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Effects of milk feeding strategies on short- and long-term productivity of Holstein heifers

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ABSTRACT

Research investigating the effects of feeding raw or pasteurized nonsaleable milk (NSM) on heifers' performance beyond the period of supplementation is limited. This study aimed to examine the effects of type of milk [NSM or milk replacer (MR)] and pasteurization of NSM on preweaning and first-lactation performance of heifers born with low (<36.3 kg) or normal birth weight (≥36.3 kg). Holstein heifers (n = 154) were sequentially assigned to 1 of 3 treatments: MR, pasteurized NSM, or raw NSM. Heifers assigned to raw NSM were fed raw colostrum, whereas heifers assigned to MR and pasteurized NSM were fed pasteurized colostrum. The low birth weight heifers were fed 1.4 L at each feeding until they reached 36.3 kg body weight, whereas normal birth weight heifers were fed 1.9 L at each feeding. A grain mix starter was offered throughout the study. Heifers were weaned ≥42 d old if consuming at least 0.9 kg/d of starter for 3 consecutive days. Data were analyzed with the MIXED procedure of SAS (SAS Institute Inc., Cary, NC), and the basic model included milk treatments, birth weight group, and treatment × birth weight group. The low birth weight heifers fed raw colostrum and NSM versus pasteurized colostrum and NSM had lower serum protein concentrations. Heifers fed MR versus NSM had or tended to have greater concentrations of hematocrit, red blood cells, and eosinophils but lesser concentrations of platelets, although some of those responses were temporary. Pasteurization tended to increase blood lymphocyte concentrations. Heifers with normal birth weight had greater concentrations of blood neutrophils, lymphocytes, and monocytes, compared with low birth weight heifers. For the first 42 d of life, low birth weight heifers fed pasteurized versus raw NSM had greater weight gain, grain intake, and feed efficiency and were weaned earlier (hazard ratio for weaning by 56 d: 2.90). These pasteurization effects for low birth weight heifers tended to be sustained through 24 wk of age, indicated by greater weight gain and hip height growth. In their first lactation, low birth weight heifers produced less mature-equivalent (MEq) protein and tended to produce less MEq milk and fat than normal birth weight heifers. However, the negative effects of low birth weight on MEq milk and fat yield was only evident in heifers fed raw NSM, whereas the performance of low birth weight heifers was similar to that of normal birth weight when fed MR or pasteurized NSM. These findings confirm that calf management practices influence future performance; in this case, failing to pasteurize milk and colostrum for low birth weight heifers had effects that remained apparent for more than 2 years.
Key words: heifers, milk replacer, pasteurization, lifelong performance

INTRODUCTION

An efficient replacement program is crucial to ensure the sustainability of the dairy industry. However, the costs associated with producing high-quality replacement heifers represent a large portion of the total cost of milk production, surpassed only by feed costs (Heinrichs, 1993). In the last decades, several research-based strategies have been successfully implemented in calf rearing, with revised nutritional and feeding strategies returning the most quantifiable benefits. One critical feeding strategy is to deliver adequate quantities of good-quality colostrum (>50 g/L IgG) to ensure proper IgG transfer. Some studies have reported that calves attaining serum IgG concentrations ≥10 g/L have better short- and long-term performance (Faber et al., 2005; Dewell et al., 2006; Berge et al., 2009; Furman-Fratczak et al., 2011). However, greater intake of nutrients during the preweaning period may also affect future productivity. Two meta-analyses concluded that strategic

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feeding for enhanced growth subsequently translated into greater milk yields, accounting for 3 to 22% of variation in first-lactation milk yield (Soberon and Van Amburgh, 2013; Gelsinger et al., 2016). During the preweaning period, the type of milk fed and its processing are among the most variable management practices, and have been reported to affect calves' health and performance (Jamaluddin et al., 1996; Shamay et al., 2005).

Milk replacer (**MR**) has been fed to calves since at least the 1950s, and its use is widespread (USDA, 2016). The traditional feeding program utilizing MR with 20% fat and 20% protein (DM basis), fed at <6 L/d, is still the dominant approach, used by 58.7% of US dairy operations (USDA, 2016). Nevertheless, studies have reported that feeding MR with more protein (e.g., 28% CP and 20% fat) at greater feeding rates improved preweaning BW gain, and its benefit was extended into the first and second lactations (Soberon et al., 2012). Although the use of MR, fed alone or in combination with milk, has increased in recent years, dairy operations also rely heavily on milk (USDA, 2016), which is often made more economical by using nonsaleable milk (**NSM**) for calf feeding. More than 50% of dairy operations feed unpasteurized milk; only 5% of small operations but 44% of large operations feed pasteurized milk (USDA, 2016). The pasteurization process has been documented to reduce microbial load in NSM (Stabel, 2008; Elizondo-Salazar et al., 2010), as well as the risk of morbidity and mortality (Armengol and Fraile, 2016). Most studies, although confirming reductions in microbial load and some health parameters, reported no effects of pasteurization on calf growth (Aust et al., 2013; Teixeira et al., 2013). In contrast, pasteurizing NSM was shown to increase weight gain in one study (Jamaluddin et al., 1996) but decreased it in another (Zou et al., 2017). A putative negative influence of pasteurization on calves' weight gain may be associated with partial destruction of colostral growth factors and vitamins (Gauthier et al., 2006; Peroni et al., 2009).

Although the effects of NSM pasteurization on calf growth remain unsettled, most studies agree that pasteurization benefits animal health during the preweaning period and may provide a longer-term benefit for overall performance. However, the effect of feeding pasteurized or raw NSM on health and performance beyond the period of supplementation has not been carefully studied. We hypothesized that feeding pasteurized NSM, relative to feeding raw NSM or a high-protein MR, would not only benefit the health and growth of heifers during the preweaning period but would also benefit the subsequent lactation.

MATERIALS AND METHODS

All experimental procedures were approved by the Kansas State University Institutional Animal Care and Use Committee (Manhattan, KS).

Enrollment, Treatments, and Management

Holstein heifers (n = 154) born at the Kansas State University Dairy Teaching and Research Center between June 2013 and March 2014 with birth BW >27.2 kg and calving ease scores <3 were enrolled in the study. Heifers were born in an open, cooled maternity barn with straw bedding and were immediately transferred to a cooled, enclosed barn bedded with wood shavings. Within 2 h of birth, heifers were weighed and ear-tagged, and the umbilical cord was disinfected. Approximately 12 h after birth, heifers were transferred to the calf rearing area and housed in individual hutches until weaning. Heifers ≥42 d old and consuming at least 0.9 kg of starter grain for 3 consecutive days were weaned.

After birth, heifers were sequentially assigned to 1 of 3 treatments: (1) MR, (2) pasteurized NSM, or (3) raw NSM. Heifers assigned to raw NSM were fed raw colostrum, whereas heifers assigned to MR and pasteurized NSM were fed pasteurized colostrum. Warm colostrum (Brix value >22%, equivalent to >50 g/L of IgG) was fed twice, at ≤2 and 6 h after birth; volumes at each feeding were 1.42 to 1.89 L, targeting a total volume of 3.78 L.

