Effect of nutritional plane on health and performance in dairy calves after experimental infection with *Cryptosporidium parvum*

Theresa L. Ollivett, DVM, DACVIM; Daryl V. Nydam, DVM, PhD;
Thomas C. Linden, BS; Dwight D. Bowman, PhD; Michael E. Van Amburgh, PhD

Objective—To evaluate the effect of nutritional plane on health and performance of dairy calves after infection with *Cryptosporidium parvum*.

Design—Randomized, controlled trial.

Animals—20 Holstein bull calves.

Procedures—Calves were assigned to a higher plane of nutrition (HPN; 0.30 Mcal intake energy/kg of metabolic body weight using a 28% protein–20% fat milk replacer) or conventional nutrition (CN; 0.13 Mcal intake energy/kg of metabolic body weight using a 20% protein–20% fat milk replacer). Calves were inoculated with *C parvum* oocysts at 3 days old. Fecal and health scores, oocyst counts, weight gain, dry matter intake, and hematologic variables were measured for 21 days. Data were analyzed with nonparametric and regression methods.

Results—Body weight (day 1), serum total protein concentration (day 3), and PCV (day 3) were not different between groups. Oocyst shedding was not different between groups. The PCV was higher in the CN group (40%), compared with the HPN group (32%) at the end of the study. Fecal scores (FS) improved faster in the HPN group (median, –0.1 FS/feeding), compared with the CN group (median, –0.06 FS/feeding). The HPN calves had better average daily gain (ADG) than did CN calves (median, 433 g/d vs –48 g/d, respectively). Feed efficiency (ADG:dry matter intake ratio) was better for HPN calves than CN calves (median, 131.9 g/kg vs –31.4 g/kg).

Conclusions and Clinical Relevance—After a pathogen challenge, calves maintained hydration, had faster resolution of diarrhea, grew faster, and converted feed with greater efficiency when fed a higher plane of nutrition. (*J Am Vet Med Assoc* 2012;241:1514–1520)

Feeding the appropriate quantity of liquid feed is crucial to the health and performance of neonatal dairy calves. However, the dairy industry has knowingly undersupplied the energy requirements for these animals.[1] Conventional milk replacer feeding programs involve providing approximately 2 L of reconstituted milk replacer (typically, 20% protein and 20% fat) twice daily. This provides minimal energy (approx 2.2 Mcal/d) above maintenance energy requirements (1.75 Mcal/d) for maintaining body temperature, mounting immune responses, and growing at desired rates of 0.5 to 0.7 kg/d (1.1 to 1.5 lb/d) in the face of environmental and pathogenic challenges. The rationale for this conventional feeding approach is multifactorial and includes industry recommendations to feed 8% to 10% of body weight to encourage early starter intake and enhance rumen maturation, which will in turn reduce expenditures on liquid feed.[1,2]

Contemporary nutrition programs involve feeding more energy- and protein-rich liquid feeds. These programs have been shown to increase body weight gain, hip height, and first-lactation production.[3–5] Reduced starter intake and increased preweaning feed costs have discouraged some producers from changing their conventional feeding programs.[6,7]

To date, minimal research investigating the effect of nutritional plane on the health of neonatal dairy calves,

Abbreviations

ADG	Average daily gain
CN	Conventional nutrition
FE	Feed efficiency
FS	Fecal score
HPN	Higher plane of nutrition
HS	Health score
MBW	Metabolic body weight
NEFA	Nonesterified fatty acids
TP	Total protein

From the Departments of Clinical Sciences (Ollivett), Population Medicine and Diagnostic Sciences (Nydam, Linden), and Microbiology and Immunology (Bowman), College of Veterinary Medicine, and the Department of Animal Science (Van Amburgh), College of Agriculture and Life Sciences, Cornell University, Ithaca, NY 14850. Dr. Ollivett's present address is Department of Population Medicine, Ontario Veterinary College, University of Guelph, Guelph, ON N1G 2W1, Canada.

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Address correspondence to Dr. Nydam (dvn2@cornell.edu).

specifically during a diseased state, is available. One report[8] documented the need for more veterinary intervention and a tendency toward higher mortality rates in calves fed a higher rate of milk replacer after a coronavirus challenge. *Cryptosporidium parvum* has been an effective model of infectious disease in neonatal calves, considering its reliability in establishing infection.[9] Supporting the immune system through nutrition is desirable in the management of dairy calves in light of the endemic nature of *C parvum* on dairy farms,[10,11] the risk of death from severe diarrhea due to *C parvum* infection, and the lack of available veterinary products for treatment. The objective of the study reported here was to evaluate the effect of nutrient intake on the health and performance of dairy calves after experimental infection with *C parvum*.

