Plane of nutrition influences the performance, innate leukocyte responses, and resistance to an oral *Salmonella enterica* serotype Typhimurium challenge in Jersey calves

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ABSTRACT

Two experiments investigated how plane of nutrition influences performance, leukocyte responses, and resistance to an oral *Salmonella enterica* serotype Typhimurium challenge. In experiment 1, 46 (2 ± 1 d of age) calves were randomly assigned to 2 diets: a low (LPN; $n = 23$) and high plane of nutrition (HPN; $n = 23$). The LPN calves were fed 409 g/d of dry matter (DM) of a 20% crude protein and 20% fat milk replacer, whereas HPN calves were fed 610 and 735 g/d of DM of a 28% crude protein and 25% fat milk replacer during wk 1 and 2 to 6, respectively. In experiment 2, 20 bull calves (LPN; $n = 11$ and HPN; $n = 9$) were orally challenged on d 80 with 1.5×10^7 cfu of *Salmonella* Typhimurium (ATCC #14028). The HPN calves had a greater incidence (87.5 vs. 45.5%) and duration of days with high fecal scores (5.5 vs. 3.5 d). The LPN calves had greater neutrophil surface expression of L-selectin on d 7, 21, and 42. Following the *Salmonella* Typhimurium challenge, calf starter DM intake was greater among the HPN calves. The percentage of neutrophils producing an oxidative burst was also greater among HPN calves on d 1 to 5 after the challenge. Similarly, the intensity of the oxidative burst tended to be greater among the HPN calves on d 2 and 3 postchallenge. The secretion of tumor necrosis factor- α from whole-blood cultures stimulated with lipopolysaccharide tended to be greater on d 1 and was greater on d 5 and 6 among HPN calves. The median ranks of haptoglobin concentrations were greater and plasma zinc concentrations tended to be decreased among LPN calves. These data indicate that feeding a HPN to Jersey calves improved average daily gain and feed efficiency, but increased the incidence of high fecal

scores during the first few weeks of life; however, the HPN Jersey calves may be more resistant to *Salmonella* Typhimurium after weaning.

Key words: calf, health, immune, plane of nutrition

INTRODUCTION

The high incidences of morbidity and mortality among calves during the first few months of life continue to plague the dairy industry. Furthermore, the well-being of a calf depends largely on its health. In general, a very common preweaning feeding program is to restrict the quantity of milk fed to calves to increase the consumption of calf starter and decrease the age at weaning. However, over the past 2 decades, interest in calf-feeding programs that offer greater quantities of milk or milk replacer (MR) has increased (Pollock et al., 1994; Nonnecke et al., 2003). The data indicate that feeding more milk increases ADG and feed efficiency, decreases age at sexual maturity and first calving, and may improve future lactation yields (Blome et al., 2003; Soberon et al., 2012; Ballou et al., 2013). The effects that plane of milk nutrition play on the health of calves during the first few months of life are not well understood.

Most of the data related to the health of calves fed varying planes of milk nutrition are limited to small, controlled experiments with fecal scores during the first few weeks of life as the major outcome variable (Nonnecke et al., 2003; Ballou, 2012). The data on fecal scores are equivocal; some studies showing that calves fed a greater quantity of milk had more loose feces (Nonnecke et al., 2003; Bartlett et al., 2006), whereas others report no differences in fecal scores (Ballou, 2012; Obeidat et al., 2013). Recently, Ollivett et al. (2012) observed that feeding greater quantities of MR to calves reduced duration of scours and improved hydration and ADG following a *Cryptosporidium parvum* challenge at 3 d of age. Larger data sets with naturally occurring disease incidence or more experimentally

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controlled relevant disease challenges are needed before definitive conclusions can be made on the role that plane of milk nutrition plays on the health of calves.

A limited amount of research has investigated the effect that plane of milk nutrition plays on various leukocyte responses. Others examined how plane of nutrition influences adaptive leukocyte responses; however, no clear consensus is evident (Pollock et al., 1994; Nonnecke et al., 2003; Foote et al., 2007). Our laboratory has focused on understanding how plane of nutrition affects innate leukocyte responses (Ballou, 2012; Obeidat et al., 2013). Ballou (2012) reported that neutrophil oxidative burst or whole-blood bactericidal capacity was not influenced by plane of MR nutrition during the preweaning period among either Jersey or Holstein calves. In contrast, Obeidat et al. (2013) observed that Holstein calves fed a restricted quantity of MR had elevated neutrophil oxidative burst and surface expression of the adhesion molecule L-selectin compared with calves fed a greater amount of MR during the preweaning period (d 0–53). Those authors speculated that the greater neutrophil activity among the calves fed the reduced quantity of MR was a result of either less stress or increased microbial exposure due to more nonnutritive oral behaviors. Ballou (2012) reported that, a month after weaning, Jersey calves previously fed a greater plane of MR had more intense neutrophil oxidative burst and whole-blood bactericidal capacities. Therefore, those Jersey calves may have improved resistance to bacterial disease.

