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Increasing the metabolizable protein supply enhanced growth performance and led to variable results on innate and humoral immune response of preconditioning beef steers[1]

P. Moriel,*[2] L. F. A. Artioli,* M. H. Poore,* A. W. Confer,† R. S. Marques,‡ and R. F. Cooke‡

*Department of Animal Science, North Carolina State University, Waynesville 28786; †Department of Veterinary Pathobiology, Center for Veterinary Health Sciences, Oklahoma State University, Stillwater 74078; and ‡Eastern Oregon Agricultural Research Center, Oregon State University, Burns 97720

ABSTRACT: We evaluated the effects of MP supply on growth performance before and after preconditioning and measurements of innate and humoral immune response of beef steers following vaccination. Angus steers (n = 36; BW = 231 ± 21 kg; age = 184 ± 18 d) were weaned on d −6, stratified by BW and age on d 0, and randomly assigned to 1 of 18 drylot pens (2 steers/pen). Treatments were assigned to pens (6 pens/treatment) and consisted of corn silage–based diets formulated to provide 85%, 100%, or 115% of the daily MP requirements of a beef steer gaining 1.1 kg/d from d 0 to 42. Steers were vaccinated against infectious bovine rhinotracheitis virus, bovine viral diarrhea (BVDV) types 1 and 2 viruses, *Mannheimia haemolytica,* and clostridium on d 14 and 28. Blood samples were collected on d 0, 14, 15, 17, 21, 28, 29, 30, 35, and 42. Body weight did not differ ($P \geq 0.17$) among treatments from d 0 to 28. On d 42, 115% MP steers were heaviest, 100% MP steers were intermediate, and 85% MP steers were lightest ($P = 0.05$; 297, 290, and 278 ± 7 kg, respectively). Overall, ADG and G:F did not differ ($P \geq 0.13$) between 100% and 115% MP steers and were least ($P < 0.01$) for 85% MP steers (1.2, 1.4, and 0.8 ± 0.07 kg/d and 0.23, 0.24, and 0.19 ± 0.008, respectively). Plasma haptoglobin (Hp) concentrations did not differ among treatments ($P \geq 0.46$), whereas plasma ceruloplasmin (Cp) concentrations were greatest ($P \leq 0.04$) for 85% MP steers, intermediate for 100% MP steers, and least for 115% MP steers on d 30, 35, and 42. Plasma cortisol concentrations were greater ($P \leq 0.03$) for 85% vs. 100% and 115% MP steers on d 14 and 28. Liver mRNA expression of Cp and Hp and muscle mRNA expression of m-calpain, mammalian target of rapamycin, and ubiquitin did not differ among treatments ($P \geq 0.17$). Serum neutralization titers to BVDV-1b titers were greater ($P \leq 0.02$) for 115% vs. 85% and 100% MP steers on d 42 (5.8, 3.0, and 3.7 ± 0.60 $\log_2$, respectively), whereas mean serum *M. haemolytica* leukotoxin titers were greater for 85% vs. 100% and 115% MP steers (3.1, 2.4, and 2.5 ± 0.21 $\log_2$, respectively). Preconditioning MP supply did not affect ($P \geq 0.26$) **s**ubsequent finishing growth performance and carcass characteristics. Thus, increasing MP supply from 85% to 115% of daily requirement of preconditioning beef steers had variable results on innate and humoral immune response and enhanced growth performance during a 42-d preconditioning period without affecting carcass characteristics at slaughter.

Key Words: acute-phase response, beef steers, humoral, innate immune, preconditioning, vaccination

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INTRODUCTION

Acute-phase response (**APR**) typically occurs following an immunological challenge, such as vaccination, weaning, and feedlot entry (Cooke et al., 2011; Arthington et al., 2013). During the APR, nu-

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[2]Corresponding author: pmoriel@ncsu.edu

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trient demand is increased to accommodate the synthesis of acute-phase proteins (**APP**), immune cells, and gluconeogenic precursors (Reeds and Jahoor, 2001; Waggoner et al., 2009). To support an immunological response, muscle protein is mobilized (Jahoor et al., 1999), and absorbed AA are shifted from growth toward hepatic uptake (Reeds et al., 1994; Reeds and Jahoor, 2001). However, increasing MP intake is capable of providing the necessary substrate for APP synthesis, energy production, and other immunological processes (Jennings et al., 1992; Moriel and Arthington, 2013), and it simultaneously alleviates muscle mobilization and improves N retention (Waggoner et al., 2009).