Materials and Methods

Challenge model, treatment solutions, and administration—The study was performed at the College of Veterinary Medicine, Cornell University, Ithaca, NY. At least 1 study author attended all calvings from June 2008 through August 2008, whereby 29 bull calves were obtained from a local dairy farm and enrolled in the study from birth. The perineum of the dam was thoroughly cleaned with povidone-iodine scrub, and calves were caught on single-use plastic to prevent on-farm manure contamination. The calves were transported to a designated vehicle designed to hold 3 calves in individual pens, be thoroughly washable, and be safe for both the animals and the operator of the vehicle. Immediately after birth, a physical examination was performed and an identification tag was placed in the ear. Within 1 hour after birth and prior to transport to the isolation facility, 4 L of warm, heat-treated colostrum[12,13,a] was fed via an esophageal tube feeder.[14] Upon arrival from the source farm, contact between calves was prevented by use of individual housing in permanent 0.9 × 1.8-m stalls with high tile walls and concrete flooring within a closed barn with an active ventilation system. Stalls were bedded daily with pine shavings and cleaned with pressurized water between calves. Gross manure contamination on the stall walls was removed daily. Pullover boots, feed buckets, milk bottles, and thermometers were labeled and not shared among calves. Calves were cared for in compliance with the Institutional Animal Care and Use Committee of Cornell University.

Calves were enrolled in the study for 21 days and fed every 12 hours. Feeding 1 was colostrum; feedings 2 through 42 were nonmedicated milk replacer, beginning 12 hours after administration of colostrum. Calves were randomized by use of a random number generator to either the HPN group or the CN group from birth. Calves in the HPN group were fed a commercial milk replacer[b] with 28% crude protein content and 20% crude fat content at 0.23 Mcal intake energy/kg of MBW (MBW = body weight in $kg^{0.75}$) as a function of birth weight for the first 7 days after birth (13 feedings after colostrum) and then 0.30 Mcal/kg of MBW as a function of birth weight for the following 14 days (28 feedings; **Table 1**). Calves in the CN group were fed a commercial milk replacer[c] with 20% crude protein and 20% crude fat content at 0.13 Mcal intake energy/kg of MBW as a function of birth weight for the duration of the study. The ash, fiber, vitamin, and mineral contents are available on the milk replacer labels. Calves in both groups were offered the calculated amount of milk replacer dry matter and water for 30 minutes. For feedings 2 to 6, the milk replacer was fed via an esophageal tube feeder, only if an entire feeding was not consumed because of a weak suckle response in an otherwise bright, apparently healthy calf (HS < 3). Any meals that were not finished by a sick (HS > 2) calf for feedings 2 to 6, or any calf for feedings 7 to 42, were weighed, recorded, and discarded. Calf starter was not provided. Free choice water was available at all times; water intake was not measured.

All calves meeting the inclusion criteria of passive transfer of maternal antibody[15] at day 3 as indicated by serum TP concentration of 5.0 mg/dL or greater were inoculated 1 hour after that feeding (**Figure 1**). Each calf was inoculated with a > 90% viable (as determined with a dye permeability assay)[16] field strain of *C parvum* at a dose of 1×10^6 oocysts.[9,17] The rigid oral portion of an esophageal feeder was used to deliver 5 mL of oocyst suspension directly into the distal esophagus, and 120 mL of water was then flushed through the feeder to ensure all of the oocyst suspension was delivered to the calf.

All study personnel making calf-level observations were blinded to treatment group. Outcomes measured included HS, FS, body temperature, TP concentration,

Table 1—Feeding algorithm for an example calf weighing 50 kg.

Treatment	Time	BW (kg)	MBW* (kg)	Feeding rate (Mcal/MBW)	MR energy† (Mcal/kg)	Dilution rate (%)	Feed energy‡ (Mcal/d)	MR§ (kg)
CN	Days 1 through 21	50	19	0.13	4.94	15	2.44	0.49
HPN	Days 1 through 7	50	19	0.23	5.06	15	4.32	0.85
	Days 8 through 21	50	19	0.30	5.06	15	5.64	1.11

* Calculated as $(body\ weight)^{0.75}$. †Energy provided from 1 kg of milk replacer. ‡Energy provided at given feeding rate. §Weight of milk replacer fed daily.

BW = Birth weight. MR = Milk replacer.

The CN group feeding rate was 0.13 Mcal/kg of MBW for the duration of the study (days 1 through 21). The HPN group was fed at 0.23 Mcal/kg of MBW for days 1 through 7 and 0.30 Mcal/kg MBW for days 8 through 21.

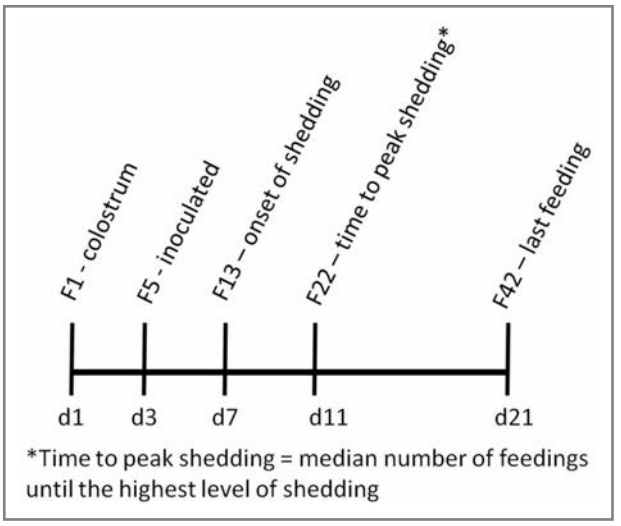

Figure 1—Time line of events starting at the first feeding (F1) on day 1 (d1) and ending at the last feeding (F42) on day 21 (d21) for calves fed either a higher plane of nutrition (HPN; n = 11) or a conventional level of intake (CN; 9) and experimentally infected with *Cryptosporidium parvum*.

