

At 5 d of age, accelerometers (UA-004-64; Onset Computer Corp., Bourne, MA) were placed on each calf’s right or left hind leg with Vetwrap cohesive bandage (3M Products, St. Paul, MN) as previously described by Calvo-Lo-

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**Table 1. The formulated nutrient content of the milk replacer and calf starter fed to preweaned Holstein calves**

| Nutrient                  | Milk replacer¹ | Starter² |
|---------------------------|----------------|----------|
|                           | LPN            | HPN      |     |
| DM, %                     | 97             | 98       | 89  |
| CP, %                     | 20             | 28       | 20  |
| Ether extract, %          | 20             | 20       | 5   |
| ADF, % (maximum), %       | 0.15           | 0.15     | 9.00|
| ME,³ Mcal/kg              | 4.65           | 4.75     | 3.08|
| Calcium, %                | 0.75           | 0.75     | 0.87|
| Phosphorus, %             | 0.7            | 0.7      | 0.12|
| Manganese, mg/kg          | —              | —        | 62  |
| Zinc, mg/kg               | —              | —        | 50  |
| Selenium, mg/kg           | —              | —        | 0.24|
| Vitamin A (minimum), IU/kg| 44,000         | 44,000   | 4,260|
| Vitamin D₃ (minimum), IU/kg| 11,000         | 11,000   | —   |
| Vitamin E (minimum), IU/kg| 220            | 330      | 27  |

¹Two commercial milk replacers were fed (Land O'Lakes Animal Milk Products Co., Shoreview, MN). LPN = low plane of nutrition; HPN = high plane of nutrition. Both milk replacers were formulated with similar macro- and microingredients and included dried whey, dried whey protein concentrate, dried whey product, dried skim milk, dried milk protein, animal fat, lecithin, polysorbate 80, dicalcium phosphate, calcium carbonate, brewers dried yeast, vitamin A acetate, d-α-tocopherol acetate, vitamin D₃, thiamine monohydrate, riboflavin, niacin supplement, folic acid, vitamin B₁₂, supplement, choline chloride, zinc methionine complex, manganese methionine complex, copper lysine complex, iron amino acid complex, ethylenediamine dihydroiodide, selenium yeast, and natural and artificial flavor. No direct-fed microbials or yeast extract was added in either milk replacer.

²Calf starter on a DM basis was 44.5% steam-flaked corn, 19.0% rolled oats, 22.0% soybean meal with 48% CP, 10.0% cane molasses, 3.0% vitamin and mineral premix, and 1.5% tallow. The vitamin and mineral premix contained on a DM basis 72% limestone, 20% dicalcium phosphate, 6% salt, 0.451% zinc sulfate, 0.4% selenium selenite 0.2%, 0.267% manganese oxide, 0.18% vitamin E—500 IU/g, 0.157% copper sulfate, 142 mg/kg vitamin A—1,000 KIU/g, and 12.5 mg/kg ethylenediamine dihydroiodide.

³Calculated based on the NRC (2001).
renzo et al. (2017). A custom-designed dummy nipple with a sensor (described by Hulbert et al., 2015; created by Metro Mall USA, Marysville, OH) was left in the bottle holder of the hutch from 5 to 68 d of age, except during MR feedings. The sensor was located in a dummy nipple, and when the calf manipulated the nipple (suck, head butt, push), the sensor activated the attached event-recording logger (UX90-001ML; Onset Computer Corp.). At midnight at 6 d of age, the accelerometers and event loggers began recording every 30 s each time the calf manipulated the dummy nipple (at a rate of 1 Hz), respectively. Every 1 to 3 wk, all logging devices were removed, data were downloaded, and loggers were refixed to the calves and hutches. Automated logger data were downloaded and processed using methods previously described (Ledgerwood et al., 2010; Hill et al., 2013; Hulbert et al., 2015); standing duration and pacifier use (min/d) were the main variables of interest. The observed dummy nipple was correlated (r = 0.98; P ≤ 0.001) with the sensor-activated event-recording logger in a previous study (Ruiz, 2019).

