Effects of chronic environmental cold on growth, health, and select metabolic and immunologic responses of preruminant calves¹

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ABSTRACT

The physiological response of the preruminant calf to sustained exposure to moderate cold has not been studied extensively. Effects of cold on growth performance and health of preruminant calves as well as functional measures of energy metabolism, fat-soluble vitamin, and immune responsiveness were evaluated in the present study. Calves, 3 to 10 d of age, were assigned randomly to cold ($n = 14$) or warm ($n = 15$) indoor environments. Temperatures in the cold environment averaged 4.7°C during the study. Frequent wetting of the environment and the calves was used to augment effects of the cold environment. Temperatures in the warm environment averaged 15.5°C during the study. There was no attempt to increase the humidity in the warm environment. Preventative medications or vaccinations that might influence disease resistance were not administered. Nonmedicated milk replacer (20% crude protein and 20% fat fed at 0.45 kg/d) and a nonmedicated starter grain fed ad libitum were fed to all calves. Relative humidity was, on average, almost 10% higher in the cold environment. Warm-environment calves were moderately healthier (i.e., lower respiratory scores) and required less antibiotics. Scour scores, days scouring, and electrolyte costs, however, were unaffected by environmental temperature. Growth rates were comparable in warm and cold environments, although cold-environment calves consumed more starter grain and had lower blood glucose and higher blood nonesterified fatty acid concentrations. The nonesterified fatty acid and glucose values for cold-stressed calves, however, did not differ sufficiently from normal values to categorize

these calves as being in a state of negative-energy balance. Levels of fat-soluble vitamin, antibody, tumor necrosis factor- α , and haptoglobin were unaffected by sustained exposure to moderate cold. These results support the contention that successful adaptation of the dairy calf to cold is dependent upon the availability of adequate nutrition.

Key words: preruminant calf, cold stress, neonatal immunity, calf physiology

INTRODUCTION

Infectious diseases of calves, especially diarrheal and respiratory diseases, are associated with significant economic losses to the US dairy cattle industry. The mortality rate for preweaned dairy calves is approximately 8 to 11% and the morbidity rate is approximately 37% (National Animal Health Monitoring Service, 2002). Infectious diseases of the calf also affect human health and food safety through preharvest contamination and zoonotic transmission. Agents causing these diseases can be transmitted to humans through direct contact or by contamination of food and water supplies.

There is a dearth of information describing the functional competency of the immune system of the neonatal calf and how management-related factors (i.e., nutrition, environmental conditions) influence the immune responsiveness and, ultimately, infectious disease resistance. In the northern United States, calves born in the late winter and early spring often experience sustained periods of cold during the first weeks of life. A recent study by Godden et al. (2005) documents the negative effects of winter calving on dairy calf health. Of the 438 calves evaluated, the morbidity rate of calves born in the winter was 52% compared with 13% for calves born in the summer. Similarly, calf mortality was 21% in the winter and 3% in the summer. Several studies suggest that reduced temperature alone is not the sole contributor to increased morbidity and mortality during winter calving. In California, calf mortality was shown to be highest in mid-winter and mid-summer, although winter losses are 1.26 times those in the summer. Losses

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in winter were associated with cold, wet, and windy weather, whereas losses in the summer were associated with hot, dry weather (Martin et al., 1975). A Danish study (Blom et al., 1984) demonstrated an increased incidence of pneumonia in calves given a milk substitute, hay, and concentrates, when ambient temperature was less than 10°C and relative humidity exceeded 85%, suggesting that low temperature in conjunction with elevated humidity decreases calf health.

