Preweaned heifer management on US dairy operations: Part VI. Factors associated with average daily gain in preweaned dairy heifer calves

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ABSTRACT

The study objective was to evaluate average daily gain (ADG) in dairy heifer calves based on health, feeding, management practices, and environmental factors. This study included 102 operations in 13 states that participated in the calf component of the National Animal Health Monitoring System's Dairy 2014 study. This 18-mo longitudinal study included 1,410 Holstein heifer calves monitored from birth to weaning. The mean ADG from birth to final weight was 0.74 kg/d. Backward elimination model selection in Proc Mixed after univariate screening determined factors that significantly affected ADG. The final model included dam lactation number, singleton versus twin birth, bedding type, *Giardia* and *Cryptosporidium* fecal shedding, disease events, a categorized average temperature-humidity index for the preweaning period (pTHI), amount of protein in the liquid diet (kg/d), milk pasteurization, direct-fed microbials, and the interaction between milk pasteurization and direct-fed microbials. After controlling for other independent variables in the model, calves born to first-lactation dams gained less (0.60 kg/d) than calves from second- (0.65 kg/d) or third- or greater-lactation (0.64 kg/d) dams. Singleton calves gained 0.07 kg/d more than twins. Calves bedded with sand or no bedding gained less (0.49 kg/d) than calves on all other bedding types. Calves negative for *Cryptosporidium* or *Giardia* at the time of sampling gained 0.03 or 0.02 kg/d more, respectively, than calves that were positive for *Cryptosporidium* or *Giardia*. Calves with no disease events gained 0.07 kg/d more

than calves with one or more disease events. Calves experiencing an average pTHI <50 gained more (0.67 kg/d) than calves experiencing an average pTHI from 50 to 69 (0.62 kg/d), or ≥70 (0.59 kg/d). Within the range of observed kilograms of protein fed per day in the liquid diet, every additional 0.1 kg of protein fed per day equated to 0.02 kg/d of gain. Calves fed milk replacer with a direct-fed microbial gained less (0.44 kg/d) than calves fed milk replacer without a direct-fed microbial (0.60 kg/d) and calves fed pasteurized or unpasteurized milk regardless of direct-fed microbial use. These results highlight the importance of feeding a quantity and quality of a liquid diet to achieve optimal growth, keeping calves free of disease, the type or status of bedding, and mitigating the effects of temperature and humidity on preweaning ADG.

Key words: average daily gain, Holstein heifer calves, liquid diet

INTRODUCTION

The preweaning phase is a critical period in the life of a dairy heifer, and optimal conditions increase the likelihood of success as a lactating cow. Growth determines the age at first breeding and age and weight at first calving, and is associated with lifetime productivity (Heinrichs, 1993; Soberon et al., 2012; Soberon and Van Amburgh, 2013). Growth during the preweaning period, and specifically ADG, is affected by many different factors, including passive transfer of immunity, disease, nutrition, management practices, and environmental factors (Place et al., 1998). Therefore, ADG is an appropriate metric to evaluate growth and health during the preweaning period.

Proper calf nutrition, defined by the energy and protein available in the diet, is essential for growth and rumen development. At birth, calves are functional

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monogastrics, and they rely exclusively on fluid milk diets for nutrients (Drackley, 2008). During the first 2 wk of life, calves typically just nibble calf starter; by about 3 wk of age, calves should increase their intake of calf starter to approximately 1.4 kg/d to stimulate rumen development. Industry recommendations state that calf starter should be offered by 4 d of age (BAMN, 2003, 2017). Calves must have access to fresh, clean water in addition to the milk diet to stimulate starter feed intake and rumen development (Kertz et al., 1984; BAMN, 2003, 2017). Protein is considered the rate-limiting nutrient for calf growth (Drackley, 2008). Feeding 4 L of a 20% protein, 20% fat milk replacer (MR) provides enough protein and energy for a 50-kg calf under thermoneutral conditions to gain 0.3 kg/d; feeding the same calf fed 4 L of a 25% protein, 20% fat MR would result in gain of 0.8 kg/d (NRC, 2001). Calves are most efficient at converting feed nutrients to body mass during the preweaning period; then, feed efficiency steadily declines as they age, and maintenance requirements increase as they grow (Owens et al., 1993; Kertz et al., 1998). Thus, it is important to try to optimize growth during this period.

Historically, calf feeding regimens included twice-daily feeding of 10% of BW total, equating to about 2 L of the liquid diet fed twice per day (Thickett et al., 1986). A rationale for limit-feeding milk has been to promote starter intake, allowing calves to be weaned earlier, but at a cost in terms of health and growth. Limiting the liquid diet fed results in decreased growth (Flower and Weary, 2001), and reducing the nutrient density may increase the incidence of disease in preweaning calves (Godden et al., 2005). More recently, enhanced feeding programs using MR with a higher percent of protein or ad libitum feeding programs using automated calf feeding systems have gained popularity (Jasper and Weary, 2002; Terré et al., 2009).

The objective of this study was to evaluate the association of different health, feeding, and management practices, as well as environmental factors on ADG in preweaned heifer calves.

MATERIALS AND METHODS

Study Design

The USDA National Animal Health Monitoring System (NAHMS) conducts national surveys to collect information on the health, management, and productivity of domestic livestock species (USDA, 2016). In 2014, a nationwide survey was conducted to collect information about the US dairy industry, including an 18-mo longitudinal preweaned dairy heifer calf study. All participation in NAHMS studies is voluntary.

However, to participate in the longitudinal calf study, operations had to have completed both NAHMS Dairy 2014 surveys and agreed to provide information on enrolled calves, whether raised onsite or offsite.