Preconditioning beef calves may experience decreased energy and MP intake (Duff and Galyean, 2007) and multiple APR induced by weaning, feedlot entry, and vaccination, which may substantially increase nutrient demand and exacerbate nutrient deficiency, leading to compromised performance and immune response. Also, MP deficiency may induce innate immune system suppression and decrease the communication between innate and humoral response (Dai and McMurray, 1998). However, there is a lack of studies evaluating the interaction between nutrition and vaccination response (Arthington et al., 2013). Thus, we hypothesized that increasing the MP supply to preconditioning beef steers would increase AA substrate to support APR, alleviate muscle mobilization, and enhance humoral immune response following vaccination. Thus, our objectives were to evaluate the growth performance and measurements of innate and humoral immune response of vaccinated beef steers provided increasing amounts of MP during a 42-d preconditioning and subsequent effects on feedlot growth performance and carcass characteristics.

MATERIALS AND METHODS

All procedures for the experiment conducted at the Butner Beef Cattle Field Laboratory (Butner, NC) were approved by the Institutional Animal Care and Use Committee of North Carolina State University (14-054-A).

Animals, Diets, and Sample Collection

Preconditioning Phase (d 0 to 42). On d −6, Angus-crossbred steers (n = 36; initial BW = 231 ± 21 kg; initial age = 184 ± 18 d) were weaned and immediately transferred to a single 2-ha tall fescue pasture (*Lolium arundinaceum*; 21% CP and 62% TDN; DM basis) with corn silage supplementation at 0.5% of BW (DM basis) for 6 d. On d 0, steers were stratified by BW and age and randomly allocated into 1 of 18 concrete slatted floor pens (11 m^2 and 2 steers/pen) in a fully covered drylot facility. Treatments were randomly assigned to pens (6 pens/treatment) and consisted of steers receiving 1 of 3 diets formulated to provide 85%, 100%, or 115% of the daily MP requirement of a beef steer gaining 1.1 kg of BW daily (NRC, 2000; Table 1). Diets were provided once daily (0800 h) at 2.01%, 2.14%, and 2.24% BW (DM basis) for 85%, 100%, and 115% MP steers, respectively, to ensure similar TDN intake from d 0 to 42. Body weight was measured on d 0 and 42 after 12 h of feed and water withdrawal and was used for calculation of overall ADG, whereas on d 14 and 28, steers were weighed before feeding (0800 h) to not disturb their DMI or elicit a physiologic stress response. Diet DM offered was estimated on the basis of average BW of each pen on d 0 and was readjusted accordingly to BW change on d 28. Accumulated diet refusal (as fed) was measured once weekly. Diet DM refusal and offered were obtained by drying weekly samples of refusal and offer in a forced-air oven at 56°C for 48 h. Daily DMI was determined by subtracting the weekly DM refusal from the weekly DM offer and then dividing by 7 d.

Before diet formulation, individual feed ingredients were analyzed in duplicate for chemical composition using wet chemistry procedures at a commercial laboratory (Dairy One Forage Laboratory, Ithaca, NY). Using the mechanistic model (level 2) of the Cornell Net Carbohydrate and Protein System (**CNCPS**, version 5.0.29; Cornell University, Ithaca, NY), diets were formulated to provide similar microbial MP but increasing RUP supply (Moriel and Arthington, 2013) to reduce differences on ruminal protein degradation among treatments that could affect the total MP supply. Samples of diet offer and refusal were collected weekly and sent in duplicate for chemical composition using wet chemistry procedures at a commercial laboratory (Dairy One Forage Laboratory, Ithaca, NY). Nutritional compositions of diets are shown in Table 1.