Effects of environmental cold on specific aspects of the immune and stress responses have been examined in other species. Relatively brief exposure (i.e., 5 d) of pigs to cold increases baseline concentrations of the stress-related hormones ACTH and cortisol and elevates proinflammatory cytokines [i.e., IL-1 β , IL-6, and tumor necrosis factor (TNF)- α] in the liver and spleen (Frank et al., 2003). A recent study demonstrated that cold stress, regardless of duration, influences cytokine gene expression (Hangalapura et al., 2005). In particular, expression of IL-1 β , IL-6, IL-12 β , and IL-4 mRNA in peripheral blood leukocytes was enhanced, suggesting that cold stress stimulates both the innate and adaptive arms of the chicken's immune system. In cold-stressed rats chronically infected with *Toxoplasma gondii*, responses of splenic lymphocytes to antigens derived from this pathogen were markedly suppressed (Aviles et al., 2004). Reduced functional capacity of this leukocyte population could lead to reactivation of the latent infection. In mice, bacterial clearance from the lungs following challenge is inhibited by cold stress in conjunction with wet fur (Green and Kass, 1964).

Nutrition is a determinant of immune function, with protein-energy balance influencing cell-mediated immunity, cytokine production, complement system, phagocytic function, and antibody concentrations (Woodward, 1998). Ensuring nutritional sufficiency during periods of cold stress may be difficult in the preruminant calf because maintenance requirements for thermoregulation are increased (Drackley, 2005). In a thermoneutral environment, the calf is not required to elicit specific heat-conserving or heat-dissipating mechanisms to maintain core body temperature (NRC, 2001). The thermoneutral zone of the young calf varies with age, weight, environmental temperature, and other stressors (Schrama et al., 1992, 1993; Scibilia et al., 1987) and ranges from 15 to 25°C. When the lower critical temperature (LCT; the effective ambient temperature dependent on wind velocity, humidity, and tissue insulation) is reached, a calf must produce more heat (i.e., expend energy) to maintain body temperature. When temperatures fall below the LCT, the energy needed to maintain core body temperature is supplied either by increased energy intake or from increased metabolism of tissue reserves. In the northern states during the

winter and early spring, many calves experience degrees of cold-stress necessitating increased dietary energy to limit the negative effects of cold on growth performance and health. Although the beneficial effects of increased nutrition in the form of intensified milk replacers on the immune response capacity and health of calves reared in a thermoneutral environment have not been demonstrated conclusively (Nonnecke et al., 2003; Foote et al., 2007b), intensified feeding programs may support optimal growth and promote infectious disease resistance during periods of sustained cold stress.

The objective of the present study was to evaluate the effects of sustained exposure to moderate cold on the growth performance, health, metabolism, and immune system of milk replacer (MR)-fed dairy calves. In an attempt to mimic typical on-farm calf nutrition programs, calves in warm and cold environments were provided fixed and equal amounts of a 20% CP, 20% fat MR with starter grain provided ad libitum.

MATERIALS AND METHODS

Calves and Environmental Treatments

Animal-related procedures were implemented following institutional guidelines for animal care and use. Twenty-nine Holstein bull calves 3 to 10 d of age and weighing between 40.8 and 45.4 kg at the beginning of the study were used. All calves had serum IgG concentrations ≥ 10 g/L as determined by ELISA (Nonnecke et al., 2003), indicative of successful acquisition of passive immunity. No preventative medications or vaccinations were administered that might influence disease resistance. Calves were examined for navel infection before the trial and navels were dipped in a 3.5% iodine solution. The evening before the trial, all calves received electrolytes orally (0.077 kg in 1.8 kg of water). During the 49-d study, all calves were fed non-medicated MR (0.45 kg/d, 20% CP and 20% fat; Table 1) and a nonmedicated calf starter (ad libitum, 18% CP, nonmedicated, Land O'Lakes-Purina Feed, Shoreview, MN) with lasalocid acid. From d 0 to 42 calves were fed MR twice daily and once daily thereafter. Calves were weaned at d 49.