Statistical Analysis

The sample size was based on a 1-sided t-test with expected proportions of calves with scours of 90% for the HPN and 50% for the LPN with a 5 and 80% protection against Type 1 and 2 error, respectively. All repeated, continuous data were analyzed by restricted maximum likelihood ANOVA for a completely randomized design using the MIXED procedure of SAS (SAS version 9.1, SAS Institute Inc., Cary, NC). A linear mixed model with the fixed effects of treatment, time, and the interaction of treatment × time were fitted. Calf nested within treatment was the subject of the repeated statement. The mean model was run with all available covariance structures for the within-subject measurements. The appropriate covariance structure was chosen for each analysis based on the Schwarz’s Bayesian information criterion. Initial BW and total serum protein at enrollment were included in the model as covariates and remained in the model even if they were not significant. Total serum protein was a significant covariant (P = 0.039) for calf starter intake. Pairwise differences were performed at each time interval for significant treatment × time interactions using a sliced effect multiple comparison approach using a Tukey-Kramer adjustment. Fecal scores were averaged by 3 consecutive days, and the residuals were normally distributed and therefore analyzed as a continuous repeated measure. Head and body score as well as incidence of scours, bloat, mortality, and antibiotic use were analyzed using a Fisher’s exact test with the FREQ procedure of SAS (SAS version 9.1, SAS Institute Inc.). Before analysis, repeated data were tested for normality of the residuals by evaluating the Shapiro-Wilk statistic, normal probability plots of the residuals, and histograms of the residuals using the UNIVARIATE procedure of SAS (SAS version 9.1, SAS Institute Inc.). In addition, all nonrepeated data were evaluated for homogeneity of variance. Least squares means (±SEM) are reported throughout. Treatment differences with P ≤ 0.05 were considered significant, and 0.05 < P ≤ 0.10 were considered tendencies.

RESULTS

Two of the 18 calves died in both treatments, which is within the expected mortality rate for high-risk calves. Nutrient contents of both diets are reported in Table 1. There was a treatment × time interaction (P ≤ 0.001) in ADG, where HPN calves had greater (P ≤ 0.001) ADG during the preweaning period but were not different (P = 0.505) from LPN calves after weaning (Table 2). There was also a treatment × time interaction (P ≤ 0.001) in feed:gain ratio, where HPN had greater (P = 0.001) efficiency from 0 to 25 d but were not different (P ≥ 0.161) from 26 to 49 d or 50 to 68 d (Table 2). Feed-to-gain ratio was calculated as [(total kg of MR + calf starter, kg of DM)/kg of BW gain]. There were treatment × time interactions (P ≤ 0.001, 0.065, and 0.001) for milk, starter, and total intakes, respectively (Figure 1). The HPN calves consumed more milk solids, whereas the LPN consumed more calf starter. The HPN consumed more total intake during the preweaning periods, whereas the LPN calves consumed more total intake during the weaning and immediate postweaning period. There was a treatment × time interaction (P = 0.017) in water intake; however, a slice effect at each week revealed LPN had greater (P ≤ 0.001) water intake throughout the study (3.6 vs. 2.3 ± 0.21 L; Table 2), which is consistent with the increased calf starter intake. Plasma glucose and urea N concentrations are reported in Table 2. Calves fed a HPN refused more (P ≤ 0.001) milk than LPN calves (Table 2).

No differences (P ≥ 0.120) in the distribution of frequencies of head or body scores were observed between treatments (data not shown). There was a tendency (P = 0.100) for more HPN calves to bloat than LPN calves during the study (29.4 vs. 6.7%; Table 2). Further, the HPN calves had greater (P = 0.007) incidence of scours (66.7 vs. 22.2%; Table 2) as well as greater (P = 0.003) fecal scores (Table 2) compared with LPN calves. However, there was no difference (P = 0.688) in the percentage of calves that received systemic antibiotics (47 vs. 40%; Table 2). Calves fed a HPN tended to have greater TNF-α concentrations in whole blood cultures compared with LPN calves (Table 2). There were no treatment × time (P = 0.156) or treatment (P = 0.346) differences for daily standing duration; however, standing duration was influenced by time (P ≤ 0.001). Treatment × time differences were observed (P = 0.013; Figure 2) in dummy nipple use after the second MR feeding was removed at 56 d of age. The HPN calves increased their use of the dummy nipple after complete weaning, whereas LPN calves did not change their dummy nipple use.
DISCUSSION

Feeding a higher PON positively affected calf performance including final BW, ADG, and the feed-to-gain ratio. However, the improved ADG was more moderate than reported in other studies where a comparable MR was fed (Bartlett et al., 2006; Ballou, 2012). Studies using calves with FPT reported reduced ADG compared with calves with appropriate passive transfer (Pithua and Aly, 2013). Calves fed HPN had greater ADG in every period, with the exception of 50 to 68 d. At this time, calves were undergoing weaning. A probable contributor to the reduced ADG among HPN calves was likely due to the lower calf starter intake as well as digestibility of that calf starter at that time. Calves fed a HPN ate less starter throughout the study. Similarly, Huber et al. (1984) reported calves fed greater MR amounts consumed less calf starter. However, once weaned, previous studies observed that the HPN rapidly increase calf starter intake and can exceed LPN calves a few weeks after weaning (Ballou, 2012; Obedat et al., 2013; Ballou et al., 2015).