PCV, WBC count, serum haptoglobin concentration, serum NEFA concentration, body weight, and fecal pathogens. At each feeding, HS, FS, and temperature were recorded. Health scores were based on a 4-point scale: 1 = normal, 2 = mildly depressed, 3 = severely depressed, 4 = moribund or dead. Fecal scores were assessed from samples taken directly from each calf into fecal cups by study personnel as they entered the stall and calves defecated or via digital stimulation of the rectal mucosa if necessary. Fecal scores were determined on the basis of a 3-point scale: 1 = sample is in patty form, has minimal water content, and does not flow across or down a surface; 2 = sample is more of a puddle, has some water content, and flows slowly across or down a surface; and 3 = sample is watery and flows across or down a surface while leaving some to no adherent material.[18] Fecal samples were obtained at every even-numbered feeding after inoculation with *C parvum* for oocyst quantification and dry weight measurements of positive samples. Each blood sample was collected from the jugular vein into evacuated tubes. Evacuated tubes containing Ca-EDTA were used for PCV and WBC count determination. Evacuated tubes without anticoagulant were used for serum protein, NEFA, and haptoglobin concentration determination. Serum TP concentration was measured by refractometery[d] after centrifugation (5 minutes at 10,062 × *g* at 20°C) on days 2, 3, 8, 11, 12, 13, 14, 15, 16, 17, and 21. Packed cell volume was measured after centrifugation (5 minutes at 10,062 × *g* at 20°C) in micro-Hct tubes on days 3, 8, 11, 12, 13, 14, 15, 16, 17, and 21. An automated WBC count[e] and serum haptoglobin concentration[f] were evaluated at days 3 and 12. Nonesterified fatty acid concentrations were measured[19] at days 8 and 16. Body weight was measured with a digital scale[g] before colostrum administration at birth and at days 2, 3, 12, and 21. Any calf that refused an entire meal and had an FS > 2 and HS > 2 was to receive flunixin meglumine (1.1 mg/kg [0.5 mg/lb], IV once) and (0.9% NaCl) solution (1 to 2 L, IV once). Any moribund calf that did not respond to treatment was to be euthanized by captive bolt and exsanguination. All calves were sold at the completion of the study.

Fecal sample analysis—Quantitative analysis of *C parvum* oocysts in the fecal samples was performed by means of immunofluorescence.[h] A 0.10-g portion of feces was mixed into 10 mL of PBS solution (pH, 7.4) in a 15-mL conical centrifuge tube. Then, 100 µL of the mixture was removed and 5 µL of IFA reagent was added. This solution was vortexed and then incubated in the dark at room temperature (approx 22°C) for at least 30 minutes and stored at 4°C until examination. Once incubated, a 10.5-µL sample was placed on a slide and covered with a coverslip. The 10× objective on a fluorescent compound binocular microscope[i] was used to read the slide and count the number of oocysts observed. The number of oocysts observed in 10.5 µL was then multiplied by 10,000 to give the number of oocysts per gram of feces. This was then standardized by the dry matter percentage. Dry matter analysis of fecal samples was obtained by taking a 10- to 20-g portion of each original fecal sample, drying it at 108°C in a forced air oven[j] for a minimum of 24 hours, then reweighing[k] it hot out of the oven. Peak shedding was calculated as the median highest number of oocysts/g of feces on a per feeding basis relative to all of the calves within the treatment group. Total shedding was calculated as the median total number of oocysts counted over the entire study period relative to all of the calves within the treatment group. Fecal culture for *Salmonella* spp and latex agglutination[l] testing for rotavirus were performed once per calf throughout the study in the Animal Health Diagnostic Center, Cornell University. Testing occurred by sampling of each calf between days 2 and 14 after the isolation facility was full of study calves.

Statistical analysis—Data were analyzed by means of descriptive and inferential methods. Continuous data were described by medians and interquartile ranges, and categorical data were summarized by use of contingency tables. Box-and-whisker plots were used to compare ADG and FE. Comparisons of continuous data between calves fed the HPN and calves fed the CN were analyzed with Wilcoxon rank sum tests because these data often had nonnormal distributions.[20] Rate of resolution of diarrhea after peak shedding and ADG were estimated for each calf using linear regression. Data were analyzed with commercially available software.[m,n] Values of $P < 0.05$ were considered significant.