Calves were assigned randomly to 2 environmental treatments at the beginning of the study and remained in their respective environments until d 49 of the study. They were housed individually on elevated stalls in 2 indoor, ventilated rooms from January to March. Calves reared in the cold (unheated) environment ($n = 14$) were exposed to temperatures maintained as close to 1.7°C (35°F) as possible. Wetting of the environment and calves twice daily was used to increase humidity and augment the effects of the cold. Wetting resulted

Table 1. Composition of milk replacer¹ fed during the experimental period

Component	DM analysis
CP, %	≥20
Crude fat, %	≥20
Crude fiber, %	≤0.15
Moisture, %	≤5.0
Calcium, %	0.75 to 1.25
Phosphorus, %	≥0.70
Ash, %	≤13.2
Added minerals, %	≤0.05
Vitamin A (retinyl acetate), IU/kg	≥9,072
Vitamin D ₃ (cholecalciferol), IU/kg	≥2,268
Vitamin E, IU/lb.	≥45.4

¹Nonmedicated; manufactured by Land O'Lakes Milk Products Co. (Black River Falls, MN).

in the hair coat being saturated with water. Calves in the warm environment ($n = 15$) were exposed to temperatures maintained as close to 15.6°C (60°F) as possible. Humidity of the warm environment was not manipulated. High and low temperature and humidity readings in both rooms were recorded manually each day.

Calves were weighed at weekly intervals beginning on d 0 of the trial. The quantities of MR consumed and refused were recorded daily and these values were converted to the amount of dry matter consumed. Similarly, the amount of starter consumed and weigh backs were recorded daily. Calf health was observed at each morning feeding. Body temperatures, scour and respiratory scores, and the type and amount of antibiotics administered were recorded for clinically ill calves. The scoring system has been described previously (Nonnecke et al., 2003). Calves with scour scores >2 were given electrolytes orally and were given lactated Ringer's solution parenterally when obviously dehydrated. The amount and frequency of electrolyte administrations were recorded.

All calves were sensitized to ovalbumin (**OVA**) to evaluate the effects of cold on the adaptive immune response. Calves were vaccinated on d 0 and 35. The vaccine consisted of crystallized OVA (Grade V, Sigma, St. Louis, MO) dissolved in sterile PBS (2 mg/mL), diluted 1:1 (vol/vol) in incomplete Freund's adjuvant (MP Biomedicals, Inc., Aurora, OH) and emulsified by sonification (model 250 Sonifier, Branson, Danbury, CT). The vaccine (4 mL) was administered subcutaneously in the left mid-cervical region.

Serum was collected by jugular venipuncture from each calf at the beginning of the study (prevaccination, d 0) and at d 35, 42, and 49. Coagulated (no additive) blood samples were collected into 10-mL vacutainers (Becton Dickinson, Franklin Lakes, NJ). Harvested serum was frozen (−20°C) until analysis.

Quantification of Glucose, NEFA, and Fat-Soluble Vitamins

Glucose and NEFA concentrations were determined in serum samples collected on d 0, 35, 42, and d 49 of the experimental period. Samples were collected in the morning before feeding. Serum NEFA concentrations were determined colorimetrically as described previously (Johnson and Peters, 1993), and glucose concentrations were determined colorimetrically using a commercially available kit (BioAssay Systems, Hayward, CA). In both assays, samples from calves in each treatment group were processed simultaneously so that interassay effects would be balanced across treatments. Intraassay coefficient of variation in both assays was <3%.

Retinol and *RRR*- α -tocopherol concentrations in d 0, 35, 42, and 49 serum samples were analyzed by reverse-phase HPLC as described previously (Ametaj et al., 2000) using a modification of the method of Kaplan et al. (1987). Serum 25-hydroxyvitamin D₃ [**25(OH)D₃**] was quantified by RIA using the methods of Hollis et al. (1993).

Quantification of Antigen-Specific IgG, TNF- α , and Haptoglobin

Antigen (i.e., OVA)-specific IgG₁ and IgG₂ concentrations in serum samples from d 0, 35, 42, and 49 were determined by capture ELISA as described by Foote et al. (2007a). Optical densities (450 nm) of individual microtiter-plate wells were measured using an ELISA plate reader (Dynatech MR7000, Dynatech Laboratories Inc., Guernsey, Channel Islands, UK). The change in optical density (**OD**) was calculated by subtracting the OD of wells containing PBS only from the OD of wells containing test samples.