The first hypothesis of our study was that feeding a lower plane of MR nutrition to Jersey calves would increase neutrophil surface expression of L-selectin and oxidative burst. The second hypothesis was that Jersey calves that were fed a high plane of MR nutrition would have improved resistance to an oral *Salmonella enterica* serotype Typhimurium challenge a month after weaning. To test these hypotheses, 2 experiments were conducted that evaluated the effects of feeding either a low or high plane of nutrition during the pre- and immediate postweaning periods on (1) the performance, health, and leukocyte responses and (2) leukocyte and the pathophysiological response to an oral *Salmonella* Typhimurium challenge a month after weaning.

MATERIALS AND METHODS

Experiment 1

The experiment was conducted from May to July 2011. All animal procedures were reviewed and approved by the Texas Tech University Animal Care and Use Committee. Forty-six Jersey calves, 23 bulls and 23 heifers (2 ± 1 d of age), were transported 155 km

from a commercial dairy farm to the Hilmar Cheese/Agri-Plastics Calf Research Facility at Texas Tech University (New Deal, TX). Approximately equal numbers of bull and heifer calves were randomly assigned to the 2 dietary treatments. All calves were fed 3.8 L of pooled colostrum at the dairy within the first 6 h of life; upon enrollment, a peripheral blood sample was taken and individual total serum protein content was recorded using a handheld refractometer (Atago USA Inc., Bellevue, WA), which averaged 6.1 ± 0.3 g/dL (mean $\pm$ SD). Calves were housed individually outside on sandy soil in commercial polyethylene calf hutches with an attached pen (2.13×1.09 m; Agri-Plastics, Tonawanda, NY) with an attached outside pen (1.83×1.09 m).

Feeding and Weaning. Upon arrival at the research facility each calf was weighed, shoulder height and length from the scapula to the pins were measured, and randomly assigned to either a low plane of nutrition (LPN) or a high plane of nutrition (HPN) treatment. Calves on the LPN were fed 409 g, DM basis, of a 20% CP and 20% fat MR (Herd Maker, Land O'Lakes Animal Protein Co., Shoreview, MN) in 4 L of water daily. Calves on the HPN were fed 610 g, DM basis, of a 28% CP and 25% fat MR (Cow's Match Jersey Blend, Land O'Lakes Animal Protein Co.) in 5 L of water daily for the first 10 d. Calves fed the HPN were then stepped up to 735 g of DM of the same 28% CP and 25% fat milk replacer in 6 L of water daily until 42 d. Calves were fed twice daily at 0730 and 1630 h for the duration of the study. After the first week, all calves had ad libitum access to a pelleted calf starter. The formulated chemical compositions of the calf starters offered to the LPN and HPN calves are shown in Table 1. The quantity of calf starter offered to each calf was adjusted daily for approximately a 10% refusal. No roughage was offered during the study. Weaning was initiated at d 42 by removing the 1630-h milk feeding. Calves were completely weaned from milk when daily consumption of calf starter exceeded 600 g, as-fed basis, for 2 consecutive days after d 49. All performance data were calculated and analyzed during the neonatal period (d 0–21), preweaning period (d 0–42), postweaning period (d 42–77), and overall period (d 0–77).

Observations. Two independent trained observers assessed sickness and fecal scores approximately 30 min before each feeding. Sickness scores were classified as 1 = normal, alert, response to stimuli quick; 2 = depressed, response to stimuli decreased; 3 = lethargic, response to stimuli greatly reduced; 4 = morbid, little or no response to stimuli (Ballou et al., 2011). Fecal scores were recorded according to the guidelines outlined by Larson et al. (1977); scores were 1 = firm, well-formed (not hard); 2 = soft, pudding-like; 3 = runny, pancake batter; and 4 = liquid, splatters, pulpy orange juice. A

Table 1. The formulated nutrient content (as-fed basis) of the milk replacer and calf starter fed to Jersey calves

Nutrient	Milk replacer ¹		Calf starter ²	
	LPN	HPN	LPN	HPN
DM, %	97	97	92	92
CP (min ³), %	20	28	18	20
Ether extract (min), %	20	25	2.2	2.2
Crude fiber (max ³), %	0.15	0.15	12.5	16.0
ADF (max), %	—	—	14.5	18.0
ME, Mcal/kg ⁴	4.78	4.91	2.55	2.66
Calcium (min), %	0.75	0.75	0.8	0.8
Calcium (max), %	1.25	1.25	1.2	1.2
Phosphorus (min), %	0.7	0.7	0.45	0.5
Selenium (min), ppm	—	—	0.3	0.3
Vitamin A (min), IU/kg	44,000	44,000	6,380	7,700
Vitamin D ₃ (min), IU/kg	11,000	11,000	—	—
Vitamin E (min), IU/kg	220	275	—	—

¹Two commercial milk replacers were fed (Land O'Lakes Animal Milk Products Co., Shoreview, MN). LPN = low plane of nutrition was a commercial milk replacer (Herd Maker) that was supplemented with dicalcium phosphate, calcium carbonate, vitamin A acetate, vitamin D₃, vitamin E, thiamine mononitrate, pyridoxine hydrochloride, folic acid, vitamin B₁₂, choline chloride, calcium silicate, manganese sulfate, zinc sulfate, ferrous sulfate, copper sulfate, cobalt sulfate, ethylenediamine dihydroiodide, and selenium yeast. HPN = high plane of nutrition was a commercial milk replacer (Cow's Match) that was supplemented with a dried *Saccharomyces cerevisiae* fermentation extract and trace minerals and vitamins at the same concentrations as the LPN milk replacer, but used mostly organic sources including zinc methionine, manganese methionine, copper lysine, iron amino acid complex, ethylenediamine dihydroiodide, and selenium yeast.