Heifers were fed the milk treatments thrice daily (0700, 1500, and 2300 h). All milk treatments were warmed and fed at 37°C. The NSM was obtained from the Dairy Teaching and Research Center, and was sampled periodically before pasteurization (4.12 ± 0.37% fat, 3.59 ± 0.28% protein, 13.1 ± 0.64% total solids, and 1,662 ± 771 somatic cells/μL; mean ± SD) and after pasteurization (4.14 ± 0.35% fat, 3.61 ± 0.29% protein, 13.1 ± 0.64% total solids, and 1,575 ± 704 somatic cells/μL). Pasteurization was carried out at 63°C for 30 min (DT-30G pasteurizer, DairyTech, Windsor, CO). The MR contained 28% protein and 18% fat on a DM basis and was mixed according to the manufacturer's recommendation (Mother's Pride, Hubbard Feeds, Mankato, MN). The reconstituted MR (14.2% total solids) provides the same metabolizable energy per unit of volume as standard whole milk; therefore, treatments were fed on an equal volume basis. Heifers born <36.3 kg (low birth weight) were fed 1.4 L at each feeding until they reached 36.3 kg, whereas heifers born ≥36.3 kg (normal birth weight) were fed 1.9 L at each feeding. Throughout the preweaning period, heifers were provided ad libitum access to a starter grain mix (18.1% CP, 3% crude fat; Calf Krunch 18%, Hubbard Feeds, Mankato, MN) containing decoquinate (50 mg/kg) as a coccidiostat. Milk and grain intakes and refusals were recorded at each feeding time.

From the 154 enrolled heifers, 2 heifers were fed the wrong colostrum for their assigned milk treatments. Thus, these heifers were omitted from all analyses. Four heifers died before 24 wk of age, and because underlying health conditions may have influenced performance at an undefined time before their death, they were not included in any analyses except for analysis of retention in the herd.

Serum Total Protein

On d 2 after colostrum feeding, blood was collected by jugular venipuncture to harvest serum. Serum total protein, as a proxy of passive IgG transfer, was determined by the biuret method with a Roche/Hitachi Cobas c501 automated analyzer (Roche Diagnostics, Indianapolis, IN).

Growth Measures

Body weight and shoulder and hip heights were recorded at birth and then once weekly until heifers were 8 wk old. Growth measures were then recorded at 12, 16, 20, and 24 wk of age. Average daily gain was calculated by the weight difference between 2 consecutive measures. Feed efficiency [BW gain/DMI (milk or MR solids + grain)] was calculated weekly for the first 6 wk.

Blood Cell Counts, Bactericidal Capacity, and Health Assessment

At 25, 39, and 53 ± 3 d of age, blood samples were collected via jugular vein into 2 Vacutainer tubes (Becton, Dickinson and Co., Franklin Lakes, NJ). Blood collected into a 3-mL K_2-EDTA tube was used to measure complete blood cell counts using a ProCyte Dx hematology analyzer (Idexx Laboratories Inc., Westbrook, ME). Blood collected into a 5-mL tube containing Na heparin was used to test bactericidal activity against a live culture of environmental *Escherichia coli* (ATCC #51813) according to methods previously described (Ballou, 2012).

Fecal scores (**FS**) were recorded twice daily on a scale from 1 to 4 (1 = normal, 2 = loose, 3+ = scours), according to Larson et al. (1977). Heifers with FS 3 were defined as having diarrhea (no scores of 4 were recorded). All diagnosed diseases and treatments were recorded.

Postweaning Management and Data Collection

Heifers were uniformly managed according to the standard procedures of the Dairy Teaching and Research Center. Briefly, if a heifer was not weaned at 42 d, it was kept in its individual hutch, and milk intake was restricted to 1 feeding per day until it reached the target starter grain intake for weaning. Weaned heifers were housed in pens with super hutches between weaning and 6 mo of age; after super hutch comingling, heifers were moved to sod-based pens. Between weaning and 6 mo of age, heifers were provided with starter grain and free-choice hay. From 6 mo of age until 250 d of gestation, heifers were fed a uniform partial mixed ration with free-choice hay. In all cases, diets were formulated to meet their nutritional requirements at that stage of life (NRC, 2001). One month before their expected calving date, heifers were moved to a maternity pen deep-bedded with straw, where they were monitored for signs of calving and where they consumed a close-up TMR diet formulated to meet their nutritional requirements. Upon calving, heifers were moved to freestall barns equipped with feedline sprinklers and fans, and fed a TMR twice per day, formulated to meet daily nutrient requirements for lactating dairy cows producing 50 kg of 3.5% fat milk (NRC, 2001). Cows were milked 3 times daily, and milk yields were automatically transferred to PCDart software (Dairy Records Management Services, Raleigh, NC). Analysis from monthly DHIA testing provided estimated 305-d mature-equivalent (**MEq**) milk, fat, and protein yields (VanRaden, 1997). The predicted transmitting ability for milk, fat, and protein yields for each heifer were obtained from PCDart records in February 2017. Cull dates were also obtained from PCDart records.

Statistical Analyses

Continuous variables were analyzed using the MIXED procedure of SAS (version 9.4, SAS Institute Inc., Cary, NC). Data were tested for normality, and the most suitable transformation, based on the distribution of residuals, was selected for statistical analysis. Outliers were removed if the absolute value of the conditional studentized residual was >4. We included birth weight category (low birth weight, <36.3 kg; or normal birth weight, ≥36.3 kg) in models to account for the differences in the amount of milk fed initially. The basic model included milk treatments, birth weight group, and milk treatments × birth weight group. The effect of season (summer: June to September 2013; or fall/winter: October 2013 to March 2014) and its 2-way interactions with milk treatments and birth weight group were also tested and removed if not significant ($P > 0.10$). For variables with repeated measures, age and its interactions with the components of the basic model, and season when significant, were included; all 3-way interactions were removed if not significant ($P > 0.10$). Time to weaning (right-censored at 56 d of age), second pregnancy (censored at 300 DIM), and culling (censored at 36 mo of age) were analyzed by Cox's proportional hazard regression using the PHREG procedure of SAS. Binary analysis of diarrhea incidence, defined as at least one FS 3, was measured by the logistic procedure. For all variables, the following orthogonal contrasts were performed: (1) type of milk: MR versus NSM (pasteurized + raw), (2) effect of pasteurization: pasteurized versus raw NSM, (3) birth weight group: low versus normal birth weight, (4) type of milk × birth weight group, and (5) pasteurization × birth weight group. For MEq milk, protein, and fat yields, the predicted transmitting ability of the corresponding variable was used as a covariate. A P-value of ≤0.05 was considered significant, and P-values >0.05 but ≤0.10 are discussed as tendencies. When 2-way interactions were or tended to be significant, they were plotted in figures and the PDIFF statement of SAS was used to separate least squares means. Experimental units are detailed in tables (refer to Supplemental Table S1 for experimental units of groups interacting with season: https://doi.org/10.3168/jds.2020-19364).