Tumor necrosis factor- α in d 0, 35, 42, and 49 samples was measured by capture ELISA as described by Nonnecke et al. (2005). The OD of standards and test samples at 405 and 490 nm were measured using an ELISA reader (Dynatech Laboratories Inc.). Cytokine concentrations in test sera, evaluated in duplicate, were determined by comparing the absorbance of test samples with the absorbance of standards (serially diluted recombinant bovine TNF- α) within a linear curve fit. Results were expressed as mean TNF- α concentrations (ng/mL) in sera samples.

Haptoglobin concentrations in sera harvested on d 0, 35, 42, and 49 were determined using a bovine haptoglobin ELISA (Immunology Consultants Laboratory Inc., Newberg, OR) following the manufacturer's instructions. Haptoglobin standards provided in the kit ranged from 62.5 to 1,000 ng/mL. The OD (450 nm)

Figure 1. Mean daily temperature (a) and relative humidity (b) in warm (■) and cold (◇) environments housing preruminant calves. Means were derived from high and low temperature and humidity readings recorded during each 24-h period from d 1 to d 49 of the experimental period.

of test samples and standards was measured using an ELISA plate reader (Dynatech Laboratories Inc.). Haptoglobin concentrations in test sera, evaluated in duplicate, were determined by comparing the absorbance of test samples with that of serially diluted standards within a linear curve fit. These data were corrected for

dilution factor to arrive at haptoglobin concentration ($\mu\text{g/mL}$) in the original sample.

Statistical Analysis

Data were analyzed as a completely randomized design (JMP, version 5.0, SAS Institute Inc., Cary, NC). Calf served as the experimental unit in the analysis of all data. Body weight; environmental temperature and humidity; serum metabolites (i.e., fat-soluble vitamins); and serum antibody, TNF- α , and haptoglobin concentrations were analyzed as a split-plot with repeated-measures ANOVA. The model included fixed effects of environmental treatments, time (week of experiment), and the treatment $\times$ time interaction. Calf was included in the model as the random effect. Fisher's protected LSD test was applied when effects ($P \leq 0.05$) were detected.

RESULTS AND DISCUSSION

Environmental Conditions

Daily room temperatures (representing mean of low and high temperatures) in warm and cold environments are shown in Figure 1a. Cold-environment temperatures averaged 4.7°C during the study period and ranged from a low of 1.2°C (d 9) to a high of 10.5°C (d 40). Warm-environment temperatures averaged 15.5°C , higher ($P < 0.0001$) than the cold-environment temperature (4.7°C), and ranged from a low of 13.6°C (d 17) to a high of 16.9°C (d 5). The stability of temperatures in the warm environment was because of thermostatically controlled heating. Because the cold environment was not heated, the more pronounced fluctuations of temperatures in the cold environment were attributable to changes in weather from January through March.

Daily room humidities in warm and cold environments are shown in Figure 1b. Relative humidity in the cold environment during the study period averaged 68.2% and ranged from a low of 52% (d 11) to a high of 84% (d 29). Relative humidity in the warm environment averaged 59% and ranged from 42% (d 11) to 73% (d 27). Average humidities in warm and cold environments during the study period were different ($P < 0.001$). This difference was not large; however, regular wetting of the fur of cold environment calves likely increased the physiological effect of the cold. The use of elevated stalls prevented the calves from nesting, which would have reduced the LCT of the calves and their caloric needs (Lago et al., 2006). Although this is a subjective observation, the coats of calves in the cold environment thickened markedly during the first week of the study suggesting a rapid adaptation to the colder