In agreement with previous studies, HPN calves had greater fecal scores throughout the study (Nonnecke et al., 2003; Bascom et al., 2007; Liang et al., 2016). Greater fecal scores in calves fed a HPN are routinely considered a consequence of greater milk solids consumed. More importantly, the HPN calves had a greater incidence of scouring than LPN calves. Scours were considered if a calf had 2 consecutive fecal scores of 4. Previously, Ballou (2011) reported that anything greater than or equal to a fecal score of 3 was considered scour. However, Liang et al. (2016) reported the DM of feces from calves with a fecal score 3 and concluded it would be unlikely for most calves to become dehydrated with a fecal score of 3 or less. The moderate increase in fecal scores reported by others when feeding HPN would not increase the risk for dehydration.

Table 2. Performance in calves fed a low (LPN) or high (HPN) plane of nutrition

| Item                     | LPN   | HPN   | Largest Treatment (Trt) | Time | Trt × Time |
|--------------------------|-------|-------|-------------------------|------|------------|
| No. of observations      | 16    | 16    |                         |      |            |
| Initial BW, kg           | 39.3  | 39.5  | 1.48                    | 0.914| —          |
| Final BW, kg             | 63.1  | 81    | 2.68                    | 0.001| —          |
| ADG, kg/d                |       |       |                         |      |            |
| 0 to 25 d                | 0.056 | 0.595 | 0.0407                  | 0.001| —          |
| 26 to 49 d               | 0.404 | 0.622 | 0.0407                  | 0.001| —          |
| 50 to 68 d               | 0.509 | 0.491 | 0.0407                  | 0.752| —          |
| 0 to 49 d                | 0.230 | 0.609 | 0.0201                  | 0.001| —          |
| 0 to 68 d                | 0.347 | 0.612 | 0.0285                  | 0.001| —          |
| Feed:gain1               |       |       |                         |      |            |
| 0 to 25 d                | 5.79  | 1.71  | 0.392                   | 0.001| —          |
| 26 to 49 d               | 2.52  | 1.9   | 0.392                   | 0.272| —          |
| 50 to 68 d               | 3.42  | 4.19  | 0.392                   | 0.167| —          |
| 0 to 49 d                | 3.76  | 1.79  | 0.403                   | 0.002| —          |
| 0 to 68 d                | 3.27  | 2.21  | 0.385                   | 0.038| —          |
| Water intake, L/d        | 3.56  | 2.34  | 0.206                   | 0.001| 0.001      |
| Milk refusal, kg         | 0.354 | 0.059 | 0.0027                  | 0.001| —          |
| Scouring,2 %             | 22.2  | 66.7  | 12.5                    | 0.007| —          |
| Mortality, %             | 11.1  | 11.1  | 8.25                    | 1     | —          |
| Bloat,3 %                | 6.7   | 29.4  | 11.5                    | 0.1   | —          |
| Antibiotic treatment,4 % | 40    | 47    | 12.5                    | 0.688| —          |
| Fecal score5             | 2.09  | 2.29  | 0.043                   | 0.003| 0.001      |
| Glucose, mg/dL           | 79.1  | 77.3  | 2.65                    | 0.639| 0.0001     |
| Urea N, mg/dL            | 6.6   | 7.1   | 0.41                    | 0.439| 0.0001     |
| Blood culture TNF-α,5 pg/mL| 141  | 102   | 14.1                    | 0.065| 0.214      |

1Feed:gain = (total kg of milk replacer + calf starter, kg of DM)/kg of BW gain.
2Consecutive fecal scores that were determined to be liquid splatters. Fecal scores were taken before each feeding and classified as 1 = firm, 2 = soft, 3 = runny, and 4 = liquid splatters.
3Bloat was assessed before and within 1 h of each feeding.
4Calves were administered Resflor Gold (Merck Animal Health, Kenilworth, NJ) if they were lying lateral, head distended, or head extended with a rectal temperature greater than 39.5°C or less than 37.5°C.
5TNF-α = tumor necrosis factor-α.
In contrast, when a calf has a fecal score of 4, the calf is at an increased risk to become dehydrated or develop metabolic acidosis. Scours can be infectious or noninfectious; however, the consequences are similar. A study completed by Quigley et al. (2006) reported findings that high-risk calves force fed a variable HPN program had greater incidence of scours and were treated with more antimicrobials than calves fed a restricted quantity of milk solids when...
raised on bedding contaminated with coronavirus. Force feeding high-risk calves has been reported to increase scours and is not a recommended nutrition strategy for sick calves and, therefore, was not included in this study design.

Liang et al. (2016) reported that healthy 1-wk-old calves fed either a low or high PON had no differences in the DE or ME efficiencies, concluding healthy neonatal calves were capable of digesting greater MR concentrations during the first week of life. In fact, healthy neonatal calves fed a high PON may be more capable of absorbing and utilizing those additional nutrients than calves fed a low PON. Further, Liang et al. (2016) reported increased digestible CP and nitrogen retention as a percentage of intake when fed the high PON. They concluded that healthy calves are able to digest and use the extra nutrients very efficiently during the first week of life; however, they constrained the inferences to healthy calves.