²Two commercial calf starters with monensin were fed using similar macroingredients, which included grain products, processed grain by-products, plant protein products, molasses and roughage products (Purina Mills, Gray Summit, MO). The LPN calf starter (Calf Startena) was formulated with trace minerals and vitamins similar to that found in the LPN milk replacer. The HPN calf starter (Cornerstone Ampli-Calf) was formulated with a proprietary blend of a yeast extract, fructooligosaccharide, anise oil, garlic oil, cassia, ethyl vanillin, and propylene glycol. The HPN calf starter also was formulated with a trace mineral and vitamins at the same concentrations as the LPN calf starter, but used mostly organic sources including zinc amino acid complex, manganese amino acid complex, copper amino acid complex, cobalt glucoheptonate, and cobalt proteinate.

³min = minimum; max = maximum.

⁴Calculated based on NRC (2001).

calf with high fecal scores was classified as consecutive fecal scores ≥ 3 .

Sampling and Blood Collection. Calves were weighed individually at arrival, and at d 7, 21, 42, 56, and 77 of the study. Shoulder height and length were measured at enrollment and at d 21, 42, and 77. Voluntary MR refusals were recorded approximately 15 min after each feeding. Peripheral blood samples (9 mL) from the jugular vein were collected at d 7, 21, 28, 42 and 77 using 3- and 6-mL evacuated tubes (Vacutainer, Becton Dickinson, Rutherford, NJ) containing K₂ EDTA and heparin, respectively. The K₂ EDTA tube was placed immediately on ice and the heparin tube was placed in an ice chest without ice. All blood samples were processed within 2 h of collection (Sellers et al., 2013). Plasma was obtained from the K₂EDTA tube after centrifugation at $1,200 \times g$ for 15 min at 20°C and stored at -40°C until analyzed. Plasma was analyzed for glucose, urea nitrogen, haptoglobin, and zinc concentrations as described by Ballou et al. (2011). All colorimetric data were measured on a SpectraMax 340PC (Molecular Devices, Sunnyvale, CA). The intraassay coefficients of variations were 5.4, 6.4, and

2.5% for plasma glucose, urea nitrogen, and haptoglobin, respectively. Interassay coefficients of variation were determined from a pooled sample and were 5.1, 6.2, and 3.4% for plasma glucose, urea nitrogen, and haptoglobin, respectively.

Ex Vivo Leukocyte Analyses. Peripheral blood from the heparinized tube was diluted 1:4 in RPMI medium at a final concentration of 1% antibiotic-antimycotic (Invitrogen Life Technologies, Grand Island, NY) and 1 µg/mL of LPS (*Escherichia coli* 0111:B4; Sigma, St. Louis, MO). Duplicate cultures were incubated for 24 h at 38.5°C in a humidified 5% CO₂ incubator. The supernatant was removed after centrifugation for 15 min at $1,200 \times g$ at 20°C and stored at -40°C until analyzed for concentrations of tumor necrosis factor-α (TNF-α) by a commercially available ELISA kit (R&D Systems, Minneapolis, MN).

The oxidative burst capacities of whole-blood neutrophils in response to an enteropathogenic *E. coli* were analyzed as described by Hulbert et al. (2011). Briefly, 200 µL of whole blood from the heparinized tube was incubated in an ice bath for 15 min. Forty microliters of a 100 µM working concentration of dihydrorhodamine

(Invitrogen, Carlsbad, CA) and the *E. coli* (10^9 cfu/mL) were added to each sample, vortexed thoroughly, and then placed in a 38.5°C water bath and incubated for 10 min. After completion of incubation, the samples were immediately placed in an ice bath for 10 min to stop the reaction at a constant rate. Erythrocytes were hypotonically lysed and washed, and the leukocytes were analyzed using a Cell Lab Quanta SC flow cytometer (Beckman Coulter, Fullerton, CA). Using flow cytometer analysis software (QuantaSC MPL, Beckman Coulter), neutrophils were gated on the scatterplot of electric volume $\times$ side scatter. The percentage of neutrophils that were positive for oxidative burst were gated as neutrophils that had a greater fluorescence intensity than neutrophils from control cultures that were incubated without *E. coli*. Data are reported as both the percentage of neutrophils positive for oxidative burst as well as the geometric mean fluorescence intensity of the positive population.

Two hundred microliters of whole blood from the K₂ EDTA tube was incubated at a final concentration of 5 μ g/mL of anti-bovine CD62L (monoclonal antibody IgG1-isotype made in mouse; VMRD, Pullman, WA) for 1 h in an ice bath. Erythrocytes were hypotonically lysed and then rinsed once. The leukocyte pellet was resuspended in fluorescein-labeled secondary antibody at a 1:400 dilution [F(ab')₂ anti-mouse IgG:FITC; AbD Serotec Raleigh, NC]. Samples were incubated on ice for an additional 1 h. Samples were then washed twice using 1 $\times$ PBS and then analyzed by single-color flow cytometry. Using the flow cytometer analysis software, neutrophils were gated on the scatterplot of electric volume $\times$ side scatter. The total geometric mean fluorescence intensity for L-selectin was analyzed (Hulbert et al., 2011).