The calf component of the NAHMS Dairy 2014 study consisted of a convenience sample of 102 dairy operations with Holstein calves, including both conventional and organic operations. These operations were located in 13 states, including California, Colorado, and Washington in the western region, and Iowa, Michigan, Minnesota, Missouri, New York, Ohio, Pennsylvania, Vermont, Virginia, and Wisconsin in the eastern region. Dairy operations were categorized based on the number of mature cows, as small (30–99 cows), medium (100–499 cows), and large (≥ 500 cows).

Data collection for the calf component of the study occurred from March 2014 through September 2015. Data collectors were trained on data and sample collection. Each operation was instructed to enroll 24 heifer calves over a 1-yr period, or an average of 2 calves per month. Farm personnel selected which calves to enroll in the study. However, a calf must have been alive at 24 h of age to be enrolled. Because fewer operations agreed to participate than originally planned, the number of calves that could be enrolled was increased to 48 to 60 calves per operation. Additionally, because enrollment of farms did not occur as quickly as anticipated, the study encompassed 18 mo instead of the 12-mo period initially planned.

Heifer Calf Health Card

Each calf enrolled in the study had a Heifer Calf Health Card (Calf Card) filled out to record information on events that occurred between birth and weaning (https://www.aphis.usda.gov/animal_health/naahms/dairy/downloads/dairy14ques/CalfHealth.pdf). The Calf Card contained questions in both English and Spanish and was filled out by farm personnel, a veterinary medical officer, extension personnel, veterinarians, or a combination of people involved with calf raising. Sections of the Calf Card included birth data (such as birth date, weight, and calving ease); colostrum feeding data (including timing, volume, and method of colostrum feeding); preweaning housing and procedure data (such as housing, ventilation, bedding, and dehorning); milk feeding (including type of liquid diet fed, any additives, and method of feeding); milk consumption record (amount and frequency of feedings); preweaning growth record (hip height and heart girth recordings every 2 wk); biologic sampling record (including serum collection and fecal sampling dates); vaccinations; disease incidence and treatment; weaning data (weaning date, primary weaning criteria); and any additional notes.

Additional information requested included starter feed labels and MR feed labels (if applicable).

Producers were provided with the Calf Health Scoring Chart (<https://www.vetmed.wisc.edu/dms/fapm/apps/chs.htm>) to guide the reporting of clinical signs. The goal of the study was to collect recognized clinical signs and causes of death, not to standardize clinical signs and causes of death across operations.

Because of the variation observed in the quantity and quality of the liquid diet fed per day, kilograms of protein and fat fed per day were calculated for each calf. Information regarding the amount of liquid diet fed per feeding and the frequency of feedings per day were recorded on the Calf Card and used to calculate the total amount of liquid diet fed. For calves fed MR, the percent protein and percent fat were obtained from the producer-reported values or the MR label. For calves fed whole or waste milk, percent protein was set at 3.1% and percent fat as 3.8% (as-fed basis; Chester-Jones and Hoffman, 2003). The total amount of protein and fat were calculated, and then divided by the number of days the liquid diet was fed to determine the average kilograms of protein and fat fed per day in the liquid diet.

Additives in the liquid diet were categorized as antibiotics, direct-fed microbials (DFM), or other additives, which included fly control, organic acids (as preservatives), and coccidiostats. Information regarding calf starter included the percent protein and the age starter was first offered, but starter intake was not recorded.

Biological Sampling and Testing

Before being enrolled in the study, calves were screened for persistent infection with bovine viral diarrhoea virus (BVDV). V-Cut ear notchers (Nasco, Fort Atkinson, WI) were used to collect ear notch samples from all calves, which were tested on-farm for BVDV using the Idexx SNAP BVDV Antigen Test (Idexx, Westbrook, ME). Calves positive for BVDV were not enrolled in the study. Colostrum samples (40–50 mL) from the first feeding of colostrum administered to the calf were collected in conical screw-top tubes by farm personnel, and then frozen until shipping. Serum samples (5 mL) from calves between 1 and 7 d of age were collected in serum separator tubes by veterinary medical officers (VMO) or animal health technicians (AHT), and samples were centrifuged if possible before shipping. Colostrum and serum samples were shipped together on ice to USDA's National Veterinary Services Laboratories (Ames, IA). Samples were then accumulated and shipped in bulk to the Saskatoon Colostrum Co. (Saskatoon, SK, Canada). Blood samples collected

within 24 h of birth or after 7 d of age were excluded from this analysis. A complete description of the methods used for Brix scoring and IgG determination can be found in Urie et al. (2018).

For the evaluation of enteric parasites, VMO and AHT collected approximately 50 g of feces directly from the rectum of calves between 2 and 4 wk of age and placed the samples in cylindrical screw-top containers. Fecal samples were shipped on ice to USDA Agricultural Research Service's Environmental Microbial Food Safety Laboratory (Beltsville, MD) as quickly as possible after sample collection. Fecal samples were tested by immunofluorescence microscopy for *Cryptosporidium parvum* and *Giardia duodenalis*. A complete description of the methods used for fecal parasite testing can be found in Urie et al. (2018).

Growth Measurements

Calves were measured approximately every 2 wk during the preweaning period to monitor growth rates. Height and weight tapes from Coburn (Nasco, Fort Atkinson, WI) were provided for measuring calves. For consistency, it was recommended that one trained VMO or AHT complete the measurements on all calves enrolled on an operation. Birth weights were estimated using either the operation's standard protocol or a weight tape. Hip height and heart girth circumference were measured approximately every 2 wk and recorded in centimeters. Heart girth circumference in centimeters was converted to kilograms using the following equation (Heinrichs et al., 1992):

$$\text{BW (kg)} = [(0.02655 \times \text{cm}^2) + (-2.876 \times \text{cm}) + 102.71].$$

Prewaning weight gain was calculated by subtracting the birth weight from the final weight. Average daily gain was calculated by taking the weight gain during the preweaning period divided by the number of days between birth weight and final weight (approximately the preweaning period). Final weights did not always occur at the same time as weaning, although only calves with final weight measurements within 14 d of weaning were included in the analysis. Average daily gain categories were determined based on field experience by one of the authors (TJE), with poor ADG <0.64 kg/d, fair ADG 0.64–0.82 kg/d, and excellent ADG >0.82 kg/d.