Results

Twenty-nine Holstein bull calves were obtained and randomly assigned to groups at birth. Nine calves were removed from the study due to failure of passive transfer. Eleven and 9 calves were successfully allocated and completed the study in the HPN and CN groups, respectively. All of the calves had clinical diarrhea and fecal shedding of *C parvum* oocysts after inoculation. One calf from each treatment group tested positive for rotavirus during the latter half of the study.

Measures of passive transfer of antibodies, initial body weight, and PCV at day 3 were not different between treatment groups ($P > 0.15$; **Table 2**). Haptoglobin concentration,

Table 2—Birth weight, PCV on day 3, TP concentration on day 3, NEFA concentration, haptoglobin concentration, and total WBC count of calves fed either a high plane of nutrition (HPN; n = 11) or a conventional level of intake (CN; 9) and experimentally infected with *Cryptosporidium parvum*.

	CN		HPN		
Variable	**Median**	**Interquartile range**	**Median**	**Interquartile range**	***P* value**
Body weight on day 1 (kg)	47.3	40–49.5	46.8	42–49.3	0.8
PCV on day 3 (%)	32	29–37	30	26–31.5	0.15
TP on day 3 (g/dL)	5.3	5–5.9	5.5	5.2–5.9	0.4
WBC count on day 3 (X 10^3 WBCs/µL)	11.8	7.1–14.3	10.7	8.3–13.5	0.65
WBC count on day 12 (X 10^3 WBCs/µL)	10.0	9.7–11.8	11.6	10.9–16.1	0.2
Haptoglobin on day 3 (mg/dL)	0.03	0.03–0.04	0.03	0.03–0.04	0.6
Haptoglobin on day 12 (mg/dL)	0.03	0.02–0.05	0.03	0.03–0.04	0.5
NEFA on day 8 (mEq/L)	0.27	0.17–0.37	0.18	0.11–0.27	0.26
NEFA on day 16 (mEq/L)	0.28	0.24–0.41	0.19	0.08–0.23	0.28

Table 3—Oocyst shedding patterns of calves fed either a high plane of nutrition (HPN; n = 11) or a conventional level of intake (CN; 9) after experimental infection at 3 days old with *C parvum*.

	CN		HPN		
Variable	**Median**	**Interquartile range**	**Median**	**Interquartile range**	***P* value**
Onset of shedding* (d)	4	3–5	4	4–4	0.8
Duration of shedding (d)	10	8–12	10	9–10	0.7
Total counted (10^7)	7.9	4.8–12.3	6.5	5.3–9.8	0.7
Peak shedding (10^7/g DM)	2.9	1.7–5.9	2.6	1.9–4.7	0.7

*Onset of shedding is the median number of days after inoculation when fecal shedding began.
DM = Dry matter.

NEFA concentration, and WBC count were also not different between groups ($P > 0.2$). Oocyst shedding patterns, including onset, duration, total counted, and peak shedding, were not different between treatment groups ($P > 0.7$; **Table 3**).

Starting at day 7, once calves were shedding *C parvum*, 330 and 270 HSs were reported for the HPN and CN calves, respectively. The breakdown of HSs was as follows: in HPN calves, 97.5% had a HS of 1, 2.1% had a HS of 2, and 0.3% had a HS of 3; 100% of CN calves had a HS of 1. Throughout the entire study, 7 of 11 HPN calves partially refused at least 1 meal, compared with 1 of 9 CN calves ($P = 0.05$). The median weight of each refused portion of the meal for the HPN calves was 0.26 kg. The weight of refused portion of the meal for the 1 CN calf was 0.26 kg. Starting at day 7, once calves were shedding oocysts, 4 of 11 calves partially refused 5 of 330 meals in the HPN group and no calves partially refused any of the 270 meals in the CN group.

The PCV data for both treatment groups were summarized (**Figure 2**). The median PCV increased between days 8 and 21 for the CN calves (34% vs 40%, respectively; $P = 0.05$), whereas the median PCV in the HPN calves did not (34% vs 32%, respectively; $P = 1$). Median TP concentration of the HPN calves decreased between days 8 and 21 (5.6 g/dL vs 4.7 g/dL, respectively; $P = 0.004$), whereas median TP concentration of the CN calves did not (5.2 g/dL vs 5.0 g/dL, respectively; $P = 0.16$). The median lymphocyte counts in the HPN calves increased between days 3 and 12 (3.75×10^6 cells/µL vs 5.75×10^6 cells/µL, respectively; $P = 0.01$), whereas the median lymphocyte counts in the CN calves did not (3.85×10^6 cells/µL vs 5.25×10^6 cells/µL, respectively; $P = 0.1$).