Table 2. Health of neonatal calves reared in warm and cold environments

Health parameter/treatment	Period						
	Wk 1	Wk 2	Wk 3	Wk 4	Wk 5	Wk 6	Wk 7
Mean respiratory score ¹							
Warm environment	1.69	3.00	0.75	0.56	1.50	0.69	0.19
Cold environment	0.86	2.43	2.57	1.71	1.86	2.00	0.00
Treatment effect, <i>P</i> -value	NS	NS	0.05	NS	NS	0.05	NS
Mean electrolyte and antibiotic costs, ¹ \$							
Warm environment	5.07	5.09	1.62	1.17	2.17	0.83	0.83
Cold environment	3.85	4.42	3.12	2.04	2.89	2.84	0.00
Treatment effect, <i>P</i> -value	NS	NS	NS	0.05	NS	0.05	NS

¹Values represent mean weekly (i.e., period) respiratory scores and combined electrolyte/antibiotic costs for calves housed in warm and cold environments. Respiratory scores = 1 respiratory day for each day antibiotic given for respiratory infection.

NS: $P > 0.05$.

environment. Coat thickening provides increased thermal insulation and effectively reduces the LCT and the required cold-induced thermogenesis during exposure to temperatures below the LCT (Young, 1988).

Lighting in warm and cold environments was comparable in terms of intensity and duration, excluding potential effects of photoperiod on metabolic and immunologic responses of the calves. Effects of photoperiod on growth and immune function in cattle have been reviewed recently (Collier et al., 2006).

Calf Health and Growth Performance

Scour scores, days scouring, and the amount of electrolytes administered were comparable for calves in warm and cold environments (data not shown). Average weekly respiratory scores and antibiotic costs, however, were affected by environmental temperature (Table 2). Respiratory scores of cold-environment calves were higher ($P < 0.05$) during wk 3 and wk 6. Antibiotic costs for cold-environment calves were higher during wk 4 ($P < 0.05$) and wk 6. By wk 7, treatment differences with regard to respiratory scores and costs associated with electrolyte and antibiotic administrations were not different. Blom et al. (1984) observed an increased incidence of pneumonia in calves given a milk substitute, hay, and concentrates when the ambient temperature was below 10°C and relative humidity exceeded 85%, suggesting that low temperature in conjunction with elevated humidity affects calf respiratory health. Anecdotal evidence from producers also supports a direct effect of cold stress on the incidence and severity of respiratory infections in preruminant calves. Wetting the fur of experimentally infected, cold-stressed mice inhibited clearance of bacteria from the lungs (Green and Kass, 1964).

Effects of environmental temperature on growth performance and starter consumption are shown in Figure

2. Body weights of calves assigned to cold and warm environments were not different at the beginning of the study (d 0) and averaged 45.6 and 45.4 kg, respectively (Figure 2a). Although treatment effect ($P < 0.71$) and treatment $\times$ time interaction ($P < 0.28$) were not significant for BW, the time effect was significant ($P < 0.0001$). For all calves, BW did not change ($P > 0.05$) during the first 14 d of the study; however, from d 14 to 49 it increased ($P < 0.05$) progressively (0.68 kg/d) achieving a maximum of 69.5 kg on d 49. Although calf BW was unaffected by environmental temperature, cold-environment calves consumed more starter than warm-environment calves during wk 5, 6, and 7 of the study (Figure 2b). Taken together, these data suggest that the extra energy associated with increased intake of starter was necessary for cold-environment calves to maintain a growth rate comparable to that of the warm-environment calves.

A summary of the effects of environment on the energy requirements of young calves (NRC, 2001) indicates that the maintenance energy requirement from birth to 4 wk of age ranges from 1,735 to 1,969 kcal of ME/d for calves at 15°C compared with 1,969 to 2,437 kcal of ME/d for calves at 5°C (calculated for a calf weighing 45.35 kg). Because temperatures in the cold environment averaged 5°C during the study, substantially below the LCT (Young, 1988), the cold-environment calves likely required increased metabolic heat production to compensate for the increased thermal demand imposed by the cold environment. The energy requirement of cold-environment calves may have been even higher because of the regular wetting of their coats. The extra starter consumed by cold-environment calves was necessary to meet the increased ME requirement at 5°C. For cold-environment calves fed only MR, an intensified MR with a higher fat percentage might provide the additional energy needed to meet the ME requirement when temperatures decreased below the LCT.