Environmental Factors

Temperature-humidity index (THI) data were obtained monthly by county from the National Oceanic

and Atmospheric Administration (NOAA, 2016); the THI accounts for the effects of temperature and relative humidity, and the equation uses the dry bulb temperature (T , °F) and the relative humidity (RH). The equation used for this analysis was $THI = T - [(0.55 - (0.55 \times RH/100)) \times (T - 58)]$ (Collier et al., 2011). Each calf was assigned a THI for each month during the preweaning period (**pTHI**) and then averaged. The average pTHI for each calf was then categorized according to the thermoneutral zone for a calf as follows: <50 (cold stress), ≥ 50 and <70 (thermoneutral zone), or ≥ 70 (heat stress) (Stull and Reynolds, 2008).

Elevation data (in meters) were obtained for each county and state from the National Oceanic and Atmospheric Administration (NOAA, 2016).

Statistical Analysis

When each calf was weaned, the Calf Card was mailed to NAHMS (Fort Collins, CO). Initial validation was performed on every Calf Card as it came in to check accuracy of dates and other information. Data were then entered into SAS (version 9.4; SAS Institute Inc., Cary, NC). Once all Calf Cards were entered, the data were validated again by the NAHMS staff and merged with the results from the colostrum, serum, and fecal testing obtained from the laboratories. Descriptive data were analyzed using the FREQUENCY and MEANS procedures of SAS for categorical and continuous variables, respectively.

A multivariable random effects mixed model was constructed to determine the factors associated with ADG, with operation included as the random variable. Predictor variables were considered for the model based on biological plausibility. To be included in the analysis, only Holstein calves were included, and calves had to have data for all of the variables included in the initial multivariable model. Univariate screening was used to determine which variables to include in the initial model, and variables with a $P < 0.20$ were considered for inclusion in the multivariable model. Stepwise backward elimination model selection in PROC MIXED of SAS was used to construct the final model, with variables with $P < 0.05$ considered significant and thus included in the model. All first-order interactions of the final model variables were evaluated for significance. Model fit was evaluated using the Akaike information criterion.

RESULTS

Complete data were available for 1,410 calves from 97 operations in 12 states (Figure 1). The mean ADG was 0.74 kg/d (SE 0.01), and calves were fed liquid

diets an average of 64.7 d (SE 0.48; Table 1). Average birth weight was 43.2 kg (SE 0.14), average final weight was 90.9 kg (SE 0.56), and average preweaning weight gain was 48.4 kg (SE 0.53). Overall, 33.3% of calves had a mean ADG of greater than 0.82 kg/d.

Initial variables included in the multivariable model for ADG included region, pTHI category, dam lactation, number of calves (single vs. twin), DFM added to liquid diet, coccidiostats in calf starter, producer-reported disease status, *Cryptosporidium* status, *Giardia* status, bedding, sex of caretaker, dehorning status, milk pasteurization, birth weight, calf serum IgG, colostrum IgG, kilograms of protein and kilograms of fat in liquid diet per day, frequency of feedings, and number of vaccines (Tables 2 and 3). Although the number of calves in the group and milk delivery system had P -values < 0.2 in the univariate screening, they were excluded from the multivariable model due to a strong correlation with kilograms of protein and fat per day.

The final model included disease status ($P < 0.001$), protein fed in the liquid diet per day (kg/d; $P = 0.004$), milk pasteurization ($P < 0.001$), addition of DFM to the liquid diet ($P = 0.123$), the interaction between DFM and milk pasteurization ($P < 0.001$), categorized average pTHI ($P < 0.001$), dam lactation number ($P < 0.001$), bedding type ($P < 0.001$), singleton versus twin birth ($P = 0.007$), *Cryptosporidium* status ($P = 0.003$), and *Giardia* status ($P = 0.020$) (Table 4). After controlling for other independent variables in the model, calves with no disease events gained 0.07 kg/d more than calves with one or more disease events. Within the range of observed kilograms of protein fed per day in the liquid diet, every additional 0.1 kg of protein fed per day equated to 0.02 kg/d of gain. The interaction between diet and DFM showed that calves fed MR with a DFM gained less (0.44 kg/d) than calves fed MR without a DFM (0.60 kg/d) and calves fed pasteurized or unpasteurized milk with or without a DFM (Table 5; Figure 2). Calves fed pasteurized milk without a DFM gained more (0.70 kg/d) than calves fed unpasteurized milk without a DFM (0.64 kg/d) and calves fed MR with or without a DFM (0.44 and 0.60 kg/d, respectively). Additionally, calves fed MR without DFM gained less than calves fed unpasteurized milk with a DFM (0.72 kg/d). Calves experiencing an average pTHI < 50 gained more (0.67 kg/d) than calves experiencing an average pTHI from 50 to 69 (0.62 kg/d), or ≥ 70 (0.59 kg/d). Calves born to first-lactation dams gained less (0.60 kg/d) than calves from second- (0.65 kg/d) or third- or higher-lactation dams (0.64 kg/d). Calves bedded with sand or no bedding gained less (0.49 kg/d) than all other bedding types (straw, shavings, or manure biosolids). Single calves gained 0.07 kg/d more than twins. Calves negative for *Cryptosporidium*

Table 1. General growth parameters for Holstein heifer calves during the preweaning period (n = 1,410)

Parameter	Mean	SE	Percentile				
			5th	25th	50th	75th	95th
Birth weight (kg)	43.2	0.14	36.3	39.9	42.6	45.8	52.2
Final weight (kg)	90.9	0.56	59.9	77.6	89.4	101.6	127.0
Preweaning weight gain (kg)	48.4	0.53	20.4	34.9	46.3	58.9	85.5
Age at weaning (d)	64.7	0.48	43.0	56.0	61.0	70.0	100.0
Age at final weight measurement (d)	64.6	0.48	43.0	56.0	61.0	71.0	93.0
ADG ¹ (kg/d)	0.74	0.01	0.4	0.6	0.7	0.9	1.1

¹Calculated by taking the weight gain during the preweaning period divided by the number of days between birth weight and final weight (approximately the preweaning period).