On d 14, all steers were implanted with 36 mg of zeranol (Ralgro, Merck Animal Health, Summit, NJ), treated with doramectin for internal and external parasites (5 mL subcutaneous [**s.c.**]; Dectomax injectable, Zoetis Inc., Kalamazoo, MI), and vaccinated against infectious bovine rhinotracheitis (**IBR**), bovine viral diarrhea types 1 and 2 viruses, *Mannheimia haemolytica* (2 mL s.c.; Bovi Shield One Shot, Zoetis Inc.), and clostridium (2 mL s.c.; Ultrabac 7, Zoetis Inc.). On d 28, steers received a 2-mL s.c. booster of Bovi Shield Gold 5 (Zoetis Inc.) and Ultrabac 7 (Zoetis Inc.). The vaccination and parasite control protocol described above was chosen to replicate the protocol established by the local preconditioning alliance (Mountain Cat-

Table 1. Nutritional composition of diets

Item	Treatment[1]		
	85% MP	100% MP	115% MP
Ingredients, % DM			
Corn silage	40.7	51.1	52.7
Ground wheat	38.9	4.3	6.5
Ground corn	9.1	23.0	0.0
Soybean meal	4.5	11.1	14.6
Limestone	1.8	1.7	1.5
Dried distillers grains	4.5	8.5	24.3
Vitamin premix[2]	0.01	0.01	0.01
Trace mineral salt[3]	0.36	0.34	0.32
Monensin[4]	0.036	0.034	0.032
Sodium selenite 1%	0.001	0.001	0
Composition (DM basis)			
DM, %	51	45	45
NDF, %	24	26	35
TDN,[5] %	79	76	74
ME, Mcal/kg	2.86	2.75	2.69
NE_m, Mcal/kg	2.14	2.05	1.98
NE_g, Mcal/kg	1.28	1.21	1.15
CP, %	11	15	19
RDP, % CP	72	66	60
Microbial MP,[6] g/d	404	392	375
RUP,[6] g/d	111	195	282
Total MP,[6] g/d	505	587	657
NSC, %	59	50	41
Ca, %	0.46	0.35	0.44
Cu, mg/kg	7.0	6.0	7.0
Fe, mg/kg	322	405	306
K, %	0.81	0.93	1.12
Mg, %	0.18	0.17	0.21
Mn, mg/kg	43	35	38
Mo, mg/kg	<1	<1	1.5
Na, %	0.19	0.18	0.22
P, %	0.39	0.37	0.48
S, %	0.16	0.17	0.28
Zn, mg/kg	46	39	51

[1]Diets were formulated to provide 85%, 100%, and 115% of daily MP requirement of a beef steer gaining 1.1 kg of BW daily and were limit fed at 2.01%, 2.14%, and 2.24% of BW (DM basis), respectively, to ensure similar TDN intake from d 0 to 42. Diets were pooled by week and sent in duplicate to a commercial laboratory for wet chemistry analysis (Dairy One Laboratory, Ithaca, NY).

[2]Premix containing 9,911,000, 2,000, and 4,405 IU/kg of vitamins A, D_3, and E, respectively (Provimi North America Inc., Brookville, OH).

[3]Guaranteed analysis: 90% NaCl, 32.9% Na, 0.91% S, 72 mg/kg Co, 5,000 mg/kg Cu, 104 mg/kg I, 2,500 mg/kg Mn, 104 mg/kg Se, and 10,000 mg/kg Zn (Cargill Animal Nutrition, Minnetonka, MN).

[4]Rumensin 90 (Elanco, Greenfield, IN).

[5]Calculated as described by Weiss et al. (1992).

[6]Microbial MP, RUP, and total MP were estimated using level 2 of Cornell Net Carbohydrate and Protein System (CNCPS, version 5.0.29; Cornell University, Ithaca, NY).

tle Alliance, Western NC Regional Livestock Center, Canton, NC). The vaccination protocol was initiated 21 d after weaning to avoid the weaning-induced inflammatory response that could interfere with the vaccination response (Richeson et al., 2008).