Figure 2. Growth performance (a) and starter intake (b) of pre-ruminant calves housed in warm and cold environments. Growth performance is represented by mean ($\pm$ SEM) BW (kg) recorded at the beginning of the study and weekly thereafter for calves in warm ($n = 15$, ■) and cold ($n = 14$, ◇) environments. Starter intake is represented by the mean ($\pm$ SEM) weight of starter consumed each wk (kg/wk) of the study by calves in warm ($n = 15$, black bars) and cold ($n = 14$, white bars) environments. Treatment effect, time effect, and treatment $\times$ time interaction for BW data were $P = 0.71$, $P < 0.0001$, and $P = 0.28$, respectively. For starter intake data, treatment effect, time effect, and treatment $\times$ time interaction for starter intake were $P = 0.033$, $P < 0.0001$, and $P < 0.0001$, respectively.

All calves in the present study were provided warm water 2 to 3 times a day. Observations by Kertz et al. (1984) suggest that availability of free water stimulates starter intake, helping to counteract cold stress.

Concentrations of Glucose, NEFA, and Fat-Soluble Vitamins

Glucose and NEFA concentrations in the circulation of calves housed in warm and cold environments are shown in Figure 3. It is generally accepted that blood glucose concentrations in young calves exceed adult values during the first weeks after birth but decrease to adult values (45–55 mg/dL) by 3 mo of age. Blood glucose concentrations in calves raised in warm (75.3 mg/dL) and cold (76.7 mg/dL) environments were comparable at the beginning of the study (Figure 3a) and were higher than adult values. Glucose concentrations in cold-environment calves tended to be lower ($P = 0.06$) than those in warm-environment calves (85.1 vs. 93.5 mg/dL) during the study period. The treatment $\times$ time interaction was not significant ($P > 0.05$); however, glucose concentrations in warm-environment calves tended to be higher ($P = 0.07$) than those in cold-environment calves (103 vs. 89 mg/dL; $P = 0.08$) on d 42. Blood glucose levels in all calves increased with time; however, this effect was more pronounced in warm-environment calves ($P < 0.01$) than in cold-environment calves ($P = 0.07$).

Blood NEFA concentrations in both groups were not different ($P = 0.99$) at the beginning of the study (d 0; Figure 3b). All calves experienced a marked decrease in NEFA concentrations from d 0 through d 49. Cold-environment calves had higher blood NEFA concentrations than warm-environment calves at d 35 ($P < 0.001$) and d 42 ($P < 0.01$). By d 49, NEFA concentrations in both groups of calves were not different ($P = 0.56$). Because both groups showed a significant increase in blood glucose levels and parallel decreases in NEFA concentrations, it is likely that the cold-environment calves were not in a state of negative energy balance on d 35 or d 42. The NEFA values for cold-environment calves at d 35 to 49 were below threshold concentrations considered indicative of prepartum negative energy balance in dairy cows (Oetzel, 2004).

The fat-soluble vitamins A, E, and D are essential micronutrients in the diet of dairy cattle and necessary for optimal growth and immune function in the newborn calf. Plasma retinol, *RRR*- α -tocopherol, and 25-(OH) D_3 concentrations in preruminant calves housed in warm and cold environments are shown in Figure 4, panels a, b, and c. Treatment effects and treatment $\times$ time interactions were not significant ($P > 0.05$) for these vitamins, indicating that sustained