RESULTS

Total Serum Protein, Preweaning Whole Blood Bactericidal Capacity, and Cell Populations

Mean concentrations of serum total protein for each of the 6 treatments were above 6.0 g/dL, and all heifers, except one born with normal body weight and fed raw colostrum, had >5.0 g/dL serum total protein. We detected no main effects of type of milk, pasteurization, or birth weight on serum total protein concentration ($P > 0.10$). However, a birth weight group × type of milk interaction ($P = 0.03$) revealed that among heifers born with normal body weight, heifers fed raw colostrum and NSM had lower serum protein concentrations compared with those fed pasteurized colostrum and NSM (Figure 1A, $P = 0.01$). Season also affected serum protein concentration but only for heifers fed raw colostrum and NSM (pasteurization × season, $P = 0.01$); those born during warmer months had lower serum protein compared with those born during cooler months (6.33 ± 0.17 vs. 6.99 ± 0.26 g/dL). Whole blood bactericidal capacity was not affected by type of milk, pasteurization, birth weight group, age, or their interactions (Table 1, $P > 0.50$). Heifers fed MR instead of NSM had greater hematocrit volume and red blood cell concentration (contrast $P < 0.01$), but this difference was no longer evident at wk 7 (type of milk × age, $P < 0.05$). Platelet concentration was greater in heifers fed NSM compared with MR (contrast $P < 0.01$), but again the difference was gone by wk 7 (type of milk × age, $P = 0.06$). Eosinophils, expressed as a concentration ($P = 0.01$) or as a proportion of blood leukocytes ($P = 0.02$), were more abundant in heifers fed MR versus NSM. Heifers fed pasteurized, rather than raw, NSM tended to have greater lymphocyte concentrations ($P = 0.08$).

Total leukocyte concentrations were greater in normal versus low birth weight heifers (contrast $P < 0.01$). Blood neutrophils, lymphocytes, and monocytes ($P < 0.05$), but not eosinophils or basophils ($P > 0.10$), were more abundant in the normal birth weight heifers. Among these normal birth weight heifers, the proportion of monocytes tended to be greater if they were fed pasteurized versus raw NSM (Figure 1B, pasteurization × birth weight group, $P = 0.09$). The type of milk fed (MR or NSM) did not affect blood lymphocyte concentration in heifers born in cold months (Figure 1C). However, among heifers born in warmer months, the lymphocyte concentration tended to be decreased by raw NSM versus MR or pasteurized NSM at 5 and 7 wk of age (type of milk × season × age interaction, $P = 0.06$, Figure 1C).

Fecal Score, Incidence of Diarrhea, and Weaning

Fecal consistency was assessed twice daily for the first 42 d of age, before the weaning protocol. The average number of observations was 80 ± 0.6 per heifer and did not differ by birth weight group, treatment, or interactions ($P > 0.48$). The cohort of heifers used in this study was relatively healthy: 0.46 ± 0.07% of fecal scores were FS 3, and this proportion did not differ by birth weight group, treatment, or interactions ($P > 0.59$). Twenty-six percent of heifers (39/148) were diagnosed with diarrhea, defined as having at least 1 FS 3 observation, and this proportion was not affected by treatment ($P = 0.87$). Because of the minimal number of observations with FS 3, the proportion of FS >1 was also analyzed. For normal birth weight heifers, those fed MR had more FS observations >1 compared with heifers fed NSM (type of milk × birth weight group, $P = 0.01$, Figure 2A).

Among low birth weight heifers, the hazard of weaning by 56 d of age was 2.90 times greater for those fed pasteurized versus raw NSM (Figure 2B). However, pasteurization did not affect hazard of weaning for normal birth weight heifers.

Growth, Intake, and Feed Efficiency

Birth weight was similar for heifers assigned to each milk treatment group ($P > 0.25$, Table 2), whereas low birth weight heifers were 19% lighter than normal birth weight heifers ($P < 0.01$). As per the design of the study, low birth weight heifers consumed less of their assigned milk treatment until they were ≥36.3 kg; this was 10.0, 9.0, and 10.4 ± 0.62 d for MR, PNSM, and RNSM, respectively, with no difference across treatments ($P > 0.15$). For the first 42 d of life, feeding NSM did not affect ADG of normal birth weight heifers; however, feeding raw NSM to low birth weight heifers had a detrimental effect on ADG (pasteurization × birth weight group, $P < 0.01$, Figure 3A). Neither shoulder nor hip height was affected by milk type, pasteurization, birth weight group, age, or their interactions ($P > 0.40$). Grain intake increased weekly ($P < 0.01$), and, regardless of age, heifers fed NSM instead of MR ate more grain ($P = 0.03$, Table 2), although the greater grain intake was driven by heifers fed pasteurized