Figure 1. Flowchart summarizing the number of calves used in the ADG model from the overall USDA National Animal Health Monitoring System's (NAHMS) Dairy 2014 study (calf component; USDA, 2016) and reasons for removing calves from the analysis.

Table 2. Percent of Holstein heifer calves for all categorical variables included in univariate screening for the multivariable ADG model, by ADG category¹

Variable	Variable level	Calves (no.)	Calves (%)	Calves by ADG ² (%)			P-value
				Poor (<0.64 kg/d)	Fair (0.64–0.82 kg/d)	Excellent (>0.82 kg/d)	
All calves		1,410	100.0	31.4	35.4	33.3	
Region	West	517	36.7	34.0	34.2	31.7	0.095
	East	893	63.3	29.8	36.1	34.2	
Herd size (adult cows)	Small (30–99 cows)	194	13.8	29.4	38.1	32.5	0.611
	Medium (100–499 cows)	408	28.9	31.4	37.7	30.9	
	Large (500+ cows)	808	57.3	31.8	33.5	34.7	
Prewaning THI³	<50 (cold stress)	647	45.9	25.3	33.5	41.1	<0.001
	≥50 and <70 (thermoneutral)	645	45.7	34.9	37.5	27.6	
	≥70 (heat stress)	118	8.4	44.9	33.9	21.2	
Dam lactation	First	541	38.4	36.4	32.5	31.1	0.000
	Second	378	26.8	27.2	37.6	35.2	
	Third or higher	491	34.8	28.9	36.9	34.2	
Calving ease	Unassisted	1,013	71.9	29.8	36.1	34.1	0.331
	Easy (1 person)	274	19.4	36.5	31.0	32.5	
	Difficult (2+ people)	62	4.4	37.1	40.3	22.6	
	Mechanical/surgical extraction	21	1.5	28.6	47.6	23.8	
	Not reported	40	2.8	27.5	32.5	40.0	
Number of calves born	Single	1,360	96.5	31.2	34.9	33.9	0.044
	Twin	50	3.5	36.0	48.0	16.0	
Milk delivery system	Bottle	280	19.9	41.4	36.4	22.1	0.005
	Bucket/pail	233	16.5	23.2	37.3	39.5	
	Milk bar	52	3.7	25.0	25.0	50.0	
	Automated feeder	36	2.6	33.3	30.6	36.1	
	Bottle and bucket	691	49.0	32.0	34.9	33.1	
	Other combinations	118	8.4	22.0	38.1	39.8	
DFM⁴ in milk/milk replacer (MR)	Yes	244	17.3	29.1	37.3	33.6	0.001
	No	1,166	82.7	31.8	35.0	33.2	
Antimicrobials in milk	Yes	192	13.6	14.1	42.7	43.2	0.396
	No	1,218	86.4	34.1	34.2	31.7	
Other additives in milk	Yes	784	55.6	29.6	35.6	34.8	0.709
	No	626	44.4	33.5	35.1	31.3	
Liquid diet type	MR	528	37.4	42.4	30.7	26.9	0.439
	Whole/waste milk	521	37.0	23.8	38.6	37.6	
	Combination	361	25.6	26.0	37.7	36.3	
Pasteurization of milk	Yes	348	24.7	20.7	36.8	42.5	0.190
	No	534	37.9	27.3	39.1	33.5	
	Not applicable (fed MR)	528	37.4	42.4	30.7	26.9	
Bacterial counts of milk	Yes	139	9.9	20.9	38.1	41.0	0.423
	No	743	52.7	25.4	38.2	36.3	
	Not applicable	528	37.4	42.4	30.7	26.9	
Fly control in calf starter	Yes	213	15.1	25.8	35.7	38.5	0.937
	No	1,197	84.9	32.3	35.3	32.3	
Coccidiostats in calf starter	Yes	555	39.4	25.2	35.0	39.8	0.056
	No	855	60.6	35.3	35.7	29.0	
Disease events	1 or more	491	34.8	36.3	31.8	32.0	<0.001
	0	919	65.2	28.7	37.3	33.9	
<i>Cryptosporidium</i>	Fecal negative	788	55.9	27.9	35.7	36.4	0.001
	Fecal positive	622	44.1	35.7	35.0	29.3	
<i>Giardia</i>	Fecal negative	980	69.5	29.1	35.9	35.0	0.003
	Fecal positive	430	30.5	36.5	34.2	29.3	
Bedding	Straw/hay	782	55.5	26.6	37.0	36.4	<0.001
	Shavings	336	23.8	25.3	36.3	38.4	
	Sand/none	135	9.6	73.3	22.2	4.4	
	Combination/other	157	11.1	31.8	36.9	31.2	
Sex of caretaker	Male	909	64.5	34.4	33.2	32.3	0.023
	Female	450	31.9	25.3	37.8	36.9	
	Both male and female	51	3.6	29.4	52.9	17.6	
Dehorning	Yes with medications	223	15.8	24.7	41.3	34.1	0.116
	Yes without medications	557	39.5	27.1	35.0	37.9	
	Not dehorned	630	44.7	37.5	33.7	28.9	
Ventilation	Natural/outside	1,118	79.3	33.5	34.4	32.1	0.446
	Positive pressure tubes	141	10.0	14.9	35.5	49.6	
	Other	151	10.7	31.1	42.4	26.5	

Continued

Table 2 (Continued). Percent of Holstein heifer calves for all categorical variables included in univariate screening for the multivariable ADG model, by ADG category¹

Variable	Variable level	Calves (no.)	Calves (%)	Calves by ADG ² (%)			P-value
				Poor (<0.64 kg/d)	Fair (0.64–0.82 kg/d)	Excellent (>0.82 kg/d)	
Weaning criteria	Starter intake	44	3.1	52.3	22.7	25.0	0.271
	Age	764	54.2	35.9	35.1	29.1	
	Lack of space	84	6.0	21.4	29.8	48.8	
	Other	25	1.8	24.0	24.0	52.0	
	Combination	493	35.0	24.5	38.5	36.9	

¹Bolded variables were included in the initial multivariable ADG model (P -value < 0.20).