Blood samples were collected via jugular venipuncture in tubes (Vacutainer, Becton Dickinson, Franklin Lakes, NJ) containing sodium heparin (158 U.S. Pharmacopeia units) for plasma harvest on d 0, 14, 15, 17, 21, 28, 29, 30, 35, and 42. In addition, blood samples from the jugular vein were collected into tubes containing no additives (Vacutainer, Becton Dickinson) for serum harvest on d 0, 28, and 42. Blood samples were immediately placed on ice following collection and were then centrifuged at 1,200 × *g* for 25 min at 4°C. Plasma and serum samples were stored frozen at −20°C until later analysis for plasma concentrations of haptoglobin, ceruloplasmin, urea nitrogen (PUN), NEFA, IGF-1, and cortisol and serum titers against IBR, bovine viral diarrhea virus type 1b (**BVDV-1b**), and *M. haemolytica* leukotoxin.

On d 0, steers within each pen were randomly assigned for a liver or LM biopsy (1 liver or 1 muscle tissue sample/steer) on d 17 and 30, which corresponds to the vaccination-induced peak of inflammatory response based on plasma concentrations of haptoglobin (Moriel and Arthington, 2013; Arthington et al., 2013). Each steer remained in its respective tissue assignment on d 17 and 30. Liver and muscle samples were collected via needle biopsy, following the procedure described by Arthington and Corah (1995). Immediately following collection, 100 mg of wet tissue were stored into 1.5 mL of RNA stabilization solution (RNAlater, Ambion Inc., Austin, TX), kept on ice for 8 h, and stored at −80°C.

Postpreconditioning Phase (d 43 to Slaughter). On d 43, steers were walked for about 100 m to an adjacent feedlot facility and were individually fed a common diet from Calan-gate feeders (American Calan Inc., Northwood, NH) until slaughter (d 263). The postpreconditioning phase was divided into receiving (d 43 to 70), growing (d 71 to 206), and finishing periods (d 207 to 262). The diet provided from d 43 to 206 (76% TDN and 11% CP; DM basis) consisted of 36.4% concentrate and 73.6% corn silage (DM basis). Concentrate consisted of a mixture of 86.4% ground corn, 11.2% soybean meal, 0.5% urea, 1.6% limestone, 0.30% vitamin premix (same as described previously), and 0.20% rumensin (Elanco Animal Health, Greenfield, IN). Steers were trained to eat from Calan-gate feeders for 10 d, and hence, DMI from d 43 to 52 was not measured. Starting on d 207, steers were adapted to the finishing diet by gradually decreasing corn silage and simultaneously increasing concentrate inclu-

sion by 6% units daily over 9 d. Hence, the finishing diet (85% TDN and 14% CP; DM basis) was provided from d 214 to slaughter and consisted of 14.7% corn silage and 85.3% of the same concentrate described above (DM basis). Diet DM was determined by drying diet samples in a forced-air oven at 55°C for 48 h. Dry matter intake from d 43 to slaughter was determined by multiplying the respective diet DM percentage by the daily diet intake (as-fed basis). Samples of concentrate and corn silage were pooled by month and sent in duplicate for chemical composition using wet chemistry procedures at a commercial laboratory (Dairy One Forage Laboratory). All steers were slaughtered on d 263 at a commercial packing facility. Longissimus muscle area, back fat thickness, KPH percentage, marbling scores, carcass quality, and yield grade were determined by a qualified USDA grader 48 h after slaughter.

Laboratory Analyses

Plasma concentrations of haptoglobin were determined in duplicate samples using a biochemical assay measuring haptoglobin-hemoglobin complex by the estimation of differences in peroxidase activity (Cooke and Arthington, 2013). Inter- and intra-assay CV of haptoglobin assays using the biochemical procedure were 3.5% and 7.9%, respectively. Plasma ceruloplasmin oxidase activity was measured in duplicate samples by using the colorimetric procedures described by Demetriou et al. (1974) and expressed as milligrams per deciliter, as described by King (1965). Inter- and intra-assay CV for ceruloplasmin assays were 2.7% and 7.0%, respectively. Commercial quantitative colorimetric kits were used to determine the plasma concentrations of PUN (B7551; Pointe Scientific Inc., Canton, MI) and NEFA (HR Series NEA-2; Wako Pure Chemical Industries Ltd. USA, Richmond, VA). Inter- and intra-assay CV for assays of PUN and NEFA were 2.7% and 3.4% and 3.2% and 7.8%, respectively. Plasma cortisol concentrations were analyzed in duplicate samples using a single chemiluminescent enzyme immunoassay (Immulite 1000; Siemens Medical Solutions Diagnostics, Los Angeles, CA). Intra-assay CV for cortisol assay was 4.8%. Plasma IGF-1 concentrations were analyzed in duplicate samples using commercial enzyme-linked immunosorbent assay kits (SG100; R&D Systems Inc., Minneapolis, MN) previously validated for bovine samples (Moriel et al., 2012). Inter- and intra-assay CV for IGF-1 assay were 1.31% and 2.65%, respectively.