Statistical Analyses. All continuous, repeated data were analyzed by restricted maximum-likelihood ANOVA using the MIXED procedure of SAS (v.9.3, SAS Inst. Inc., Cary, NC). An initial model that included the fixed effects of treatment, sex, time, and all possible combinations of those main effects was analyzed for each variable. The effects of sex or sex $\times$ treatment were not significant for any model; therefore, sex was removed from all subsequent statistical models. Calf nested within treatment was the subject of the repeated statement. Compound symmetry and anteregressive (1) were the covariance structures tested for the within-calf measurements; the one with the best fit determined by the lowest Bayesian Information Criteria was used. Prior to statistical analyses, repeated data were tested for normality of the residuals by evaluating the Shapiro-Wilk statistic using the UNIVARIATE procedure of SAS (v.9.3). Pairwise differences were performed at each time interval using a sliced effect multiple comparison ap-

proach with a Tukey-Kramer adjustment. The incidence of scours was analyzed using a Chi-squared goodness of fit test with the FREQ procedure of SAS (v.9.3). Neither the duration of scours nor the day of the study that the scours started could be transformed into a normal distribution; therefore, the data were analyzed using the nonparametric Mann-Whitney-Wilcoxon test with the NPAR1WAY procedure of SAS (v.9.3). Differences of $P \leq 0.05$ were considered significant and $0.10 \geq P > 0.05$ was considered a tendency.

Experiment 2

Experimental Design, Calves, and Salmonella Typhimurium Challenge. All animal care was conducted according to the *Guide for the Care and Use of Agricultural Animals in Research and Teaching* (FASS, 2010) and approved by the Institutional Animal Care and Use Committee of the USDA-Agricultural Research Service. Twenty Jersey bull calves (77 ± 1 d of age) that were previously fed either the LPN (409 g/d of a 20% CP and 20% fat MR; $n = 11$) or HPN (610 and 735 g/d of a 28% CP and 25% fat MR for wk 1 and 2–6, respectively; $n = 9$) were acquired from experiment 1 and transported 11.5 km to the USDA Livestock Issues Research Unit calf facility in Liberty, Texas. Calves were housed in individual stainless steel pens (1 $\times$ 2 m) with slotted rubber mat flooring in a temperature-controlled room (ranged from 20.3 to 21.9°C). Upon arrival, all calves were fitted with rectal temperature monitoring devices as described by Ballou et al. (2011). Briefly, rectal temperatures were collected via a DST micro-T small thermo logger (Star Oddi, Reykjavik, Iceland) at 5-min intervals and averaged by hour from –18 to 240 h relative to the challenge.

Following a 72-h acclimation period, all calves were challenged orally with approximately 1.5×10^7 cfu of *Salmonella* Typhimurium (ATCC # 14028). An individual colony from a streak plate was incubated overnight at 37°C in trypticase soy broth at 200 rpm. One hundred microliters of the overnight culture was added to 20 mL of fresh trypticase soy broth and incubated at 37°C at 200 rpm until it reached an optical density of 0.8 at 450 nm, which is mid-logarithmic growth. The broth was diluted to approximately 10^6 cfu per mL in 10 mL of sterile PBS. Each calf was given the challenge dose by oral gavage using a 25-mL syringe. The target oral dose was 1.0×10^7 cfu per calf. The actual challenge dose was determined by serial diluting and spread plating and it was determined that each calf was challenged with approximately 1.5×10^7 cfu. Each calf was observed for 240 h (10 d) following the challenge.

Observations and Sampling. Sickness scores and appetite was assessed twice daily at 0800 and 1600 h.

The sickness scores were recorded as described in experiment 1. Appetite was assessed through DMI. Ten milliliters of peripheral blood were collected by jugular venipuncture at 0, 1, 2, 3, 4, 5, 6, 7, 8, and 9 d relative to the challenge into heparinized Vacutainer for whole blood cultures to measure TNF- α secretion when co-cultured with LPS and to measure neutrophil oxidative burst capacities. All blood was processed within 1 h of collection. Plasma was collected from whole blood after centrifugation at $1,200 \times g$ for 15 min at 20°C and stored at -40°C. Plasma was analyzed for glucose, urea nitrogen, haptoglobin, and zinc concentrations as described by Ballou et al. (2011). The intraassay coefficients of variations were 4.2, 4.0, 1.7, and 3.1% and the interassay coefficients of variation were 4.4, 5.1, 2.1, and 4.3% for glucose, urea nitrogen, haptoglobin, and zinc, respectively.