²Calculated by taking the weight gain during the preweaning period divided by the number of days between birth weight and final weight (approximately the preweaning period). The cutoff values were determined by one of the authors (TJE).

³Temperature-humidity index (THI) = $T - [0.55 - (0.55 \times RH/100) \times (T - 58)]$, where T = dry bulb temperature (°F); and RH = relative humidity. Each calf was assigned a THI for each month during the preweaning period (pTHI) and then averaged. The average pTHI for each calf was then categorized according to the thermoneutral zone for a calf as follows: <50 (cold stress), ≥50 and <70 (thermoneutral zone), or ≥70 (heat stress).

⁴Direct-fed microbials.

or *Giardia* at the time of sampling gained 0.03 or 0.02 kg/d more, respectively, than calves that were positive for *Cryptosporidium* or *Giardia*.

DISCUSSION

The results of this study showed that ADG of dairy heifer calves during the preweaning period was influenced by many factors, including disease, kilograms of protein fed per day in the liquid diet, milk pasteurization, the interaction between DFM and milk pasteurization, THI for the preweaning period, dam lactation, bedding type, the number of calves born (single vs. twin), *Cryptosporidium* status, and *Giardia* status. As expected, calves with one or more disease events during the preweaning period gained less than calves with no disease events. Previous research has also shown a negative relationship between disease and growth (Virtala et al., 1996; Donovan et al., 1998; Bach, 2011). The 2 most commonly reported clinical signs of disease in this study were digestive and respiratory signs. Disease during the preweaning period can affect ADG by nutrient loss from the diet via diarrhea, diversion of energy to the immune system and away from growth, dehydration, and suppressed appetite and feed intake (Donovan et al., 1998). Disease events were self-reported by producers for this study, and it is possible that some operations were more vigilant about detecting and reporting disease than others. Therefore, the real impact of disease on ADG was probably greater than the results of this study indicate. Ensuring calves are fed the recommended amount of high-quality colostrum immediately after birth for adequate passive transfer of immunity, housing calves in a clean environment, and providing proper nutrition can all help reduce the

incidence of disease in calves (LeBlanc et al., 2006), thus improving ADG.

Nutrition during the preweaning period is critical for growth, with both the quantity and the quality of the liquid diet having significant effects (Drackley, 2008; Soberon et al., 2012; Soberon and Van Amburgh, 2013). In one study, calves fed milk ad libitum consumed twice as much milk and gained 4 times as much compared with restricted-fed calves, who also displayed signs of hunger (De Paula Vieira et al., 2008). Increasing the amount of milk fed during the preweaning period can also increase future milk yield (Rincker et al., 2011; Soberon et al., 2012; Soberon and Van Amburgh, 2013; Van Amburgh et al., 2014), as well as decrease the time to first calving (if bred based on weight; Curtis, 2015). According to the National Research Council's energy requirements for calves (NRC, 2001), the ME required for maintenance of a 45-kg calf under thermoneutral conditions is 1.75 Mcal/d, which equates to about 2.5 L of whole milk or 3.0 L of an average MR (20% protein, 20% fat), because most MR have lower levels of fat than milk (Drackley, 2008). Protein requirements are determined by the desired rate of growth (Drackley, 2008). Because calves in this study were fed varying amounts of liquid diets of differing compositions, the average amount of protein and fat fed to the calf per day were used in the ADG model. The protein and fat per day (kg) were highly correlated, with only protein per day staying in the final model. The results of this study showed that ADG increased with greater amounts of protein fed per day. Similarly, Li et al. (2008) showed that calves fed a high-protein diet had greater ADG than calves fed mid- or low-protein diets. Increasing protein in the diet appears to have a positive linear relationship with BW gain (Blome et al., 2003). Calves fed a higher plane of

nutrition are able to convert the additional nutrients into lean tissue at high efficiency (Liang et al., 2015b). Increasing the amount of protein in the liquid diet, by either increasing the overall amount of liquid diet fed or increasing the concentration of protein in the liquid diet, improves ADG in dairy heifer calves during the preweaning period.

In this study, calves fed MR gained significantly less than calves fed pasteurized or unpasteurized milk. A survey of on-farm pasteurizers showed that although waste milk composition is highly variable, concentrations of protein and fat are greater than in conventional MR (Jorgensen et al., 2006). Concerns with feeding waste milk include inconsistent composition, bacterial contamination, and antimicrobial residues. A controlled study comparing calves fed pasteurized waste milk versus a 20% CP, 20% fat conventional MR found that calves fed waste milk had greater weaning weights, higher ADG, and lower morbidity and mortality, possibly due to the higher levels of protein provided (Godden et al., 2005). The higher fat content of milk can have a detrimental effect on starter intake, potentially reducing ADG, as seen by Hill et al. (2009). Not all MR are created equal, and supplementing MR with amino acids and fatty acids can improve ADG (Hill et al., 2007). Perhaps more important than the source of the liquid diet is the amount of total solids consumed by the calf as an indicator of the overall quantity and quality of the liquid diet. Although the quality of MR in terms of percent fat and protein were accounted for in the model, the sources of fat and protein were not controlled for, nor were additional supplements.