exposure to cold did not influence fat-soluble vitamin status. When considering all calves, mean retinol concentrations increased ($P < 0.0001$) from a minimum of 109.7 ng/mL on d 0 to a maximum of 205.7 ng/mL on d 49. Vitamin D concentration increased modestly from 30.1 ng/mL on d 0 to 36.2 ng/mL on d 35 ($P < 0.01$), 38.0 ng/mL on d 42 ($P < 0.001$), and 37.0 ng/mL on d 49 ($P < 0.01$). The increases in plasma retinol and 25-(OH)D₃ concentrations are typical of those observed in calves fed MR containing a minimum of 20,000 IU of vitamin A (Nonnecke et al., 1999) and 5,000 IU of vitamin D (B. Nonnecke; unpublished data) per pound of dry matter. Plasma tocopherol concentrations in all calves decreased ($P < 0.001$) from a maximum of 525 ng/mL on d 0 to a minimum of 310 ng/mL on d 49. Reasons for this unexpected decline are not known. Calves supplemented with vitamin E at levels comparable to the level provided in the present study typically undergo a pronounced age-related increase in plasma vitamin E concentrations (Nonnecke et al., 1999; Ametaj et al., 2000). Because of the known role of vitamin E in ensuring optimal function of the immune system, it is conceivable that both groups of calves may have experienced more health-related issues than calves with normal plasma vitamin E concentrations.

Concentrations of Ovalbumin-Specific IgG, TNF- α , and Haptoglobin

Stress is known to influence both adaptive and innate immune responses in both young and adult animals. Because calves are vaccinated at a very early age, it is important to determine the effects of environmental stress on vaccine efficacy in the preruminant calf. Antibody (i.e., OVA-specific IgG₁) responses of warm and cold environment calves to vaccination with OVA on d 0 and d 35 are shown in Figure 5a. Sensitization with OVA, an antigen not seen in the natural environment of the dairy cow, precluded the possibility that colostral antibody would negatively affect B-cell responses of calves to vaccination. Ovalbumin has been used successfully to evaluate B-cell function in the neonatal calf (Foote et al., 2007a). Treatment effect and treatment $\times$ time interaction were not significant for this variable, indicating that moderate cold exposure did not affect the adaptive (i.e., antigen-specific) immune response. All calves responded to primary vaccination, resulting in increased ($P < 0.0001$) antibody levels by d 35. Responses at d 42 and d 49 exceeded ($P < 0.001$) those at d 35, suggesting that the second (i.e., booster) vaccination on d 35 induced an anamnestic or secondary response. Ovalbumin-specific IgG₂ responses, although lower in magnitude, followed a similar pattern and were not affected by treatments (data not shown).

Figure 3. Glucose (mg/dL) and NEFA (mmol/L) concentrations (mean $\pm$ SEM) in peripheral blood from preruminant calves housed in warm ($n = 15$, ■) and cold ($n = 14$, ◇) environments. Analyses were performed on samples collected on d 0, 35, 42, and 49 of the study. Treatment effect, time effect, and treatment $\times$ time effect for the glucose data were $P = 0.02$, $P < 0.001$, and $P = 0.31$, respectively. For the NEFA data, treatment effect, time effect, and treatment $\times$ time interaction were $P = 0.05$, $P < 0.0001$, and $P = 0.08$, respectively.

Figure 4. Vitamin A (retinol, ng/mL), vitamin E (*RRR*- α -tocopherol, ng/mL), and vitamin D (25-OH-vitamin D₃, ng/mL) concentrations (mean $\pm$ SEM) in peripheral blood from calves housed in warm ($n = 15$, ■) and cold ($n = 14$, ◇) environments. Analyses were performed on samples collected at d 0, 35, 42, and 49 of the study. Treatment effects and treatment $\times$ time interactions were not significant for retinol, vitamin E, or vitamin D. However, time effects were significant for each variable ($P < 0.01$ for all variables).

Figure 5. Ovalbumin (OVA)-specific IgG₁ [optical density (OD) at 450 nm, panel a] and tumor necrosis factor (TNF)- α (ng/mL, panel b) in peripheral blood from calves housed in warm ($n = 13$, ■) and cold ($n = 18$, ◇) environments. All calves were vaccinated subcutaneously in the left mid-cervical region with OVA in incomplete Freund's adjuvant on d 0 and 35. Analyses were performed on samples collected at d 0, 35, 42, and 49 of the study. Analysis of OVA-specific IgG₁ data indicated that the treatment effect, time effect, and treatment $\times$ time interaction were $P = 0.35$, $P < 0.0001$, and $P = 0.42$, respectively. For the TNF- α data, treatment effect, time effect, and treatment $\times$ time interaction were $P = 0.64$, $P < 0.0001$, and $P < 0.10$, respectively.