Serum antibody titers against BVDV-1b and IBR were analyzed by the Oklahoma Animal Disease and Diagnostic Laboratory using a virus neutralization test, as described by Rosenbaum et al. (1970). Serum titers against IBR and BVDV-1b were reported as the $\log_2$ of the greatest serum dilution that provided complete protection of the cells (lowest and greatest tested dilutions were 1:4 and 1:256, respectively). For the seroconversion analysis, samples with a serum neutralization value of <4 were considered negative and assigned a value of 0, whereas samples with a serum neutralization value ≥4 were considered positive and assigned a value of 1. Then the assigned values (0 or 1) were used to calculate the positive seroconversion (percentage of steers with positive serum neutralization) to IBR and BVDV-1b. Serum antibodies titers against leukotoxin for a formalin-killed *M. haemolytica* S1 were determined in singles by an ELISA test as described by Confer et al. (1996). Leukotoxin titers were reported as nanograms of immunoglobulin binding based on a set of IgG standards present in each plate, as described by Burciaga-Robles et al. (2010). Serum titers against BVDV-1b, IBR, and *M. haemolytica* leukotoxin on d 0 were included as covariates, even though no treatment effects ($P \geq 0.30$) were detected on d 0.

A detailed description of procedures for mRNA isolation and tissue gene expression is given in Cappellozza et al. (2014). Briefly, total RNA was extracted from liver tissue samples using the TRIzol Plus RNA Purification Kit (Invitrogen, Carlsbad, CA). Extracted RNA was quantified via UV absorbance (UV Mini 1240; Shimadzu Scientific Instruments, Inc., Columbia, MD) at 260 nm, incubated (2.5 μg) at 37°C for 30 min in the presence of RNase-free (DNase; New England Biolabs Inc., Ipswich, MA), and reverse transcribed using the High Capacity cDNA Reverse Transcription Kit with random hexamers (Applied Biosystems, Foster City, CA). Real-time PCR was completed using the SYBR Green PCR Master Mix (Applied Biosystems) and gene-specific primers (20 p*M* each; Table 2) with the StepOne Real-time PCR system (Applied Biosystems). All primers were used and validated by previous research (Table 2) except for ceruloplasmin, which was designed using the Basic Local Alignment Search Tool (Ye et al., 2012). At the end of each real-time PCR, amplified products were subjected to a dissociation gradient (95°C for 15 s, 60°C for 30 s, and 95°C for 15 s) to verify the amplification of a single product by denaturation at the anticipated temperature. A portion of the amplified products was purified with the QIAquick PCR purification kit (Qiagen Inc., Valencia, CA) and was sequenced at the Oregon State University Center for Genome Research and Biocomputing (Corvallis, OR) to verify the specificity of amplification. All amplified products represented only the genes of interest. In addition, ceruloplasmin primers yielded a single-peak dissociation curve with an average melting point of 79.64°C ± 0.02°C, which is similar to that

Table 2. Nucleotide sequence of bovine-specific primers used in the quantitative real-time reverse transcription PCR to determine the hepatic mRNA expression of ceruloplasmin, haptoglobin, and LM mRNA expression of m-calpain, mammalian target of rapamycin (mTOR) and ubiquitin