Direct-fed microbials, or probiotics, are nonpathogenic microbial species that are added to the diet to improve gastrointestinal flora (BAMN, 2011). In this study, the addition of DFM to the liquid diet resulted in a lower ADG for calves fed MR, but calves fed DFM with unpasteurized milk had higher ADG than calves fed MR without DFM. Although several studies have shown improved growth rates when feeding DFM (Abe et al., 1995; Timmerman et al., 2005; Adams et al., 2008), other studies have shown that DFM had no effect on growth or health (Jenny et al., 1991; Morrill et al., 1995; Abu-Tarboush et al., 1996; Higginbotham et al., 1998). Direct-fed microbials may be most important for calves exposed to pathogens or environmental challenges that lead to stress, disrupting the intestinal flora (BAMN, 2011). Because of this, perhaps calves that were fed DFM in this study were already experiencing stress, which is why the producer decided to add DFM to the diet. The resulting lower ADG detected in this study associated with feeding DFM and MR might be reflecting other challenges on the operation.

Table 3. Continuous variables initially included in univariate screening for the ADG model, by ADG category (n = 1,410)¹

Variable	Mean	SE	Percentile					Poor (<0.64 kg/d)			Fair (0.64–0.82 kg/d)			Excellent (>0.82 kg/d)			P-value
			5th	25th	50th	75th	95th	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	
ADG (kg/d)	0.74	0.01	0.39	0.59	0.73	0.87	1.08	0.49	0.00	0.73	0.00	0.97	0.01	0.97	0.01	0.97	
Elevation (m)	507.4	11.1	112	258	290	868	1513	442	18.5	555	19.5	517	19.0	517	19.0	517	0.369
Birth weight (kg)	43.2	0.14	36.3	39.9	42.6	45.8	52.2	42.5	0.21	43.1	0.23	43.8	0.27	43.8	0.27	43.8	0.009
Colostrum IgG (g/L)	73.7	0.83	25.7	52.0	72.3	91.5	130.2	72.3	1.48	72.1	1.40	76.6	1.41	76.6	1.41	76.6	0.150
Serum IgG (g/L)	21.4	0.27	6.2	14.4	20.8	27.9	38.8	19.9	0.48	21.9	0.46	22.4	0.46	22.4	0.46	22.4	0.032
Protein per day (kg/d)	0.2	0.00	0.1	0.2	0.2	0.2	0.4	0.2	0.01	0.2	0.00	0.2	0.01	0.2	0.01	0.2	0.006
Fat per day (kg/d)	0.2	0.00	0.1	0.1	0.2	0.3	0.3	0.2	0.01	0.2	0.00	0.2	0.00	0.2	0.00	0.2	0.011
Frequency fed (no./d)	2.7	0.06	1.7	1.9	2.0	2.0	9.0	2.5	0.08	2.7	0.09	3.0	0.11	3.0	0.11	3.0	0.085
Amount per feeding (L)	2.6	0.02	0.9	1.9	2.6	3.2	3.8	2.5	0.04	2.6	0.04	2.6	0.04	2.6	0.04	2.6	0.935
Days fed starter	59.5	0.46	37.0	51.0	57.0	66.0	93.0	56.9	0.77	60.0	0.88	61.4	0.68	61.4	0.68	61.4	0.395
Age offered water (d)	6.3	0.34	0.0	1.0	3.0	5.0	34.0	5.8	0.54	7.1	0.60	5.9	0.60	5.9	0.60	5.9	0.879
Starter percent protein	20.3	0.06	17.0	18.0	20.0	22.0	24.0	20.2	0.10	20.4	0.10	20.2	0.11	20.2	0.11	20.2	0.426
Calves in group (no.)	2.5	0.12	1.0	1.0	1.0	1.0	12.0	2.1	0.20	2.4	0.19	3.1	0.24	3.1	0.24	3.1	0.145
Age at weaning (d)	64.7	0.48	43.0	56.0	61.0	70.0	100.0	62.2	0.87	65.5	0.88	66.0	0.72	66.0	0.72	66.0	0.661
Number of vaccines	1.4	0.04	0.0	0.0	1.0	2.0	4.0	1.2	0.06	1.5	0.06	1.6	0.06	1.6	0.06	1.6	0.079

¹Bolded variables were included in the initial multivariable ADG model (P -value < 0.20).

²Calculated by taking the weight gain during the preweaning period divided by the number of days between birth weight and final weight (approximately the preweaning period). The cutoff values were determined by one of the authors (TJE).

Increasing the plane of nutrition during the preweaning period not only affects ADG but can also lead to better disease resistance. Until recently, few studies evaluating the effects of nutrition on disease had been conducted, though previous work has shown that calves fed less energy and protein than necessary for growth also have higher death losses (Goff, 2006). One study found that the plane of nutrition might modulate functions associated with cell-mediated immune responses, though the study was conducted via ex vivo cell function assays (Nonnecke et al., 2003). Another study showed that Jersey dairy heifers fed a higher plane of nutrition had a higher ADG and were more resistant to *Salmonella* postweaning (Ballou et al., 2015). In a controlled trial comparing a low plane of nutrition with a high plane of nutrition during the preweaning period, calves were subsequently challenged postweaning with *Mannheimia haemolytica* or a combined bovine herpesvirus-1 and *M. haemolytica*. The calves fed a low plane of nutrition preweaning responded with more severe disease to the combined viral and bacterial challenge postweaning (Sharon et al., 2015a,b). Lower planes of nutrition during the preweaning period appeared to

increase the risk of respiratory disease 1 mo after weaning, indicating that early life nutrition can influence resistance to and pathology of disease (Sharon et al., 2015a,b). Another study found that calves fed a high plane of nutrition during the preweaning period had a greater acute phase response to an opportunistic bacterial enteric infection compared with calves fed a low plane of nutrition (Liang et al., 2015a). Nutrition of calves during the preweaning period is important for immune function and disease susceptibility, in addition to improving growth rates.