Once shedding *C parvum* at day 7, 330 and 269 fecal samples were scored for the HPN and CN calves, respectively. The breakdown of FSs was as follows: in HPN calves, 34% had an FS of 1, 31% had an FS of 2, and 35% had an FS of 3; in CN calves, 29% had an FS of 1, 46% had an FS of 2, and 24% had an FS of 3. Before peak shedding of oocysts, the daily change in FS from days 6 to 10 was not different between groups. However, after peak shedding (day 11), FS improved at a faster rate in the HPN group (median slope, –0.1 FS/feeding), compared with the CN group (median, –0.06 FS/feeding; $P = 0.03$). From peak shedding onward, the median percentage of feedings associated with severe diarrhea (FS = 3) was not different between groups (29% in HPN calves vs 19% in CN calves; $P = 0.2$).

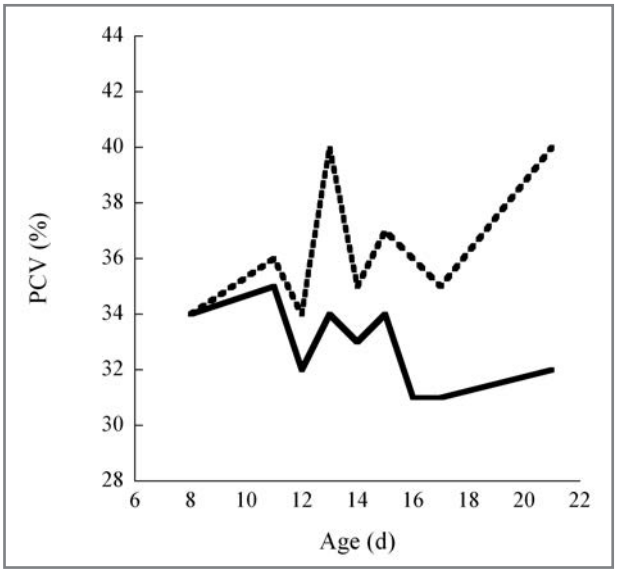

Figure 2—Packed cell volume throughout the duration of the study for calves fed either a high plane of nutrition (HPN; n = 11; solid line) or a conventional level of intake (CN; 9; dashed line) and experimentally infected with *C parvum*.

The median weight of the calves in the HPN group at the end of the study was 55.9 kg, whereas it was 43.2 kg in

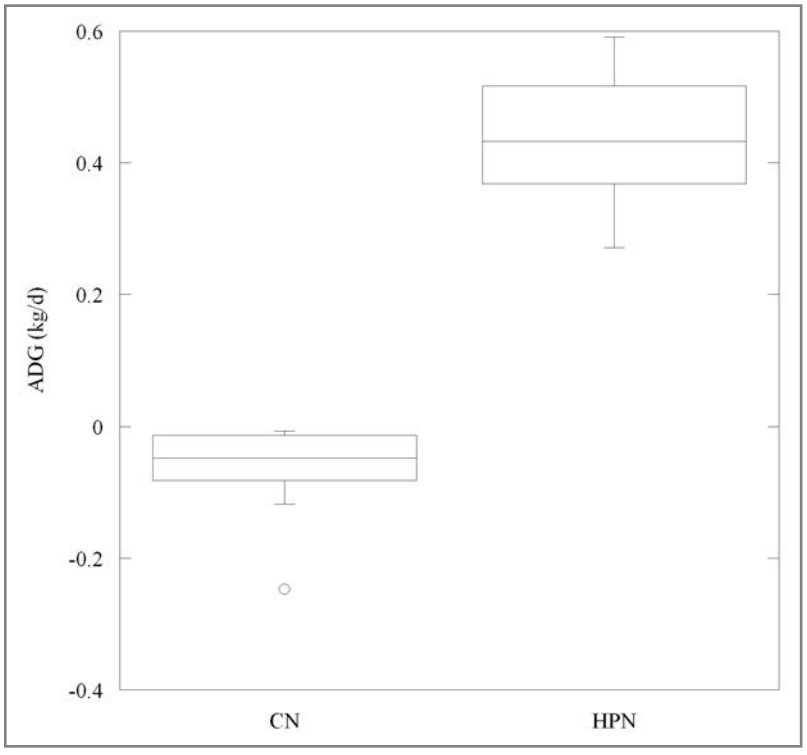

Figure 3—Box-and-whisker plots reflecting the ADG (kg/d) of calves fed either a high plane of nutrition (HPN; n = 11) or a conventional level of intake (CN; 9) and experimentally infected with *C parvum*. The box encloses 50% of the data, (ie, first quartile to third quartile, with the horizontal line representing the median value of the variable). The whisker lines extending from the top and bottom of each box mark the minimum and maximum values (ie, the range) of the data set that fell within an acceptable range. Any value outside of this range, called an outlier, is displayed as an individual point and calculated as either greater than the third quartile plus 1.5 times the interquartile range or less than the first quartile minus 1.5 times the interquartile range.