Target gene	Primer sequence[1]	Accession number
Ceruloplasmin		XM_002685007
Forward	AGCAGAGACTGGAGACCTCAT	
Reverse	GCCCGTAGTGTTGTCTGGAT	
Cyclophilin		NM_178320.2
Forward	GGTACTGGTGGCAAGTCCAT	
Reverse	GCCATCCAACCACTCAGTCT	
Haptoglobin		AJ_271156
Forward	GTCTCCCAGCATAACCTCATCTC	
Reverse	AACCACCTTCTCCACCTCTACAA	
m-Calpain		XM_864105
Forward	GGAGGAAGAGGACGAGGAC	
Reverse	TTGCTGAGGTGGATGTTGG	
mTOR		NM_004958.2
Forward	CGGGACTACAGGGAGAAAAA	
Reverse	CCTCAAAGCAGTCCCCAAAG	
Ubiquitin		NM_174133
Forward	GCCGCACTCTTTCTGATTACAAC	
Reverse	CGTTCTCGATGGTGTCACTGG	

[1]Primer sequences obtained for ceruloplasmin (Ye et al., 2012), cyclophilin (Cappellozza et al., 2014), haptoglobin (Hiss et al., 2004), mTOR (Hayashi et al., 2009), m-calpain, and ubiquitin (Chibisa et al., 2008).

predicted by the Basic Local Alignment Search Tool (79.7°C). Responses were quantified on the basis of the threshold cycle (**CT**) and normalized to cyclophilin CT (Δ**CT**) examined in the same sample and assessed at the same time as the targets. Within each target gene, results are expressed as relative fold change ($2^{-\Delta\Delta CT}$) using the sample with the greatest ΔCT as reference, as described by Ocón-Grove et al. (2008).

Statistical Analyses

Except for seroconversion analysis, all data were analyzed as a completely randomized design using the MIXED procedure of SAS (version 9.3; SAS Inst. Inc., Cary, NC) with Satterthwaite approximation to determine the denominator degrees of freedom for the test of fixed effects. During the preconditioning phase, pen was the experimental unit and steer (pen) and pen (treatment) were included as random variables. Average daily gain and G:F were tested for fixed effects of treatment. Body weight, plasma and serum measurements (except for seroconversion), and tissue mRNA expression were analyzed as repeated measures with steer (pen) as the subject. The covariance structure was chosen using the lowest Akaike information criterion. The unstructured covariance structure was used for the analysis of mRNA expression and plasma and serum measurements, whereas compound symmetry covariance structure was used for the BW analysis throughout the study. Plasma measurements obtained on d 0 were not significant covariates ($P \geq 0.15$) and were removed from the model. Serum conversion to IBR and BVDV-1b was analyzed as repeated measures using the GLIMMIX procedure of SAS and pen (treatment) and steer (pen) as random variables. The previous preconditioning pen assignment remained the experimental unit for statistical analysis during the postpreconditioning phase. Body weight, ADG, DMI, G:F, and carcass characteristics were tested for the fixed effects of treatment using steer (treatment) as random effect. All results are reported as least squares means. Data were separated using PDIFF if a significant preliminary F test was detected. Significance was set at $P \leq 0.05$, and tendencies were indicated if $P > 0.05$ and ≤ 0.10.

RESULTS

Preconditioning Phase. A treatment × day effect was detected ($P < 0.0001$) for BW from d 0 to 42 (Table 3). Body weight did not differ ($P \geq 0.17$) among treatments from d 0 to 28. However, 115% MP steers were heaviest, and 85% MP steers were lightest ($P = 0.05$), whereas 100% MP steers were intermediate on d 42 ($P \geq 0.21$). Average daily gains from d 0 to 14, d 28 to 42, and d 0 to 42 were similar ($P \geq 0.13$) between 100% and 115% MP steers, but both had greater ($P \leq 0.05$) ADG than 85% MP steers (Table 3). Overall G:F (d 0 to 43) was similar ($P = 0.28$) between 100% and 115% MP steers, but both had greater ($P \leq 0.003$) G:F than 85% MP steers (Table 3).