Environmental factors were evaluated for influences on ADG, and the average THI during the preweaning period was associated with growth. Calves experiencing an average pTHI of <50 gained more than calves experiencing an average pTHI of 50 to 69 or ≥70. Previous research has shown that calf growth is negatively affected by periods of heat stress, with lower starter consumption and lower BW at weaning (Broucek et al., 2009). Decreased blood flow to the digestive tract during periods of heat stress led to decreased absorption of nutrients and decreased feed intake (Beede and Collier, 1986). Any deviation from the thermoneutral

Table 4. Results of multivariable modeling of factors associated with ADG for Holstein heifer calves (n = 1,410)

Variable ¹	Factor P-value	Variable level	Model- predicted ADG (kg/d)	SE	Estimate	SE	P-value for pairwise comparisons
Intercept					0.548	0.05	<0.001
Dam lactation	<0.001	First	0.60	0.02	-0.037	0.01	0.001
		Second	0.65	0.02	0.008	0.01	0.521
		Third or higher	0.64	0.02	Referent		
Number of calves	0.007	Single	0.66	0.02	0.070	0.03	0.007
		Twin	0.59	0.03	Referent		
Bedding	<0.001	Straw/hay	0.68	0.02	0.004	0.03	0.868
		Shavings	0.67	0.03	-0.000	0.03	0.999
		Sand/none	0.49	0.04	-0.180	0.04	<0.001
		Combination/other	0.67	0.03	Referent		
<i>Giardia</i> status	0.020	Fecal negative	0.64	0.02	0.025	0.01	0.020
		Fecal positive	0.62	0.02	Referent		
<i>Cryptosporidium</i> status	0.003	Fecal negative	0.64	0.02	0.030	0.01	0.003
		Fecal positive	0.61	0.02	Referent		
Pasteurization of milk	<0.001	Yes	0.69	0.03	0.095	0.03	0.002
		No	0.68	0.03	0.033	0.03	0.203
		Not applicable (fed MR)	0.52	0.03	Referent		
DFM in milk/MR	0.123	Yes	0.61	0.03	-0.162	0.03	<0.001
		No	0.65	0.02	Referent		
Interaction between DFM and milk pasteurization	<0.001	Pasteurized milk w/ DFM	0.67	0.04	0.137	0.05	0.007
		Pasteurized milk w/o DFM	0.70	0.03	0		
		Unpasteurized milk w/ DFM	0.72	0.04	0.242	0.05	<0.001
		Unpasteurized milk w/o DFM	0.64	0.02	0		
		MR w/ DFM	0.44	0.04	0		
Disease events	<0.001	MR w/o DFM	0.60	0.02	0		
		1 or more	0.59	0.02	-0.069	0.01	<0.001
		0	0.66	0.02	Referent		
Preweaning temperature- humidity index	<0.001	<50 (cold stress)	0.67	0.02	0.077	0.02	<0.001
		≥50 and <70 (thermoneutral)	0.62	0.02	0.031	0.02	0.120
		≥70 (heat stress)	0.59	0.03	Referent		
Protein per day (kg/d)	0.004		0.21	0.07	0.213	0.07	0.004

¹DFM = direct-fed microbial; MR = milk replacer; w/ = "with"; w/o = "without."

zone for a calf causes some degree of thermal stress (Roland et al., 2016), yet calves seem better able to handle cold stress than heat stress if provided enough nutrition. Maintenance requirements for calves outside of the thermoneutral zone increase (Drackley, 2008), although an increase in starter intake can help the calf meet maintenance requirements. Nonnecke et al. (2009) showed that calves raised in a cold environment had similar gains to calves raised in a warm environment, but the cold calves consumed more starter. When ADG is compared over different seasons, calves in the winter tended to gain the most and calves in the summer tended to gain the least, providing further support for calves adjusting better to cold than to heat stress (Place et al., 1998). This may also be due to increased liquid feeding in cold weather, although this is a more frequent practice now than 20 yr ago, when that study was conducted (Drackley, 2008). Heifers that experience heat stress have increased metabolic requirements, yet this is compounded by decreased appetite and decreased feed intake (West, 2003). The current study was unable to quantify starter intake, but calves raised during colder periods might have consumed more starter than calves raised in the thermoneutral zone or in hot periods, accounting for their increased ADG.

The bedding type used was associated with ADG in this study, with calves bedded with sand or no bedding gaining significantly less than all other bedding types used (including straw, shavings, or manure biosolids). It is interesting that sand bedding was not a good choice for preweaned calves but appears to be the best bedding for cows (Lombard et al., 2010; Andreassen and Forkman, 2012). When given a choice, calves preferred sawdust over concrete, even if the sawdust was wet (Camiloti et al., 2012). Another study showed that calves preferred sawdust over all other bedding types (Worth et al., 2015). One study showed that calves bedded with straw gained more than calves bedded with sand (Hill et al., 2011). Although Panivivat et al. (2004) found no differences in growth between different bedding types, calves housed on sand had more scour treatments and were scored as dirtiest compared with calves on all other bedding types. Calves with more lying bouts tended to have higher gains than calves with fewer lying bouts (Mogensen et al., 1997). By providing calves with a more comfortable environment with bedding substrates other than sand or no bedding, calves likely spend more time lying down, thus improving their growth.