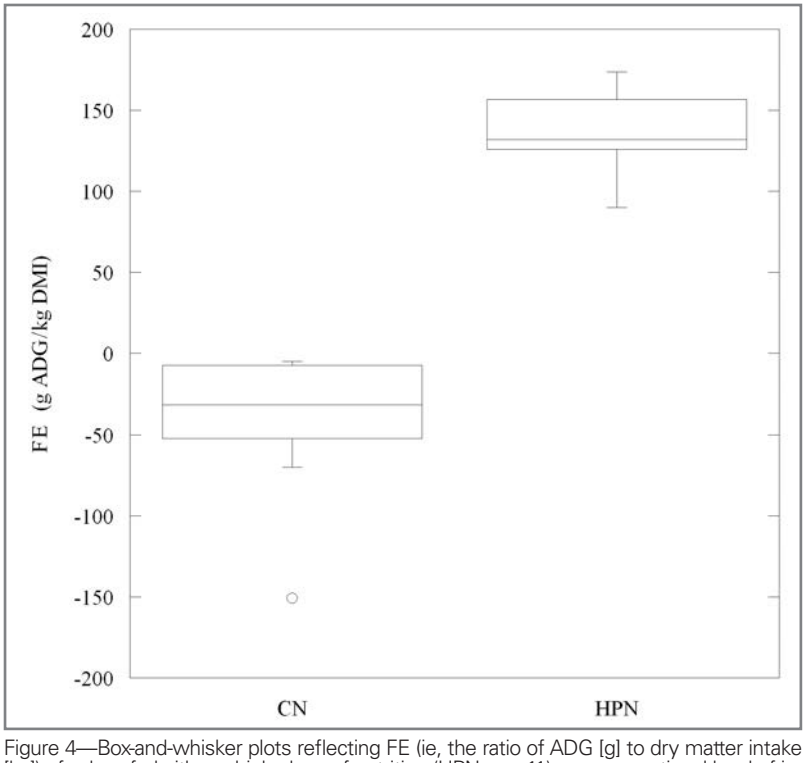

Figure 4—Box-and-whisker plots reflecting FE (ie, the ratio of ADG [g] to dry matter intake [kg]) of calves fed either a high plane of nutrition (HPN; n = 11) or a conventional level of intake (CN; 9) and experimentally infected with *C parvum*. *See* Figure 3 for remainder of key.

the CN group ($P = 0.06$). The calves in the HPN group had higher ADG than CN calves (median, 433 g vs –48 g, respectively; $P < 0.001$; **Figure 3**). Feed efficiency (ADG-to-dry matter intake ratio) was higher for the calves in the HPN group than the CN group (median, 131.9 g/kg vs –31.4 g/kg, respectively; $P < 0.001$; **Figure 4**).

Discussion

In the present study, our challenge model successfully created clinical cryptosporidiosis in every calf. No deaths occurred, and no supportive care was required for calves in either the HPN or CN groups. Calves fed the HPN diet had faster resolution of diarrhea, maintained hydration, grew better, and converted feed with greater efficiency than the calves fed the CN diet, despite not finishing an occasional meal.

In a previous study,[8] higher morbidity and mortality rates were documented in calves fed an HPN after a coronavirus challenge. In that study,[8] passive transfer of maternal antibody was not an inclusion criterion, which likely contributed to higher rates of disease and death. Secondly, the nutritional plane was increased by 50% in that study,[8] compared with a 30% increase in the present study. Lastly, differences in treatment criteria and treatment protocols might have contributed to the differences in morbidity and mortality rates. Specifically, in the older study,[8] treatment with oral rehydration solution was initiated in calves with diarrhea whenever FS > 2, antimicrobials were administered whenever body temperature increased above 39.4°C, and all calves that refused a portion of a meal were fed via an esophageal feeder. In the present study, treatments were administered only if an entire meal was missed and FS > 2. Calves that refused a portion of a meal were not tube fed once the animals were infected. Force feeding milk or milk replacer to calves that are anorexic is controversial and might be a contradiction of the communications between the brain and immune system where an immune response triggers a proinflammatory cytokine response, whereas the normal physiologic response is to decrease or cease food intake for a period of immune response.[21] Further, in experimental studies in mice,[22] force feeding diseased animals resulted in higher number of deaths, consistent with results of the previous study.[8]

Although the rate of resolution of diarrhea has not previously been reported, we believe it might have been related to the PCV and TP concentration changes observed in the present study. One study[23]

that evaluated the effect of feeding higher protein and energy diets on blood metabolites in Holstein calves found that calves (in all treatment groups) had a decrease in PCV throughout the study but that the PCV was not correlated with the concurrent linear decrease in FS. This is different from our study in that the HPN calves had a decrease and the CN calves had an increase in PCV. The authors[23] proposed that the differences were not expected but were possibly due to the daily feeding of electrolyte solutions to every calf that had an FS > 2 and twice-a-day feeding to every calf with an FS > 3. Another difference between that study[23] and the present study is the presence of a pathogen challenge, which perhaps created more severe and consistent diarrhea. This may have led to a greater difference between treatment groups. In addition, orally administered electrolytes provided earlier in our protocol might have eliminated the observed increase in PCV.