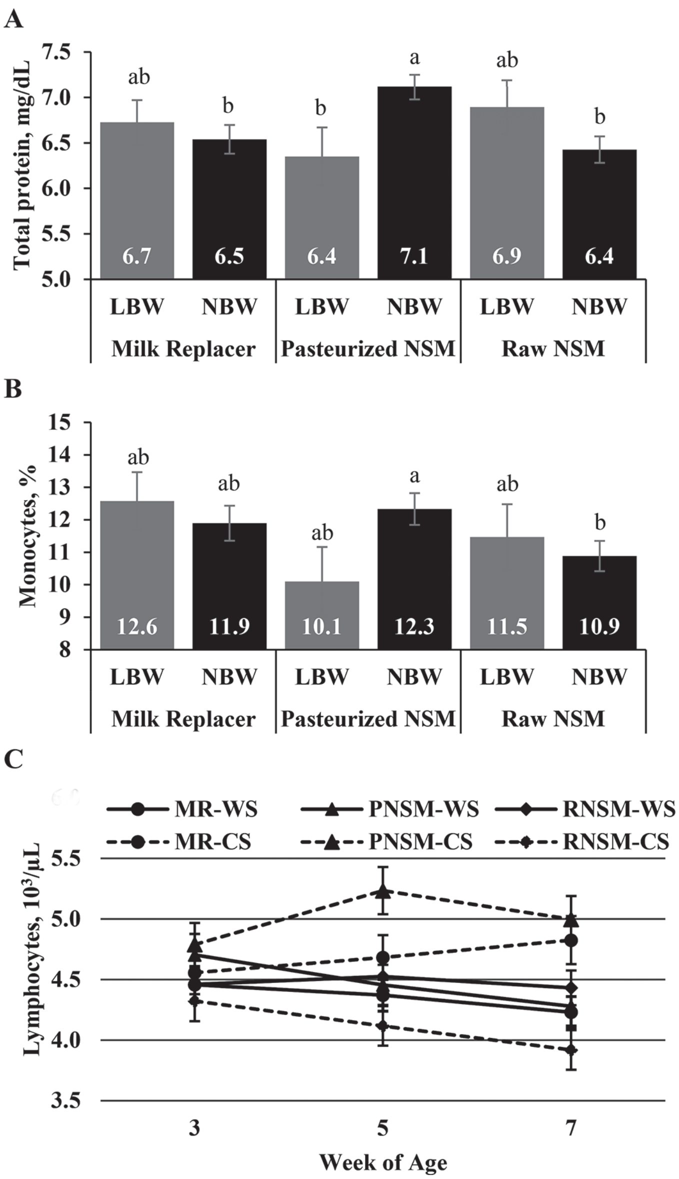

Figure 1. Serum total protein (A), blood monocytes (B, % of total leukocytes), and blood lymphocytes (C, $10^3/\mu L$) for heifers fed milk replacer (MR) or pasteurized or raw nonsaleable milk (PNSM or RNSM, respectively) from birth to weaning (≥6 wk). Heifers born <36.3 kg (LBW) were fed 4.2 L/d of milk until they reached 36.3 kg, whereas heifers born ≥36.3 kg (NBW) were fed 5.7 L/d. Heifers were born in warm (WS, June to September) or cold seasons (CS, October to March). Contrast of pasteurized vs. raw NSM × birth weight group, $P = 0.01$ (A) and $P = 0.09$ (B). Interaction of type of milk × season × week of age, $P = 0.06$ (C; wk 5 and 7, $P < 0.05$). Values are LSM ± SEM, and means that do not share a common letter differ ($P \le 0.05$).

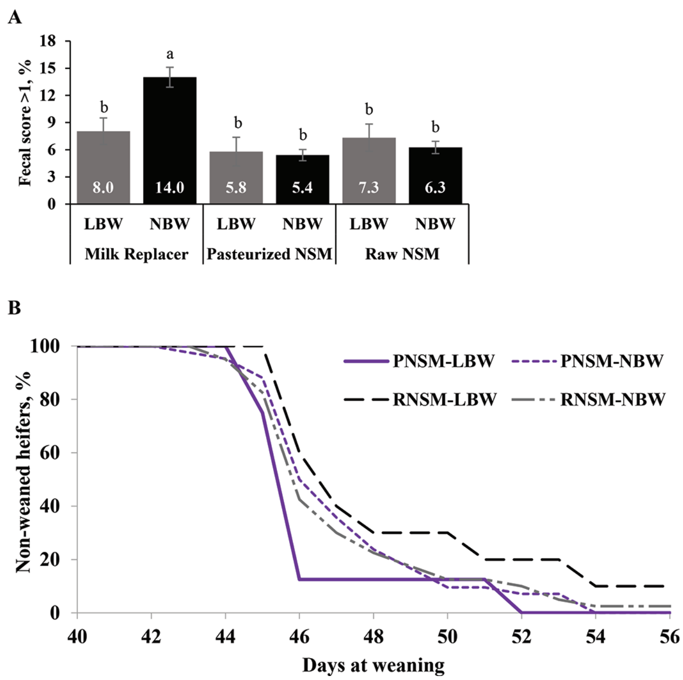

Figure 2. Percentage of observed fecal scores greater than 1 on a scale from 1 to 3 (A; 1 = normal, 2 = loose, 3 = scours) and survival curve age for weaning age (B) for heifers fed milk replacer (MR) or pasteurized or raw nonsaleable milk (PNSM or RNSM, respectively) from birth to weaning (≥6 wk). Heifers born <36.3 kg (LBW) were fed 4.2 L/d of milk until they reached 36.3 kg, whereas heifers born ≥36.3 kg (NBW) were fed 5.7 L/d. Fecal scores were recorded twice daily. Incidence of scours was minimal (0.11% across treatments) and was not affected by treatments, but among NBW heifers, those fed MR had higher scores than those fed nonsaleable milk (NSM). Contrast of MR vs. NSM × birth weight group, $P = 0.01$. Values are LSM ± SEM, and means that do not share a common letter differ ($P \le 0.05$; A). LBW heifers fed PNSM were weaned at earlier age ($P = 0.03$) than LBW heifers fed RNSM (adjusted hazard ratio = 2.90, 95% CI = 1.11 to 7.57).

rather than raw NSM ($P = 0.05$). Furthermore, the effect of pasteurization differed by birth weight: low birth weight heifers fed pasteurized NSM ate more grain than did low birth weight heifers fed raw NSM (pasteurization × birth weight group, $P = 0.01$, Figure 3B). Feed efficiency decreased as heifers aged (time, $P < 0.01$), and normal birth weight heifers were more efficient in wk 3 and 5 compared with low birth weight heifers (birth weight group × age, $P = 0.02$). Throughout the 42-d preweaning period, feed efficiency was greater for normal birth weight heifers fed raw NSM than for low birth weight heifers fed raw NSM (pasteurization × birth weight group, $P = 0.01$, Figure 3C).

The negative growth effects of feeding raw NSM to low birth weight heifers was maintained through 24 wk (pasteurization × birth weight group, $P = 0.04$, Figure 3D, PDIFF = 0.10 for low birth wight heifers fed pasteurized versus raw NSM). Milk type, pasteurization, birth weight group, age, and their interactions did not affect shoulder height growth ($P > 0.50$). However, a trend for a greater hip height growth was observed in low birth weight heifers when fed pasteurized NSM (pasteurization × birth weight group, $P = 0.10$, Figure 3E, PDIFF = 0.10 for low birth weight heifers fed pasteurized versus raw NSM).

Long-Term Productive and Reproductive Performance

The number of AI required to achieve first pregnancy was 1.78 ± 0.17 (SEM), and was not affected by milk type, birth weight group, or their interactions ($P > 0.15$, Table 2). Neither milk type, pasteurization, birth weight group, nor their interactions ($P > 0.20$) affected age at first calving (22.6 ± 0.3 mo). The interval from calving to second pregnancy was not affected by any factor or their interactions ($P > 0.30$) and was 111, 107, or 123 ± 13 d, for MR, pasteurized NSM, and raw NSM, respectively. Sixty-three percent of enrolled heifers (100/152) remained in the herd beyond 36 mo of age, and culling hazard, censored at 36 mo of age, was not altered by any factor or the interactions between factors ($P \geq 0.25$, Figure 4).

Heifers with low birth weights tended to produce 5% less MEq milk ($P = 0.06$, Table 2) and MEq fat ($P = 0.08$) compared with normal birth weight heifers. However, the negative influence of low birth weight on MEq milk and fat yield was only evident in heifers fed raw NSM (pasteurization × birth weight group, $P \leq 0.05$, Figure 5). Low birth weight heifers also produced less MEq protein ($P = 0.02$). Although the contrast for

Table 1. Whole blood bactericide (WBB) activity and blood cell counts of heifers fed milk replacer (MR) or pasteurized or raw nonsaleable milk (PNSM or RNSM, respectively) from birth to weaning (≥6 wk); heifers born <36.3 kg (LBW) were fed 4.2 L/d of milk until they reached 36.3 kg, whereas heifers born ≥36.3 kg (NBW) were fed 5.7 L/d, and heifers were uniformly managed after weaning