Statistical Analyses. All performance, immunological, rectal temperature, glucose, urea nitrogen, and zinc data were analyzed by restricted maximum-likelihood ANOVA using the MIXED procedure of SAS (v.9.3, SAS Inst. Inc.). The model included the fixed effects of treatment, time, and treatment $\times$ time interaction. Calf nested within treatment was the subject of the repeated statement. Compound symmetry and anteregressive (1) were the covariance structures tested for the within-calf measurements; the one with the best fit determined by the lowest Bayesian information criterion was used. Prior to statistical analyses, repeated data were tested for normality of residuals by evaluating the Shapiro-Wilk statistic using the UNIVARIATE procedure of SAS (v.9.3). Not all calves showed clinical signs of disease; therefore, morbidity data was analyzed using a Chi-squared goodness of fit test with the FREQ procedure of SAS (v.9.3). Plasma haptoglobin concentrations were unable to be transformed into a normal distribution; therefore, the ANOVA of ranks was analyzed using the nonparametric Friedman test with the FREQ procedure of SAS (v.9.3). Pairwise differences were performed at each time interval using a sliced effect multiple comparison approach with a Tukey-Kramer adjustment. Least squares means ($\pm$ SEM) are reported throughout. Differences of $P \leq 0.05$ were considered significant and $0.10 \geq P > 0.05$ was considered a tendency.

RESULTS

Experiment 1

Intake, Performance, and Fecal Score. Three HPN calves died within the first 14 d from complications associated with gastrointestinal disease. A gross necropsy and clinical signs at the time of death in-

dicated that 1 of the calves died due to *Clostridium perfringens* bloat whereas the other 2 calves showed no other apparent signs of disease except severe scouring. These calves were removed before data analysis. The LPN calves consumed more ($P = 0.004$; Table 2) calf starter throughout the study, but the HPN calves had greater ADG during the preweaning period because they consumed more MR ($P = 0.001$; Table 2). A lag in calf starter intake was observed among the HPN calves immediately following weaning, which caused the HPN to have a lower ADG ($P = 0.025$) for the 2 wk postweaning (0.126 vs. 0.246 ± 0.033 kg for HPN and LPN, respectively). The HPN calves were more efficient at converting feed into gain during both the pre- and postweaning periods, as evidenced by the reduced feed-to-gain ratio ($P = 0.001$; Table 2). A high incidence of scouring was seen among calves during the first 3 wk of the study. The HPN calves had an increased incidence of high fecal scores ($P = 0.002$, Table 2). Among calves that had high fecal scores, no difference was noted between LPN and HPN calves in the median days of age that the high fecal scores started, but the high fecal scores persisted for more days among the HPN calves ($P = 0.026$, Table 2).

Plasma Metabolites. There was a treatment $\times$ time interaction ($P = 0.001$) for plasma glucose concentrations (data not shown). No difference was observed on d 7 or 77 ($P > 0.144$), but HPN calves had increased ($P \leq 0.002$) plasma glucose concentrations throughout the preweaning period compared with the LPN calves. A treatment $\times$ time interaction ($P = 0.001$) was observed for plasma urea nitrogen concentrations. The HPN calves tended to have elevated concentrations on d 7 (14.1 vs. 11.7 ± 1.02 mg/dL; $P = 0.087$), but the LPN calves had increased concentrations ($P \leq 0.017$) immediately before weaning (15.0 vs. 12.2 ± 0.85 mg/dL) and on d 77 (15.0 vs. 13.1 ± 0.52 mg/dL).

Ex Vivo Leukocyte Responses. A tendency ($P = 0.078$) was seen for a treatment $\times$ time interaction on the secretion of TNF- α from LPS whole-blood-stimulated cultures. The HPN calves had greater ($P = 0.027$) TNF- α secretion on d 7 (712 vs. 479 ± 70.5 pg/mL), but no differences were observed on d 21, 42, and 77. The LPN calves had greater ($P = 0.031$) neutrophil L-selectin protein concentrations on d 7, 21, and 42 (Figure 1). No differences ($P > 0.225$) were observed between treatments in either the percentage of neutrophils positive for an oxidative burst or the intensity of the oxidative burst (data not shown). The only difference observed for plasma haptoglobin concentrations ($P = 0.012$) was on d 21 where the LPN calves had increased concentrations (1.03 vs. 0.92 ± 0.035 optical density $\times 100$).

Table 2. Influence of plane of nutrition on milk replacer intake, calf starter intake, growth performance, feed efficiency, and scouring of Jersey calves (experiment 1)

Item	Nutrition ¹		Largest SEM	P-value
	LPN	HPN		
Initial BW, kg	24.2	25.3	0.71	0.297
Final BW, kg	49.9	60.2	2.1	0.0001
Milk replacer, ² kg	18.9	32.3	0.42	0.0001
Starter, kg/d				
d 0 to 21 (neonatal period)	0.054	0.019	0.0401	0.548
d 0 to 42 (preweaning)	0.173	0.067	0.0401	0.0001
d 42 to 77 (postweaning)	1.099	0.864	0.0401	0.0001
d 0 to 77 (overall)	0.595	0.429	0.0401	0.004
ADG, kg/d				
d 0 to 21	0.224	0.498	0.0326	0.0001
d 0 to 42	0.292	0.492	0.0326	0.0001
d 42 to 77	0.383	0.396	0.0326	0.914
d 0 to 77	0.334	0.445	0.0326	0.016
Feed-to-gain ratio				
d 0 to 21	2.19	1.49	0.112	0.0001
d 0 to 42	2.05	1.49	0.112	0.0008
d 42 to 77	3.26	2.67	0.112	0.0004
d 0 to 77	2.61	1.99	0.112	0.0001
High fecal scores ³				
Incidence, %	45.5	87.5	—	0.002
Initiated, ⁴ d	7.1	7.5	—	0.698
Duration, ⁴ d	3.5	5.5	—	0.026

¹LPN = low plane of nutrition (n = 23); HPN = high plane of nutrition (n = 20).