Researchers have found that serum TP concentration did not change over time with respect to dietary treatment.[23,24] However, in our study, we observed that TP concentration decreased with time in calves fed the HPN diet, compared with that in calves fed the CN diet, which did not. We postulate that dehydration might have obscured the change in TP concentration for the CN-fed calves and that the decrease seen in the HPN calves was the natural process of losing maternal antibody over time. We propose that the well-fed calves with the ability to resolve their diarrhea faster spent less time losing fecal water, contributing to their overall lower PCV and TP concentration.

The effects of nutrient intake on calf immune function have been previously evaluated.[25,26] In those studies,[25,26] the calves fed an HPN had increased nitric oxide production and reduced interferon-γ production by peripheral blood mononuclear cells (factors both involved in cell-mediated immunity) but there were no effects on lymphocyte numbers or composition, IgM secretion, or mitogen-induced DNA synthesis. We observed an increase in lymphocyte numbers over time in the calves fed the HPN diet. This may be a normal biological event as the calf is maturing toward adult lymphocyte counts. The CN diet might have prevented maturation toward adult lymphocyte counts.

Despite an increase in number of feed refusals, concentration of the inflammatory protein haptoglobin was not elevated, suggesting that failure to finish a meal was partially the result of satiation and not a direct result of an inflammatory process or illness. In our study, ADG and FE had similar patterns relative to plane of nutrition (median ADG, 433 g/d vs –48 g/d for HPN and CN, respectively; median FE, 131.9 g/kg vs –31.4 g/kg for HPN and CN, respectively). The relationship between dry matter intake, ADG, and FE has been demonstrated in several previous studies[3,5,8,27] in which the HPN calves gained more weight (468 to 790 g/d vs 211 to 560 g/d, respectively) and were more efficient (425 to 700 g/kg vs 332 to 590 g/kg, respectively), compared with the CN-fed calves. However, in our study, the ADG and FE were markedly decreased and the disparity between treatment groups was much more dramatic, which we attribute to the presence of the *C parvum* pathogen.

Mobilization of body fat might have contributed to weight loss and reduced FE in the CN calves; however, this was not supported by the NEFA concentrations in our study. In fact, at the level of intake energy of calves fed the CN diet, it would be highly unlikely that any adipose tissue would have been accreted; thus, there would not be enough adipose tissue to turnover into the NEFA pool.[28] Cryptosporidiosis appears to have considerably reduced the nutrient absorption and associated feed efficiencies observed in conventionally fed calves as compared with the above mentioned studies,[3,5,8,27] which likely affected the ADG in those calves of the present study.

In some ways, the feeding methods used in the present study might have departed from some farm practices in North America, such as feeding on the basis of MBW, tube feeding refusals in calves with weak suckle that are otherwise healthy, and not providing calf starter in the first weeks after birth. Feeding based on MBW was performed to reduce bias by ensuring that all calves were getting proportionally the same amount of nutrients because calf was the unit of analysis. On most farms, small calves are fed the same as large calves and therefore have relatively more energy and protein provided for maintenance and growth and might actually gain more weight during a specified time period. Conversely, large calves may be underfed, compared with the average calf. Lastly, calf starter was not provided because of the previously documented negligible intakes in calves during their first 3 weeks after birth.[7,28] However, in commercial settings, it is still recommended to offer starter in this early period so the calves are accustomed to eating it when liquid feed is decreased and then removed.

a. Dairy Tech Inc, Windsor, Colo.
b. Instant Cow's Match, Land O'Lakes Animal Milk Products Co, Shoreview, Minn.
c. Herd Maker 20-20, Land O'Lakes Animal Milk Products Co, Shoreview, Minn.
d. Kernco Instruments Inc, El Paso, Tex.
e. Siemens, Tarrytown, NY.
f. Tridelta Development Ltd, Maynooth, Ireland.
g. Digi-star, Fort Atkinson, Wis.
h. Meridian Diagnostics, Cincinnati, Ohio.
i. Olympus BX41, Olympus America Inc, Center Valley, Pa.
j. Barnstead International, Dubuque, Iowa.
k. Precision Standard Scale, Ohaus Corp, Pine Brook, NJ.
l. Wampole Laboratories, Cranbury, NJ.
m. Epi Info, version 6, CDC, Atlanta, Ga.
n. SAS, version 9, SAS Institute Inc, Cary, NC.

References

1. National Research Council. Nutrient requirements of the young calf. In: *Nutrient requirements of dairy cattle*. 7th ed. Washington, DC: National Academy Press, 2001;214–243.
2. Davis CL, Drackley JK. Introduction. In: The *development, nutrition, and management of the young calf*. Ames, Iowa: Iowa State University Press, 1998;5–6.
3. Diaz MC, Van Amburgh ME, Smith JM, et al. Composition of growth of Holstein calves fed milk replacer from birth to 105-kilogram body weight. *J Dairy Sci* 2001;84:830–842.
4. Bartlett KS, McKeith FK, VandeHaar MJ, et al. Growth and body composition of dairy calves fed milk replacers containing different amounts of protein at two feeding rates. *J Anim Sci* 2006;84:1454–1467.
5. Raeth-Knight M, Chester-Jones H, Hayes S, et al. Impact of conventional or intensive milk replacer programs on Holstein heifer performance through six months of age and during first lactation. *J Dairy Sci* 2008;92:799–809.
6. Jasper J, Weary DM. Effects of ad libitum milk intake on dairy calves. *J Dairy Sci* 2002;85:3054–3058.