	MR		PNSM		RNSM			P-value[2]		
Item	LBW	NBW	LBW	NBW	LBW	NBW	SEM[1]	TM	EP	BWG
Heifers, n	12	33	7	41	9	41				
WBB activity,[3] %	23.5	20.5	20.0	20.3	23.5	21.3	3.1	0.80	0.51	0.56
Hematocrit,[3] %	32.4	32.8	28.0	27.5	27.4	28.1	1.5	<0.01	0.98	0.89
RBC,[3] $\times 10^6/\mu L$	8.72	8.65	7.76	7.81	7.75	7.82	0.21	<0.01	0.99	0.94
Cell number,[3] $\times 10^3/\mu L$										
Platelets	395	427	494	510	458	501	15	<0.01	0.38	0.11
Leukocytes	9.38	10.41	9.17	10.44	8.63	10.66	0.35	0.68	0.75	<0.01
Neutrophils	3.61	4.19	3.33	3.94	3.52	4.13	0.21	0.56	0.60	0.04
Lymphocytes	4.38	4.65	4.50	4.98	3.73	4.94	0.15	0.96	0.08	<0.01
Monocytes	1.16	1.20	0.95	1.25	0.99	1.19	0.06	0.21	0.95	0.02
Eosinophils	0.04	0.05	0.02	0.03	0.03	0.04	0.004	0.01	0.12	0.12
Basophils	0.02	0.02	0.02	0.02	0.02	0.02	0.002	0.39	0.74	0.68
Cells,[3] % of leukocytes										
Neutrophils	39.2	40.5	36.9	38.3	41.6	39.7	1.8	0.61	0.12	0.86
Lymphocytes	46.1	45.1	50.2	47.6	44.8	47.2	1.6	0.19	0.11	0.77
Monocytes[4]	12.6	11.9	10.1	12.3	11.5	10.9	0.7	0.11	0.98	0.60
Eosinophils	0.57	0.60	0.26	0.37	0.45	0.43	0.10	0.02	0.21	0.61
Basophils	0.11	0.09	0.10	0.05	0.08	0.04	0.03	0.17	0.70	0.09

[1]Pooled SEM.

[2]Contrasts: TM = type of milk, MR vs. pasteurized + raw NSM; EP = effect of pasteurization, pasteurized vs. raw NSM; BWG = birth weight group, low vs. normal birth weight. Interaction contrasts of TM × BWG or EP × BWG were not significant, unless footnoted.

[3]Square root (WBB activity, hematocrit, and individual cell percentages) and $\log_{10}$ [red blood cells (RBC), platelets, and total and individual leukocytes] transformations were used to attain normality, and reported LSM and SEM are back-transformed. SEM for $\log_{10}$ and square root were back-transformed according to Bland and Altman (1996) and Jørgensen and Pedersen (1998), respectively.

[4]Contrast EP × BWG: $P = 0.09$; see Figure 1A for details.

Table 2. Performance of heifers fed milk replacer (MR) or pasteurized or raw nonsaleable milk (NSM) from birth to weaning (≥6 wk); heifers born <36.3 kg (LBW) were fed 4.2 L/d of milk until they reached 36.3 kg, whereas heifers born ≥36.3 kg (NBW), were fed 5.7 L/d, and heifers were uniformly managed after weaning

Item	MR		Pasteurized NSM		Raw NSM			*P*-value[2]		
	LBW	NBW	LBW	NBW	LBW	NBW	SEM[1]	TM	EP	BWG
Birth to 42 d of age[3]										
Heifers, n	12	35	7	43	10	41				
Birth BW, kg	32.5	39.9	31.7	40.2	33.3	40.2	0.64	0.81	0.28	<0.01
ADG, kg/d	0.63	0.65	0.70	0.63	0.54	0.66	0.04	0.69	0.13	0.48
Shoulder height gain, cm/d	0.23	0.22	0.24	0.21	0.24	0.21	0.03	0.91	0.99	0.42
Hip height gain, cm/d	0.25	0.26	0.26	0.25	0.22	0.25	0.03	0.84	0.54	0.70
Grain intake, kg/d	0.37	0.34	0.59	0.40	0.36	0.43	0.04	0.03	0.05	0.18
Feed efficiency, kg/kg	0.60	0.65	0.60	0.57	0.48	0.63	0.03	0.04	0.43	0.03
Birth to 24 wk of age[4]										
Heifers, n	12	35	7	43	10	41				
ADG, kg/d	0.80	0.81	0.83	0.81	0.75	0.84	0.02	0.89	0.35	0.35
Shoulder height gain, cm/d	0.21	0.22	0.22	0.19	0.22	0.20	0.02	0.76	0.91	0.56
Hip height gain, cm/d	0.23	0.23	0.26	0.21	0.21	0.21	0.02	0.56	0.18	0.14
First pregnancy										
Heifers, n	12	34	6	38	9	38				
Number of AI	1.56	1.79	1.73	2.10	1.56	1.92	0.17	0.54	0.56	0.17
First lactation[5]										
Heifers, n	12	32	6	37	9	36				
Age at calving, mo	22.3	22.8	22.6	22.7	22.4	22.8	0.3	0.83	0.90	0.21
305-MEq milk, kg	13,798	14,328	14,396	14,149	13,068	14,774	399	0.92	0.46	0.06
305-MEq fat, kg	539	557	553	544	496	565	16	0.54	0.37	0.08
305-MEq protein, kg	407	419	402	411	387	426	10	0.46	0.99	0.02

[1]Pooled SEM.

[2]Contrasts: TM = type of milk, MR vs. pasteurized + raw NSM; EP = effect of pasteurization, pasteurized vs. raw NSM; BWG = birth weight group, low vs. normal birth weight. Interaction contrasts of TM × BWG or EP × BWG were not significant, unless footnoted.

[3]Contrast EP × BWG for ADG ($P = 0.03$), grain intake ($P = 0.01$), and feed efficiency ($P = 0.01$; BW gain/DMI); see Figure 3 for details.

[4]Contrast EP × BWG for ADG ($P = 0.04$) and hip height gain ($P = 0.10$); see Figure 3 for details.

[5]MEq = mature equivalent. Contrasts EP × BWG for 305-MEq milk ($P = 0.04$) and 305-MEq fat ($P = 0.05$) were significant. See Figure 5 for details.