²Milk replacer intake is reported as the total kilograms of milk replacer consumed from enrollment until completely weaned off milk replacer onto calf starter.

³A calf with high fecal scores was classified as having at least 2 consecutive days with a fecal score of either a (3) = runny, pancake batter or (4) = liquid, splatters, pulpy orange juice.

⁴Data are reported as the median days.

Experiment 2

Intakes and Plasma Metabolites. The HPN calves consumed more calf starter (DM) following the *Salmonella* Typhimurium challenge ($P = 0.039$; Figure 2) compared with the LPN calves. The treatment effects sliced by time indicated that the difference in calf starter intake began on d 3 postchallenge and persisted through the end of the observation period at d 9. Intakes of ME and CP when expressed as a percentage of metabolic BW were not different between treatments during the study (Table 3). The HPN calves had greater plasma concentrations of glucose following the challenge ($P = 0.007$; Table 3). In contrast, the HPN calves had reduced plasma concentrations of urea nitrogen following the challenge ($P = 0.004$; Table 3).

Clinical Disease, Ex Vivo Leukocyte Responses, and Measures of Health. No treatment difference was observed in the percentage of calves that developed clinical disease (anorexia, decreased response to stimuli, or distended head) following the *Salmonella* Typhimurium challenge ($P = 0.279$; Table 3). All calves survived the entire observation period. The HPN calves tended to have elevated ($P = 0.079$) TNF- α concentrations on d 1 and were increased ($P = 0.024$) on d 5

Figure 1. Effects of plane of nutrition for experiment 1 (LPN = low plane of nutrition, n = 23; HPN = high plane of nutrition, n = 20) on expression of L-selectin protein (CD62L) on the surface of peripheral blood neutrophils at 7, 21, 42, and 77 d after initiation of treatments. A treatment $\times$ time effect was observed ($P = 0.031$); sliced time effects are denoted with an asterisk (* $P \leq 0.05$). Error bars represent SEM. GMFI = geometric mean fluorescence intensity.

Table 3. Influence of plane of nutrition on morbidity, calf starter intake, plasma glucose concentrations, and plasma urea nitrogen concentrations of Jersey bull calves challenged with *Salmonella enterica* serotype Typhimurium (experiment 2)

Item	Nutrition ¹		Largest SEM	P-value		
	LPN	HPN		Treatment	Time	Treatment × time
Morbidity, %	45.5	22.2	—	<0.279	—	—
DMI, kg/d	1.81	2.05	0.075	<0.031	<0.001	<0.321
CP intake, g/d	360.7	437.8	24.23	<0.029	<0.001	0.891
ME intake, Mcal/d	5.1	5.8	0.33	<0.131	<0.001	<0.923
CP intake per kilogram of metabolic BW, g/BW ^{0.75}	18.39	19.33	0.956	<0.479	<0.001	<0.958
ME intake per kilogram of metabolic BW, Mcal/BW ^{0.75}	0.261	0.257	0.0132	<0.845	<0.001	<0.972
Plasma glucose, mg/dL	71.7	77.3	1.39	<0.007	<0.001	<0.715
Plasma urea nitrogen, mg/dL	13.8	12.1	0.38	<0.004	<0.097	<0.373

¹LPN = low plane of nutrition (n = 11); HPN = high plane of nutrition (n = 9).

and 6 compared with the LPN calves (Figure 3a). A treatment effect ($P = 0.005$) was also observed for the percentage of neutrophils positive for an oxidative burst response, whereas HPN calves had a greater percentage on d 1, 2, 3, 4, and 5 (Figure 3b) compared with LPN calves. When measuring geometric mean fluorescence intensity of oxidative burst positive neutrophils, the HPN calves tended to be elevated on d 3 and 4 ($P = 0.097$; Figure 3c) compared with the LPN calves. There was no treatment × time or treatment effect on rectal temperatures ($P > 0.841$). The rectal temperatures in both treatments peaked at 48 h postchallenge and

returned to circadian rhythm at approximately 120 h; however, a larger circadian fluctuation was observed throughout the duration of the observation period (Figure 4). Median ranks of plasma haptoglobin concentrations were lower ($P = 0.037$) among the HPN calves throughout the study. The mean plasma haptoglobin concentrations after the challenge were 2.63 vs 1.72 ± 0.838 optical density × 100 for the LPN and HPN, respectively. Plasma concentrations of zinc tended to be decreased among LPN calves ($P = 0.098$, Figure 5). The treatment effects sliced by time indicated that the LPN calves tended ($P = 0.096$) to have reduced plasma

Figure 2. Effects of plane of nutrition in experiment 2 (LPN = low plane of nutrition, n = 11; HPN = high plane of nutrition, n = 9) on calf starter DMI from −6 to 9 d relative to an oral challenge with 1.5×10^8 cfu of *Salmonella enterica* serotype Typhimurium ATCC #14028 on d 80 after initiation of treatments. No treatment × time effect was observed ($P = 0.321$), but treatment ($P = 0.039$) and time ($P = 0.001$) effects were seen; sliced time effects were denoted by an asterisk (* $P \leq 0.05$, # $0.05 > P \leq 0.10$). Error bars represent $\pm$ SEM.

zinc on d 1 and 4 and were lower ($P = 0.033$) on d 3 after the challenge.