Effects of treatment × day were detected for intake of total DM ($P = 0.0003$; Fig. 1) and NE_g ($P = 0.01$). Total DMI of 85% MP steers was less ($P \leq 0.005$) than that of 100% and 115% MP steers on wk 1 and 2 and less than that of 115% MP steers ($P \leq 0.004$) but not different ($P \geq 0.10$) from that of 100% MP steers from wk 3 to 6. Total DMI was greater ($P \leq 0.02$) for 115% MP steers than for 100% MP steers on wk 1 and 3 but did not differ ($P \geq 0.11$) on wk 2, 4, 5, and 6 (Fig. 1). Intake of NE_g on wk 1 did not differ ($P = 0.45$) between 100% and 115% MP steers, but both had greater ($P \leq 0.01$) NE_g intake than 85% MP steers (5.26, 5.48, and 4.50 ± 0.200 Mcal/d, respectively). Intake of NE_g did not differ ($P \geq 0.19$) among treatments from wk 2 to 6 (mean = 4.74, 5.27, 5.96, 6.29, and 6.51 ± 0.200 Mcal/d for wk 2, 3, 4, 5, and 6, respectively). However, overall NE_g intake did not differ ($P = 0.27$) among treatments (5.90, 6.17, and 6.32 ± 0.176 Mcal/d, respectively). A treatment × day effect was not detected ($P = 0.50$) for CP

Table 3. Growth performance of steers provided increasing supply of MP (85%, 100%, and 115% of MP requirements; n = 6 pens/treatment; 2 steers/pen) during a 42-d preconditioning period

	Treatment[1]				*P*-value	
Item	85% MP	100% MP	115% MP	SEM	Treatment	Treatment × day
BW,[2] kg						
d 0	243[a]	240[a]	240[a]	6.6	0.52	<0.0001
d 14	250[a]	257[a]	263[a]			
d 28	265[a]	271[a]	278[a]			
d 42	278[a]	290[a,b]	297[b]			
ADG, kg/d						
d 0 to 14	0.48[a]	1.22[b]	1.61[b]	0.231	0.02	—
d 14 to 28	1.09[a]	1.05[a]	1.09[a]	0.108	0.95	—
d 28 to 42	0.92[a]	1.33[b]	1.34[b]	0.111	0.02	—
d 0 to 42	0.83[a]	1.20[b]	1.35[b]	0.068	<0.0001	—
G:F[3]	0.19[a]	0.23[b]	0.24[b]	0.008	0.002	—

[a,b]Within a row, means without a common superscript differ ($P \leq 0.05$).

[1]Diets were formulated to provide 85%, 100%, and 115% of daily MP requirement of a beef steer gaining 1.1 kg of BW daily and were limit fed at 2.01%, 2.14%, and 2.24% of BW (DM basis), respectively, to ensure similar TDN intake from d 0 to 42.

[2]Steers were weighed on d 0 and 42 following a 12-h period of feed and water withdrawal, whereas on d 14 and 28, steers were weighed before feeding (0800 h).

[3]Estimated by dividing total BW gain by total DMI (d 0 to 42).

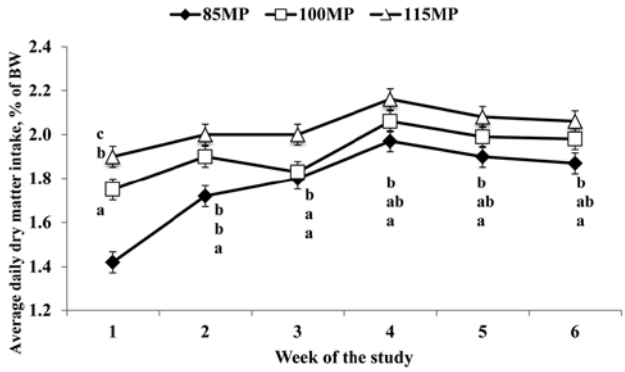

Figure 1. Total daily DMI of beef steers provided 85%, 100%, and 115% of MP requirements of a beef steer gaining 1.1 kg of BW daily (NRC, 2000). Effects of treatment × day were detected for intake of total DM (P = 0.0003). Total DMI of 85% MP steers was less ($P \leq 0.005$) than that of 100% and 115% MP steers in wk 1 and 2 and less than that of 115% MP steers ($P \leq 0.004$) but not different ($P \geq 0.10$) from that of 100% MP steers from wk 3 to 6. Total DMI was greater ($P \leq 0.02$) for 115% vs. 100% MP steers in wk 1 and 3 but did not differ ($P \geq 0.11$) in wk 2, 4, 5, and 6.[a–c]Within day, means without a common superscript differ ($P \leq 0.05$).

intake, but overall CP intake was greatest ($P < 0.0001$) for 115% MP steers, intermediate for 100% MP steers, and least for 85% MP steers (0.19%, 0.29%, and 0.39% ± 0.004% of BW, respectively).