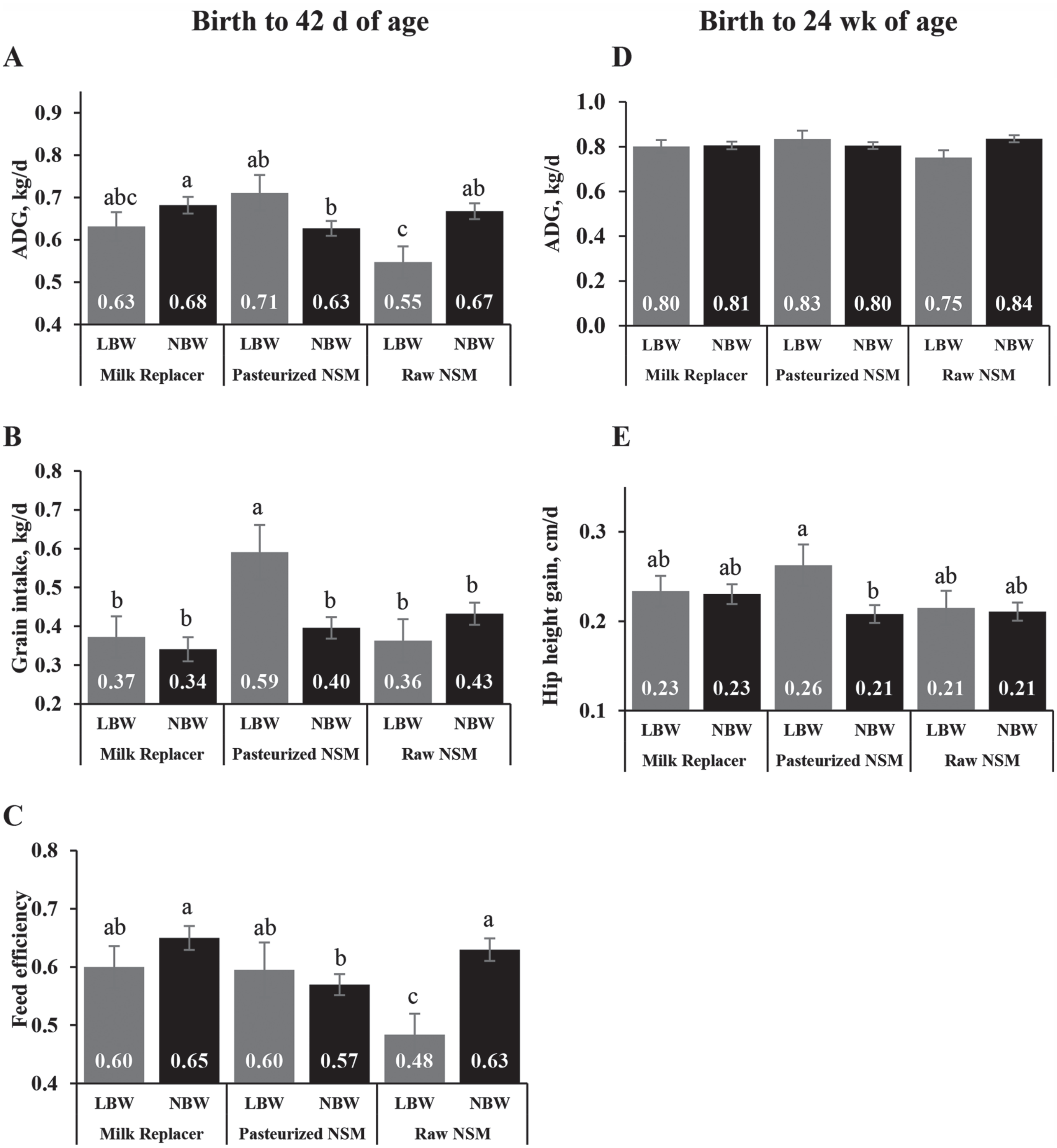

Figure 3. Birth to 42 d of age ADG (A), grain intake (B), and feed efficiency (C; BW gain/DMI) and birth to 24 wk ADG (D) and hip height gain (E) of heifers fed milk replacer or pasteurized or raw nonsaleable milk (NSM) from birth to weaning (≥6 wk). Heifers born <36.3 kg (LBW) were fed 4.2 L/d of milk until they reached 36.3 kg, whereas heifers born ≥36.3 kg (NBW) were fed 5.7 L/d. Contrast of pasteurized NSM vs. raw NSM × birth weight group, $P < 0.03$ (A), $P = 0.01$ (B), $P = 0.01$ (C), $P = 0.04$ (D), and $P = 0.10$ (E). Values are LSM ± SEM, and bars that do not share a common letter differ at $P \leq 0.05$.

interaction of milk treatments with birth weight group ($P > 0.15$) was not significant, treatment means for MEq protein yield in low versus normal birth weight heifers fed raw NSM followed the same pattern.

DISCUSSION

In the current study, 32 heifers out of the 152 that remained on the study were born with low birth weight. In a recent nationwide study in the US (Shivley et al., 2018b), regardless of sex, calves weighing ≤36.3 kg accounted for only 5% of dairy calves, substantially different than the 25% in our cohort of heifers. The high proportion of low birth weight heifers observed in our current study, coupled with the farm's standard procedure of restricting milk intake among lighter heifers, led us to account for this factor in the statistical model. Somewhat surprisingly, this cohort proved to be a relevant subset of heifers for observing short- and long-term responses to milk feeding strategies.

A few studies have evaluated the influence of birth weight on heifer productivity later in life. A small (n = 65) controlled study (Swali and Wathes, 2006) reported that low birth weight did not have adverse effects on productivity or fertility at first lactation. In contrast, more recent publications using observational data concluded that heifers with low birth weight produce less first-lactation 305-d milk (Rahbar et al., 2016), and lower levels of milk components over their first 3 lactations (Ghoraishy and Rokouei, 2013). Furthermore, low birth weight affected some fertility traits, such as age at calving and services per pregnancy, but did not affect all-service conception rate or calving rate (López et al., 2018). Although these observational studies are consistent with an effect of low birth weight on future performance, our study reports novel findings about the influence of preweaning dietary strategies on low birth weight heifers.

Performance During Preweaning and First 24 Wk of Life

The efficacy of any preweaning strategy is commonly measured by its impact on heifers' health and growth. Strategic feeding of colostrum has long been known to provide health benefits. Among heifers on this study, serum total protein averaged 6.67 ± 0.17 g/dL, far greater than the 5 g/dL cutoff point used to diagnose failure of passive transfer (Tyler et al., 1996; Garcia et al., 2014; Garcia et al., 2015; Bianco et al., 2017). Heifers born with low birth weight had similar serum total protein concentrations, regardless of the type of milk fed or pasteurization. In contrast, normal birth weight heifers fed pasteurized colostrum and NSM had greater serum protein concentration compared with normal birth weight heifers fed pasteurized colostrum and MR or raw colostrum and NSM. A recent nationwide study (Shivley et al., 2018a) listed several factors significantly associated with serum IgG. In addition to the well-known factors of colostrum quantity, IgG con-