Calves from single births gained more during the preweaning period than calves from twin births. Twin

Table 5. Results of multivariable modeling of interaction between pasteurization of milk and direct-fed microbials and association with ADG in Holstein heifer calves (n = 1,410)

Combination of practices ¹			Model-predicted ADG (kg/d)	SE
Pasteurized milk w/ DFM			0.673	0.04
Pasteurized milk w/o DFM			0.698	0.03
Unpasteurized milk w/ DFM			0.715	0.04
Unpasteurized milk w/o DFM			0.636	0.02
MR w/ DFM			0.441	0.04
MR w/o DFM			0.603	0.02
Pairwise comparisons ²			Model-predicted difference in ADG (kg/d)	
Pasteurized milk w/ DFM (B)	vs.	Pasteurized milk w/o DFM (A)	−0.025	0.04 0.525
Pasteurized milk w/ DFM (B)	vs.	Unpasteurized milk w/ DFM (C)	−0.043	0.05 0.373
Pasteurized milk w/ DFM (B)	vs.	Unpasteurized milk w/o DFM (D)	0.037	0.04 0.387
Pasteurized milk w/ DFM (B)	vs.	MR w/ DFM (F)	0.232	0.05 <0.001
Pasteurized milk w/ DFM (B)	vs.	MR w/o DFM (E)	0.070	0.04 0.108
Pasteurized milk w/o DFM (A)	vs.	Unpasteurized milk w/ DFM (C)	−0.018	0.04 0.677
Pasteurized milk w/o DFM (A)	vs.	Unpasteurized milk w/o DFM (D)	0.062	0.03 0.034
Pasteurized milk w/o DFM (A)	vs.	MR w/ DFM (F)	0.257	0.04 <0.001
Pasteurized milk w/o DFM (A)	vs.	MR w/o DFM (E)	0.095	0.03 0.002
Unpasteurized milk w/ DFM (C)	vs.	Unpasteurized milk w/o DFM (D)	0.080	0.04 0.055
Unpasteurized milk w/ DFM (C)	vs.	MR w/ DFM (F)	0.274	0.05 <0.001
Unpasteurized milk w/ DFM (C)	vs.	MR w/o DFM (E)	0.112	0.04 0.009
Unpasteurized milk w/o DFM (D)	vs.	MR w/ DFM (F)	0.195	0.04 <0.001
Unpasteurized milk w/o DFM (D)	vs.	MR w/o DFM (E)	0.033	0.03 0.203
MR w/ DFM (F)	vs.	MR w/o DFM (E)	−0.162	0.03 <0.001

¹DFM = direct-fed microbial; MR = milk replacer; w/ = “with”; w/o = “without.”

²Letters (A–F) correspond to combination of practices in Figure 2.

Figure 2. Graphical representation of the interaction between pasteurization of milk and direct-fed microbials for multivariable modeling of factors associated with ADG for Holstein heifer calves ($n = 1,410$). Letters correspond to combinations of practices summarized in Table 5; NA/MR = not applicable/milk replacer. Color version available online.

calves generally have significantly lower birth weights (Kertz et al., 1998; Hossein-Zadeh, 2010). Birth weight was included in the calculation for ADG, so it was not included in the model. The average birth weight for single calves was 43.3 kg (SE 0.14) and average birth weight for twin calves was 39.2 kg (SE 0.74). Research in beef cattle has shown that twin calves have lower birth weights and lower gains than single calves (Gregory et al., 1996). Another study showed that birth weight was a predictor for growth rates in calves (Lundborg et al., 2003). Although the calculation for ADG accounts for birth weight, it appears that twin calves are not able to maintain the same growth rate during the preweaning period as single calves.

Dam lactation was associated with ADG, with calves from first-calf heifers having significantly lower ADG than second-lactation or third- and higher-lactation dams. Because birth weight is positively associated with parity (Linden et al., 2009), larger cows tend to have larger calves (Swali and Wathes, 2006). As heifers are still growing during their first pregnancy, they typically have smaller calves at birth than second- or higher-lactation cows (Losinger and Heinrichs, 1997). Calves with lower birth weights tended to gain less throughout

the preweaning period and the first lactation (Swali and Wathes, 2006).

Calves that were fecal positive for *Cryptosporidium* and calves fecal positive for *Giardia* at the time of fecal sampling had significantly lower ADG than calves negative for *Cryptosporidium* or *Giardia*. Individual calf fecal samples were collected only once between 2 and 4 wk of age, so some of the calves that were not shedding *Cryptosporidium* or *Giardia* at the time of sampling might have been positive at some point during the preweaning period, likely decreasing the observed effect of *Cryptosporidium* and *Giardia* on ADG. *Cryptosporidium* infections occur early during the preweaning period (Santín et al., 2004), typically between 1 and 3 wk of age (average: 16 d of age; O'Handley et al., 1999). A longitudinal study showed that the prevalence of *Cryptosporidium* peaked at 2 wk of age (Santín et al., 2008). *Cryptosporidium* infections cause damage to the brush border of enterocytes, leading to decreased absorptive capacity and osmotic diarrhea (Constable, 2014). Decreased absorption and diarrhea lead to decreased nutrient absorption and decreased ADG. Infections with *Cryptosporidium* can also be accompanied by depression, weakness, and anorexia. *Giardia* infections

generally occur later in a calf's life (approximately 4 to 5 wk of age; Santín et al., 2009), and are associated with diarrhea (O'Handley et al., 1999). *Cryptosporidium* is considered endemic in dairy herds in the United States (Garber et al., 1994), making on-farm hygiene important in disease control.

Because this was a nationwide field study, there were some limitations in study design. More producers were expected to participate in the study than actually did, making this a convenience sample rather than a nationally representative sample of dairy heifer management across the US dairy industry. Data collected on farm included self-reporting on the Calf Card, with some operations potentially reporting standard operating procedures and not necessarily individual calf-level data. One important piece of information that is associated with ADG is starter intake. This study did not collect information on individual calf starter intake due to the intense resources that would be needed to capture this information on a routine and consistent basis.

These results highlight the importance of feeding an appropriate quantity and quality of a liquid diet, keeping calves free from disease and comfortable, and mitigating the effects of temperature and humidity during the preweaning period, on ADG.

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