7. Brown EG, VandeHaar MJ, Daniels KM, et al. Effect of increasing energy and protein intake on body growth and carcass composition of heifer calves. *J Dairy Sci* 2005;88:585–594.
8. Quigley JD, Wolfe TA, Elsasser TH. Effects of additional milk replacer feeding on calf health, growth, and selected blood metabolites in calves. *J Dairy Sci* 2006;89:207–216.
9. Ollivett TL, Nydam DV, Bowman DD, et al. Effect of nitazoxanide on cryptosporidiosis in experimentally infected neonatal dairy calves. *J Dairy Sci* 2009;92:1643–1648.
10. Garber LP, Salman MD, Hurd HS, et al. Potential risk factors for *Cryptosporidium* infection in dairy calves. *J Am Vet Med Assoc* 1994;205:86–91.
11. Trotz-Williams LA, Martin SW, Leslie KE, et al. Association between management practices and within-herd prevalence of *Cryptosporidium parvum* shedding on dairy farms in southern Ontario. *Prev Vet Med* 2008;83:11–23.
12. McMartin S, Godden S, Metzger L, et al. Heat treatment of bovine colostrum. I: effects of temperature on viscosity and immunoglobulin G level. *J Dairy Sci* 2006;89:2110–2118.
13. Godden S, McMartin S, Feirtag J, et al. Heat-treatment of bovine colostrum. II: effects of heating duration on pathogen viability and immunoglobulin G. *J Dairy Sci* 2006;89:3476–3483.
14. Johnson JL, Godden SM, Molitor T, et al. Effects of feeding heat treated colostrum on passive transfer of immune and nutritional parameters in neonatal dairy calves. *J Dairy Sci* 2007;90:5189–5198.
15. Calloway CD, Tyler JW, Tessman RK, et al. Comparison of refractometers and test endpoints in the measurement of serum protein concentration to assess passive transfer status in calves. *J Am Vet Med Assoc* 2002;221:1605–1608.
16. Jenkins, MB, Anguish LJ, Bowman DD, et al. Assessment of a dye permeability assay for determination of inactivation rates of *Cryptosporidium parvum* oocysts. *Appl Environ Microbiol* 1997;63:3844–3850.
17. Peeters JE, Villacorta I, Naciri M, et al. Specific serum and local antibody responses against *Cryptosporidium parvum* during medication of calves with halofuginone lactate. *Infect Immun* 1993;61:4440–4445.
18. Moore DA, Atwill RE, Kirk JH, et al. Prophylactic use of decoquinate for infections with *Cryptosporidium parvum* in experimentally challenged neonatal calves. *J Am Vet Med Assoc* 2003;223:839–845.
19. Stokol T, Nydam DV. Effect of anticoagulant and storage conditions on bovine nonesterified fatty acid and betahydroxybutyrate concentrations in blood. *J Dairy Sci* 2005;88:3139–3144.
20. Rosner BA. Nonparametric methods. In: *Fundamentals of biostatistics*. Duxbury Press, 1986;278–293.
21. Dantzer R. Cytokine, sickness behavior, and depression. *Neurol Clin* 2006;24:441–460.
22. Johnson RW. Immune and endocrine regulation of food intake in sick animals. *Domest Anim Endocrinol* 1998;15:309–319.
23. Daniels KM, Hill SR, Knowlton KF, et al. Effects of milk replacer composition on selected blood metabolites and hormones in preweaned Holstein heifers. *J Dairy Sci* 2008;91:2628–2640.
24. Terosky TL, Heinrichs AJ, Wilson L. A comparison of milk protein sources in diets of calves up to eight weeks of age. *J Dairy Sci* 1997;80:2977–2983.
25. Nonnecke BJ, Foote MR, Smith JM. et al. Composition and functional capacity of blood mononuclear leukocyte populations from neonatal calves on standard and intensified milk replacer diets. *J Dairy Sci* 2003;86:3592–3604.
26. Foote MR, Nonnecke BJ, Waters WR, et al. Effects of increased dietary protein and energy on composition and functional capacities of blood mononuclear cells from vaccinated, neonatal calves. *Int J Vitam Nutr Res* 2005;75:357–368.
27. Cowles KE, White RA, Whitehouse NL, et al. Growth characteristics of calves fed an intensified milk replacer regimen with additional lactoferrin. *J Dairy Sci* 2006;89:4835–4845.
28. Tikofsky JN, Van Amburgh ME, Ross DA. Effect of varying carbohydrate and fat content of milk replacer on body composition of Holstein bull calves. *J Anim Sci* 2001;79:2260–2267.