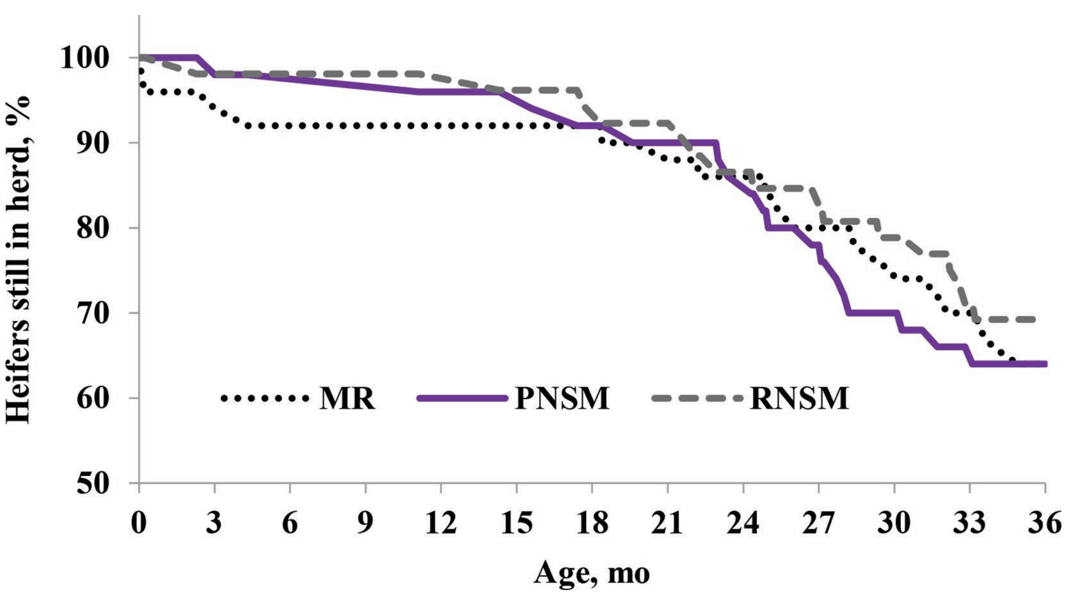

Figure 4. Retention in the herd of heifers fed milk replacer (MR) or pasteurized or raw nonsaleable milk (PNSM or RNSM, respectively) from birth to weaning (≥6 wk). Heifers born <36.3 kg (LBW) were fed 4.2 L/d of milk until they reached 36.3 kg, whereas heifers born ≥36.3 kg (NBW) were fed 5.7 L/d. Heifers were uniformly managed after weaning. Treatment, birth weight group, or their interaction did not affect time to removal from the herd ($P \geq 0.25$).

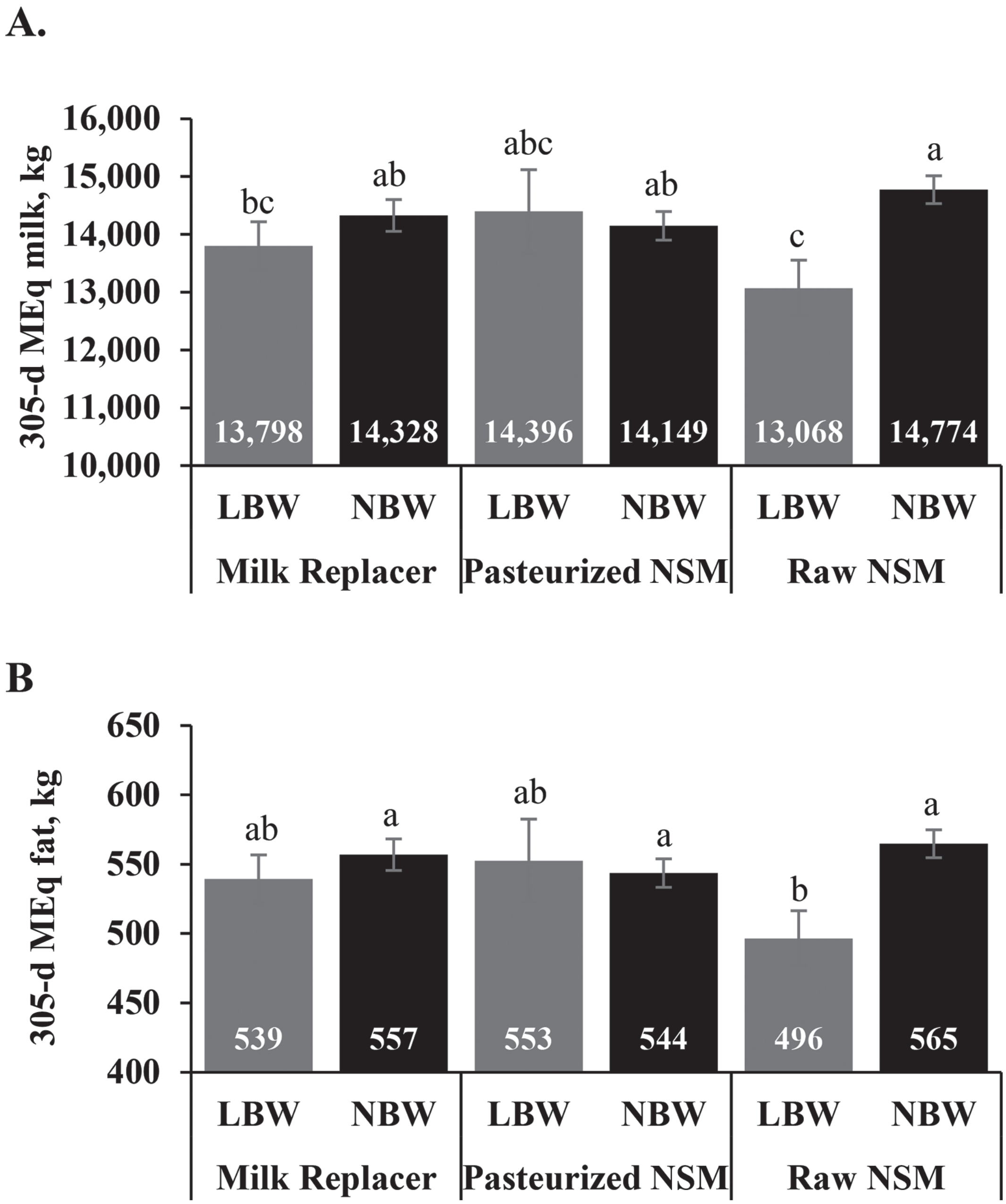

Figure 5. Yields of 305-d mature-equivalent (MEq) milk (A) and fat (B) in lactation 1. Heifers were fed milk replacer or pasteurized or raw nonsaleable milk (NSM) from birth to weaning (≥6 wk). Heifers born <36.3 kg (LBW) were fed 4.2 L/d of milk until they reached 36.3 kg, whereas heifers born ≥36.3 kg (NBW) were fed 5.7 L/d. Contrast of pasteurized NSM vs. raw NSM × birth weight group, $P = 0.04$ (A) and $P = 0.05$ (B). Values are LSM ± SEM, and bars that do not share a common letter differ at $P \leq 0.05$.

centration, and time to feeding, they also found that region, heat treatment of colostrum, and age at time of blood sample have significant effects. The same group reported a 5% average mortality during the preweaning period, and the factors associated with mortality were BW at birth, serum IgG concentration, daily milk fat intake, and morbidity. In our study, only 1.3% of heifers died during the preweaning period (2/152), and the incidence of diarrhea was 26%, demonstrating the relative health of heifers across treatments.

A factor less commonly associated with serum IgG concentrations is environmental temperature. Studies at the University of Florida (Gainesville, FL) found that calves exposed to heat stress during the last 6 wk of gestation have lower concentrations of serum protein and IgG or impaired ability to acquire passive immunity, or both (Tao et al., 2012; Monteiro et al., 2014). Our findings suggest that heifers born during warm months are more prone to failure of passive immune transfer if they are fed raw colostrum and raw NSM. Furthermore, heifers born during warmer months and fed raw NSM tended to have fewer circulating lymphocytes. Therefore, our findings support the recommendation that feeding raw colostrum or raw NSM should be avoided, particularly during the summer months.