DISCUSSION

Plane of nutrition positively influenced ADG during the preweaning period and improved the efficiency of gain during both the pre- and immediate postweaning periods. Similar data were reported when calves were fed a plane of nutrition that achieved an ADG greater than 0.45 kg/d (Bartlett et al., 2006; Bascom et al., 2007; Ballou, 2012). The greater ADG of the HPN calves was not continued through the immediate postweaning period. In fact, the starter intake among HPN calves lagged behind that of the LPN calves immediately following weaning, which accounted for the reduced ADG in the HPN calves from 42 to 56 d. This slump in starter intake and ADG after weaning among calves fed a HPN was reported previously (Ballou et al., 2013). Hill et al. (2012) reported that gradually weaning calves over a 14- to 21-d period for calves that were previously fed 0.88 kg of DM of milk solids per day did not result in reductions in ADG or calf starter intake. Additional weaning strategies that prevent a slump in ADG following weaning among calves fed higher planes of nutrition need to be investigated. Despite the immediate slump in ADG for the 2 wk during weaning, no difference in ADG was observed over the entire immediate preweaning period, from 42 to 77 d.

There was a high incidence of calves with high fecal scores in the current study, and calves fed the HPN had a greater incidence as well as duration of high fecal scores. Calves fed HPN were previously reported to have greater fecal scores (Nonnecke et al., 2003; Bartlett et al., 2006). Future research should determine if calves fed HPN have reduced fecal DM percentage and whether the greater fecal scores in the first few weeks of life could be attributed to reduced digestibility of milk solids or are the result of increased consumption of water, as suggested by Nonnecke et al. (2003). If the digestibility of milk solids is reduced, then calves fed HPN would be at a greater risk for both nutritional and infectious scours because of increased substrate for microbial growth in the lower gastrointestinal tract. In contrast, if digestibility is not influenced then the increased fecal scores could be protective against gastrointestinal disease because the greater volume of solids that move through the gastrointestinal tract may reduce pathogen colonization or dilute any potential enterotoxin. In agreement, Ollivett et al. (2012) observed that feeding greater quantities of MR to calves reduced duration of scours and improved hydration following a *Cryptosporidium parvum* challenge at 3 d of age.

Increased plasma glucose concentrations are commonly reported among HPN calves over most of the preweaning period (Quigley et al., 2006; Foote et al., 2007; Obeidat et al., 2013). The greater plasma glucose concentrations are likely due to the increased consumption of lactose in the milk solids. The greater plasma urea nitrogen concentrations among the HPN calves at d 7 may be due to the increased intake of CP that is not being deposited into lean tissue during the first week of life. In agreement, Obeidat et al. (2013) reported that Holstein calves fed an HPN had greater plasma urea nitrogen concentrations at d 3. However, on d 42 and 77 the HPN had reduced plasma urea nitrogen concentrations, which may be associated with the greater deposition of lean tissue. The HPN calves had greater ADG in the periods immediately before those sample collections. In contrast, Ballou et al. (2013) reported no difference in plasma urea nitrogen concentrations between Jersey calves fed either 454 g/d of a 20% CP and 20% fat MR or 680 g/d of a 28% CP and 25% fat MR. The difference observed between the 2 studies may be due to the quantity of the MR fed, because Ballou et al. (2013) fed 680 g/d, which was less than the 735 g/d of the same 28% CP and 25% fat MR.

Enteric disease is common among dairy calves during the first few weeks of life, as they adapt to the *ex utero* environment. Passively derived immunoglobulins and the innate immune system are important in protecting the calf from potential pathogens. The innate immune system of LPN calves may have been primed or more active during the preweaning period, as suggested by the elevated neutrophil L-selectin protein concentrations at d 7, 21, and 42. In agreement, Obeidat et al. (2013) reported that Holstein calves fed an LPN of MR had elevated neutrophil L-selectin protein concentrations during the preweaning period when compared with Holstein calves fed an HPN of MR. In addition, Obeidat et al. (2013) observed more neutrophils producing an oxidative burst, and the intensity of the oxidative burst was greater among the Holstein calves fed the LPN of MR. Those authors suggested that the more active neutrophil responses of Holstein calves fed the LPN may be due to more immunogenic stimulation because these calves had more nonnutritive suckling following MR feedings (Rushen and de Passillé, 1995). In support, microbial immunogenic stimulation in the gastrointestinal tract increased the activity of peripheral blood neutrophils in rodent models (Clarke et al., 2010). Our study agrees with Obeidat et al. (2013) that neutrophils of calves fed lower planes of nutrition are more active, and future research should determine the underlying mechanisms and health implications of this altered immune response phenotype during the preweaning period.