Table 3. Serum haptoglobin concentrations ($\mu\text{g/mL}$; mean $\pm$ SEM) in blood samples collected on d 0, d 35, d 42, and d 49 of the study

Treatment group	Day of study			
	d 0	d 35	d 42	d 49
Warm environment (n = 15)	2.36 $\pm$ 1.48	0.24 $\pm$ 0.22	2.37 $\pm$ 1.72	4.19 $\pm$ 3.98
Cold environment (n = 14)	0.45 $\pm$ 0.345	8.78 $\pm$ 6.05	5.51 $\pm$ 3.77	2.30 $\pm$ 2.22

Tumor necrosis factor- α , produced by monocytes and macrophages, is a key mediator of the inflammatory response and it promotes T- and B-lymphocyte proliferation. Blood TNF- α concentration is frequently elevated during acute and chronic inflammation associated with the body's response to infection and is considered a marker for immune activation. Cytokine concentrations in sera from warm- and cold-environment calves are shown in Figure 5b. Although TNF- α concentrations in warm-environment calves did not change during the study period, levels in cold-environment calves were lower ($P < 0.0001$) on d 35, 42, and 49 than at the beginning of the study. Blood TNF- α levels in warm- and cold-environment calves, however, were not different ($P > 0.05$) at any sampling time during the 7-wk trial, suggesting that the level of immune activation in both groups of calves was comparable. The significant time effect, characterized by lower ($P < 0.001$) TNF- α levels in all calves on wk 5, 6, and 7 compared with wk 0, may reflect to some degree immunological adaptation of the rapidly developing calf to pathogen exposure during the first months of life.

Haptoglobin is one of several acute phase proteins produced in response to inflammatory changes associated with infection, making it a potentially useful indicator of calf health. Previous research indicates that serum haptoglobin levels range from very low to undetectable in healthy calves to >1 mg/mL during *Mannheimia hemolytica*-induced respiratory disease (Godson et al., 1996). The study demonstrated that haptoglobin concentrations are highly correlated with sick score (subjective clinical examination), body temperature, weight change, and plasma zinc concentration. Experimental infection of calves with bovine respiratory syncytial virus also results in marked induction of haptoglobin, IL-6, and IFN- γ mRNA (Grell et al., 2005). Serum haptoglobin concentrations in warm- and cold-environment calves are presented in Table 3. Treatment ($P = 0.47$) and time ($P = 0.52$) effects as well as a treatment $\times$ time interaction ($P = 0.16$) were not significant for this variable, suggesting that the level of immune activation in warm- and cold-environment calves was comparable. There was a trend ($P = 0.08$) toward increased haptoglobin concentrations in cold-environment calves between d 0 and 35. In contrast, changes in haptoglobin

concentrations in warm-environment calves during the same period were negligible ($P = 0.45$). Haptoglobin concentrations (<10 $\mu\text{g/mL}$) in the serum of calves in the present study were substantially lower than those (>3 mg/mL) observed 4 d after experimental infection with *Mannheimia hemolytica*, suggesting that both warm- and cold-environment calves in this study were relatively healthy.

In conclusion, the results from the present study indicate that preruminant calves provided adequate nutrition exhibit a remarkable degree of adaptability to sustained, moderate cold. When compared with warm-environment calves, cold-environment calves had similar growth rates likely maintained by their increased intake of starter grain. Immune response variables also were unaffected by cold. With the exception of a moderate increase in respiratory scores, the health of cold-stressed calves was comparable to that of warm-environment calves. These results suggest that successful adaptation of the dairy calf to sustained cold may be linked to the availability of adequate nutrition. Because this study considered the effects of moderate cold stress, additional research is needed to evaluate effects of sustained, severe cold on limit-fed calves (i.e., calves fed no more than warm-environment calves).

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