Healthy calves may be more capable of absorbing nutrients than high-risk calves; therefore, a HPN may not be as advantageous among high-risk calves as it is in healthier low-risk calves. Calves failing to receive colostrum were reported to have reduced gastrointestinal integrity, as reflected in decreased mucosal thickness, as well as decreased villus length and width compared with calves that received colostrum (Yang et al., 2015). Further, increased colostrum intake improved intestinal epithelial cell proliferation and maturation as well as enzymatic activity (Blättler et al., 2001). Colostrum not only influences gut integrity but also contains immune components and nutrients that are considered a major factor in preweaning health. Failure of passive transfer is associated with poorer preweaning performance and health (Naylor et al., 1977; Donovan et al., 1998; Pithua and Aly, 2013). These high-risk calves receiving the HPN may have had reduced gut integrity and local immune protection and, as a result, were less tolerant to the additional milk solids, which increased the risk for scours.

Calves fed the HPN also had a tendency for increased incidence of abomasal bloat. Although the incidence of bloat is often sporadic and the etiology not completely understood, a reduced abomasal emptying rate and increased fermentable substrates in the abomasum are risk factors (Burgstaller et al., 2017). Increasing the solids content or osmolality of MR fed can delay abomasal emptying, and feeding a high PON also may increase the nutrients available for rapid fermentation by *Clostridium perfringens*, a microorganism often suspected in abomasal bloat. More research is needed to understand how PON influences abomasal dynamics and health.

The expression of nonnutritive oral behaviors early in life may be important to establish mature feeding behaviors and reduce the incidences of unwanted behaviors (e.g., cross sucking) after calves are weaned and transitioned from individual housing to group housing (Veissier et al., 2002; Horvath and Miller-Cushon, 2017). Before weaning, PON did not influence dummy-nipple use, except at 15 d, when LPN calves tended to use the nipple more than HPN calves. The day before this (14 d), daily standing duration was increased among all calves, which was likely caused by research personnel spending most of the day setting up video recording equipment. Nonetheless, after weaning was completed, the HPN calves increased dummy-nipple use to over 3 times their preweaning frequencies until 64 d of age. This is an indicator that the stress of weaning was likely greater among HPN calves. The HPN calves had MR as the main component of that diet, and the HPN calves were only motivated to use the dummy nipple when that source of satiety was removed. The HPN calves returned to their preweaning dummy-nipple use by 64 d, and by this age the calves may have been consuming enough starter to become satiated. This is in contrast to data reported by Bortoluzzi (2019), where step-down weaning calves fed a HPN at 50 d did not increase their use of the dummy nipple, whereas HPN calves either step-down weaned at 39 d or gradually weaned by decreasing the quantity of milk offered from 43 to 57 d had relatively greater use of the dummy nipple. The authors concluded it may have been less stressful to wean those calves from HPN at the older age, 50 d in that study, because they were consuming more starter. Even though the calves in the present study were weaned at approximately the same age and in the same manner as the calves in the study by Bortoluzzi (2019), the high-risk nature of the calves resulted in reduced calf starter intake and, therefore, required either a later or possibly an even more gradual weaning.

Benefits of feeding programs that increase preweaning ADG are established. Improvements in calf performance were reported in stressed calves fed 3 times per day versus 2 times a day (Schingoethe et al., 1986). Starter intake was improved with increased feeding frequency (Knickiewycz et al., 2010). Further, it is possible the issues observed with HPN in high-risk calves could be reduced with increased feeding frequencies and should be considered in future research. No difference in either serum glucose or urea nitrogen was observed between treatments. Similarly, Obeidat et al. (2013) and Smith et al. (2002) reported no difference in these metabolites in calves fed varying levels of MR. Conversely, calves fed greater levels of MR had greater plasma glucose concentrations in previous studies (Smith et al., 2002; Quigley et al., 2006; Ballou et al., 2015). Others have reported lower urea nitrogen concentrations in calves fed greater MR levels (Ballou et al., 2015). Calves in the present study did not appear to have any differences in protein or glucose metabolism.

**APPLICATIONS**

Calves fed a HPN had greater BW compared with LPN calves; however, they also had increased risk for preweaning scours and abomasal bloat as well as increased dummy-nipple activity during weaning. Differences in gut integrity may influence the ability of high-risk calves to handle high levels of MR. Therefore, when feeding high-risk calves, al-
ternal strategies may need to be considered in comparison with more healthy or low-risk calves. Potential issues with high-risk calves being fed a HPN may be reduced with increased feeding times per day, a gradual step-up method, or other nutritional strategies that improve gastrointestinal development and function.

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