Figure 3. Effects of plane of nutrition in experiment 2 (LPN = low plane of nutrition; $n = 11$ and HPN = high plane of nutrition; $n = 9$) on the secretion of tumor necrosis factor- α from diluted whole-blood cultures stimulated with $1 \mu\text{g/mL}$ of lipopolysaccharide from *Escherichia coli* 0111:B4 (a), on the percentage of neutrophils producing an oxidative burst when cocultured with an *Escherichia coli* (b), and on the intensity of the oxidative burst produced by neutrophils cocultured with an *Escherichia coli* (c) from immediately before to 9 d after an oral challenge with 1.5×10^8 cfu of *Salmonella enterica* serotype Typhimurium ATCC #14028 on d 80 after initiation of treatments. No treatment $\times$ time effect was noted ($P = 0.829$), but treatment ($P = 0.014$) and time ($P = 0.001$) effects were observed; sliced time effects are denoted by an asterisk ($*P \leq 0.05$) and a pound sign ($\#0.05 > P \leq 0.10$). Error bars represent SEM. GMFI = geometric mean fluorescence intensity.

The greater neutrophil L-selectin protein concentrations observed among the LPN calves during the preweaning period was not observed during the immediate postweaning period, at d 77. In fact, no differences in any of the innate leukocyte responses were observed at d 77. The data reported by Obeidat et al. (2013) are consistent with the finding that previous plane of nutrition during the preweaning period did not have any carryover effects on neutrophil activity during the immediate postweaning period. However, Ballou (2012) reported that both neutrophil oxidative burst and whole-blood bactericidal capacity of Jersey calves previously fed an LPN were reduced during the immediate postweaning period relative to Jersey calves previously fed an HPN. Experiment 2 of our study was designed to test the hypothesis that Jersey calves fed an HPN would have improved resistance to an oral *Salmonella* Typhimurium challenge during the immediate postweaning period. If an infection evades the physical barriers of the immune system and cannot be controlled by humoral factors, the ideal cellular innate immune response is a rapid increase in the activity of phagocytes to clear the infection, followed by a rapid return to homeostasis. A delay in the response of phagocytes increased the risk and severity of disease (Heyneman et al., 1990). The infection model used in the present study was a mild challenge with an expected clinical disease dose₅₀ (i.e., where 50% of the calves show clinical signs of disease) and was chosen because the objective was to test the hypothesis that feeding an HPN would improve disease resistance and was not designed to evaluate the response to disease. Data from the current study indicate that calves that were fed a HPN had a more rapid increase in many cellular innate leukocyte responses, including the secretion of TNF- α when whole blood was stimulated with LPS as well as neutrophil oxidative burst to an *E. coli*. It cannot be completely ruled out that the more rapid response among the HPN calves was due to greater immunogenic stimulation caused by an impaired physical barrier of the gastrointestinal mucosa or humoral factors among those calves. However, the attenuated plasma concentrations of haptoglobin, the greater plasma zinc concentrations, improved calf starter intake, and the numerical decrease in the frequency of calves classified as having clinical signs of disease among the HPN calves suggests that HPN calves had less systemic inflammation following the oral *Salmonella* Typhimurium challenge.

In addition to macronutrient differences between the planes of nutrition or a carryover affect from the preweaning period, disparities in micronutrient sources or other feed additives between the 2 dietary treatments could not be ruled out. Obeidat et al. (2013) pointed out that future research studying different planes of

Figure 4. Effects of plane of nutrition in experiment 2 (LPN = low plane of nutrition, $n = 11$; HPN = high plane of nutrition, $n = 9$) on rectal temperatures taken every 5 min and averaged by hour from -18 to 240 h relative to an oral challenge with 1.5×10^8 cfu of *Salmonella enterica* serotype Typhimurium ATCC #14028 on d 80 after initiation of treatments. No treatment $\times$ time ($P = 0.841$) or treatment ($P = 0.912$) effects were observed, but there was a time effect ($P = 0.001$). The SEM was 0.073°C .

nutrition should control for micronutrient sources and additional feed additives. However, in the current study the organic micromineral sources and the dried *Saccharomyces cerevisiae* fermentation extract that was used in the formulation of HPN treatment and not in the LPN treatment did not increase leukocyte responses at any time before the *Salmonella* Typhimurium challenge. It was only after the *Salmonella* Typhimurium challenge that innate leukocytes were more active among the HPN calves.

CONCLUSIONS

The plane of milk or milk replacer nutrition fed to preweaning dairy calves influences leukocyte responses and disease resistance. The present data indicate that calves fed lower planes of milk replacer have more active neutrophil responses during the preweaning period only. More data are needed to understand the underlying mechanisms behind the more active neutrophils and the significance of this to the development of the immune system of the calf. These data also indicate that the innate leukocytes of Jersey calves fed the higher plane of nutrition increased more rapidly after an oral

Figure 5. Effects of plane of nutrition in experiment 2 (LPN = low plane of nutrition, $n = 11$; HPN = high plane of nutrition, $n = 9$) on plasma zinc concentrations from immediately before to 9 d after an oral challenge with 1.5×10^8 cfu of *Salmonella enterica* serotype Typhimurium ATCC #14028 on d 80 after initiation of treatments. No treatment $\times$ time effect was seen ($P = 0.196$), but treatment ($P = 0.098$) and time ($P = 0.001$) effects were observed; sliced time effects are denoted with an asterisk ($*P \leq 0.05$) and a pound sign ($\#0.05 > P \leq 0.10$). Error bars represent SEM.

challenge with *Salmonella* Typhimurium. Future research needs to determine if the improved leukocyte responses and health among calves fed the higher plane of nutrition were a carry-over effect from the preweaning period or due to differences in the diets at the time the calves were challenged.

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