Blood leukocyte populations were all within expected physiological ranges, and the ex vivo whole blood bactericidal activity assay performed at 25, 39, and 53 d did not uncover any influence of treatment or body weight group. Nevertheless, heifers fed MR instead of NSM had 11 and 17% greater hematocrit and red blood cell concentrations, respectively, coupled with the greater percentage of FS >1 for the normal birth weight heifers fed MR versus NSM. Our findings suggest that feeding MR reduced fecal consistency and slightly reduced hydration status of normal birth weight heifers. Others have reported that greater intake of MR increases fecal fluidity without necessarily affecting health status (Stamey et al., 2012; de Paula et al., 2017). Differences in hematocrit concentrations observed in heifers fed MR, particularly those born with normal weight, could be due to the greater osmolality (14.2% vs. 13.1% total solids) in MR than in NSM (Floren et al., 2016).

An interesting finding of this study is the sustained greater concentration of total leukocytes, including neutrophils, monocytes, and lymphocytes, in high versus low birth weight heifers. Low birth weight may have resulted from suboptimal in utero development, which may have had carryover effects on hematopoietic activity (Cairo et al., 1995; Omar et al., 2000), although a greater migration out of circulation into pathogen-invaded tissues cannot be ruled out. Regardless of the mechanism, the reduced populations of circulating immune cells in low birth weight heifers presumably would have made them more susceptible to infection or reinfection and may help to explain the dichotomous response to raw NSM feeding.

A recent study (Johnson et al., 2018) evaluated 11 South England dairy farms and found that the primary predictors of ADG from 1 to 63 d of age were total intake of milk solids and serum IgG concentration at enrollment (4.1 ± 2.2 d), whereas negative factors were birth weight (possibly due to restrictive milk feeding practices), weeks with diarrhea, and the incidence of respiratory disease. A more recent study collected data from 102 US operations and highlighted the importance of feeding an appropriate quantity and quality of liquid diet, keeping calves comfortable and free from disease, and mitigating the negative effects of environmental factors (Shivley et al., 2018b).

Our findings highlight an important interaction with the type of milk and the effect of pasteurization. Indeed, we identified that lighter heifers are prone to have reduced ADG when they are fed raw NSM rather than MR or pasteurized NSM. Some studies have documented that the pasteurization process reduces microbial load in NSM (Stabel, 2008; Elizondo-Salazar et al., 2010) and reduces the risk of morbidity and mortality (Armengol and Fraile, 2016). Our findings suggest that normal birth weight calves in a healthy environment may not show detrimental effects of raw NSM on preweaning ADG. Of course, this finding depends on the farm-specific circumstances, especially the microbial load and composition of NSM, limiting our ability to broadly generalize this conclusion. The major limitation of studies conducted nationwide or involving several operations is the lack of information on grain intake, which is known to influence ADG. Indeed, the US nationwide field study highlights the lack of records on grain intake as the major limitation of their study (Shivley et al., 2018b). We found that grain intake was greater for calves born with lighter BW, but only if they were fed pasteurized NSM and not if they were fed MR or raw NSM. Low birth weight heifers fed pasteurized NSM appeared to compensate for limited initial milk intake with greater grain intake. On the other hand, low birth weight heifers fed raw NSM did not consume as much grain and also showed the poorest feed efficiency, perhaps reflecting effects of mild microbial insults on both appetite and nutrient utilization.

The preweaning growth benefit from pasteurizing NSM for low birth weight heifers extended beyond the period when treatments were imposed. We continued to measure BW weekly until 8 wk of age and then every 4 wk after, until 24 wk of age. The ADG and hip height growth of low birth weight heifers was greater when NSM was pasteurized.

Performance Through the First Lactation

As hypothesized, type of milk and pasteurization not only influenced short-term responses, but they also affected productivity in the first lactation. It has been previously reported that in utero but not a preweaning nutritional strategy (supplementation of essential fatty acids) affected number of AI and age at first calving (Garcia et al., 2016). Similarly, others have reported that although greater intake of milk solids preweaning improved ADG, it did not affect age at puberty (Lage et al., 2017). Nevertheless, others found that an intensive MR feeding system providing high protein and energy intake not only promoted faster growth but also reduced age at conception and at calving (Raeth-Knight et al., 2009; Davis Rincker et al., 2011). A recent study

(Wilson et al., 2017) reported that a drastic restriction on MR feeding during 8 wk (0.44 vs. 1.08 kg of MR per animal per day) delayed uterine development by 8 wk of age; however, by 10 wk of age, when both groups of heifers had consumed similar diets for 2 wk, the delay was corrected and no differences were observed. This finding suggests that preweaning nutritional strategies do not have a drastic influence on uterine development. However, an indirect role of preweaning strategies on future reproductive efficiency could be mediated by differences in growth, health, and immune competence.

Physiologists have sought for many decades to discover the relationships between mammary gland development early in life and its impact on future productivity, but this question remains incompletely resolved (Akers, 2017). Indeed, recent meta-analyses reported a relationship between preweaning nutrition and management on milk production for one or more lactations (Soberon et al., 2012; Gelsinger et al., 2016). Although the physiological connections between improved AGD and future milk production have not been fully elucidated, improved development of the mammary parenchymal tissue by a higher plane of nutrition is a reasonable explanation (Garcia et al., 2016). Brown et al. (2005) fed a higher plane of nutrition via MR and grain to preweaned heifers from 2 to 8 wk of age and reported increased ADG (0.67 kg/d) compared with heifers fed a lesser plane of nutrition (0.38 kg/d). Brown et al. (2005) also reported that heifers with improved growth had greater mammary parenchymal mass, DNA, and RNA, and concluded that this increased rate of development of mammary parenchyma might translate into more milk production. Our first-lactation performance findings are consistent with those of the meta-analyses. Low birth weight heifers, offered less milk or MR over the first 1 to 2 wk, performed similarly to normal birth weight heifers when fed MR or pasteurized milk, but showed both slower growth rates and decreased milk yield in the first lactation when fed raw NSM (as well as raw colostrum). As reported by Gelsinger et al. (2016), management practices clearly influenced future performance; in this case, failing to pasteurize milk and colostrum for low birth weight heifers had effects that remained apparent for more than 2 yr.

CONCLUSIONS

Feeding nonsaleable milk to calves is a common practice in the dairy industry, but not all farms pasteurize this milk. Our findings demonstrate that heifers with low birth weights (<36.3 kg) are negatively affected by consuming unpasteurized colostrum and milk. These heifers, compared with those born with normal birth weight (≥36.3 kg), had reduced body weight gain, starter grain intake, and feed efficiency during the first 42 d of life. The negative impact of feeding raw nonsaleable milk to low birth weight heifers remained evident in growth rates through 24 wk of age and extended through the first lactation, as evidenced by reduced 305-d MEq milk and fat yields. Based on our findings, we recommend pasteurization of nonsaleable milk used for calves, particularly when fed to heifers with low birth weights.

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