Effects of treatment × day were detected for plasma concentrations of PUN ($P < 0.0001$; Fig. 2a) and ceruloplasmin (P = 0.01; Fig. 2b). Plasma concentrations of PUN were always greater ($P \leq 0.0001$) for 115% vs. 85% and 100% MP steers, except for on d 0, on which concentrations did not differ among treatments ($P \geq 0.39$). Plasma PUN concentrations were greater ($P \leq 0.009$) for 100% vs. 85% MP steers on d 17, 30, 35, and 42 but did not differ ($P \geq 0.27$) on d 0, 14, 21, and 28. On d 21, plasma concentrations of ceruloplasmin did not differ (P = 0.76) between 85% and 100% MP steers, whereas steers provided 115% MP diet had (P = 0.04) and tended to have (P = 0.09) greater plasma concentrations of ceruloplasmin than 85% and 100% MP steers, respectively. On d 30, 35, and 42, plasma concentrations of ceruloplasmin were greater ($P \leq 0.04$) for 85% vs. 115% MP steers and intermediate ($P \geq 0.09$) for 100% MP steers.

A tendency for treatment × day effect was detected (P = 0.07) for plasma concentrations of cortisol (Fig. 2c). On d 14, plasma concentrations of cortisol were greater (P = 0.03) for 85% vs. 100% MP steers and intermediate ($P \geq 0.17$) for 115% MP steers. On d 28, plasma concentrations of cortisol were greater (P = 0.02) for 85% vs. 115% MP steers and intermediate ($P \geq 0.22$) for 100% MP steers. Treatment × day effects were detected (P = 0.001) for plasma IGF-1 (Fig. 2d). On d 14, plasma IGF-1 was greater for 115% vs. 85% MP steers (P = 0.05) and intermediate for 100% MP steers ($P \geq 0.31$). On d 21, plasma IGF-1 was greatest for 115% MP, least for 85% MP ($P \leq 0.02$), and intermediate for 100% MP steers ($P \geq 0.13$). On d 35 and 42, plasma IGF-1 did not differ ($P \geq 0.66$) between 85% and 115% MP steers, but both had greater plasma IGF-1 than 100% MP steers ($P \leq 0.05$). Effects of treatment and treatment × day were not detected ($P \geq 0.20$) for plasma concentrations of haptoglobin and NEFA (Fig. 3).

Effects of treatment ($P \geq 0.17$) or treatment × day ($P \geq 0.20$) were not detected for liver mRNA expression of ceruloplasmin, haptoglobin, and muscle mRNA expression of m-calpain, mammalian target of rapamycin (**mTOR**), and ubiquitin (Table 4). Effects of treatment ($P \geq 0.61$) and treatment × day ($P \geq 0.42$) were not detected for seroconversion to BVDV-1 and IBR and serum IBR titers (Table 5). A treatment × day interaction was detected (P = 0.03) for covariately adjusted serum BVDV-1b titers, which did not differ among treatments from d 0 to 28 ($P \geq 0.17$) but was greater ($P \leq 0.02$) for 115% vs. 85% and 100% MP steers on d 42 (Table 5). Effects of day were detected ($P \leq 0.0001$) for seroconversion to BVDV-1b (36.1%, 13.9%, and 86.1% ± 6.78% on d 0, 28, and 42, respectively) and IBR (2.8%, 32.1%, and 52.8% ± 7.07% on d 0, 28, and 42, respectively) and serum titers against BVDV-1b (1.58, 0.44, and 4.16 ± 0.344 $\log_2$ on d 0, 28, and 42, respectively) and IBR (0.39, 0.91, and 1.59 ± 0.193 $\log_2$ on d 0, 28, and 42, respectively). Effects

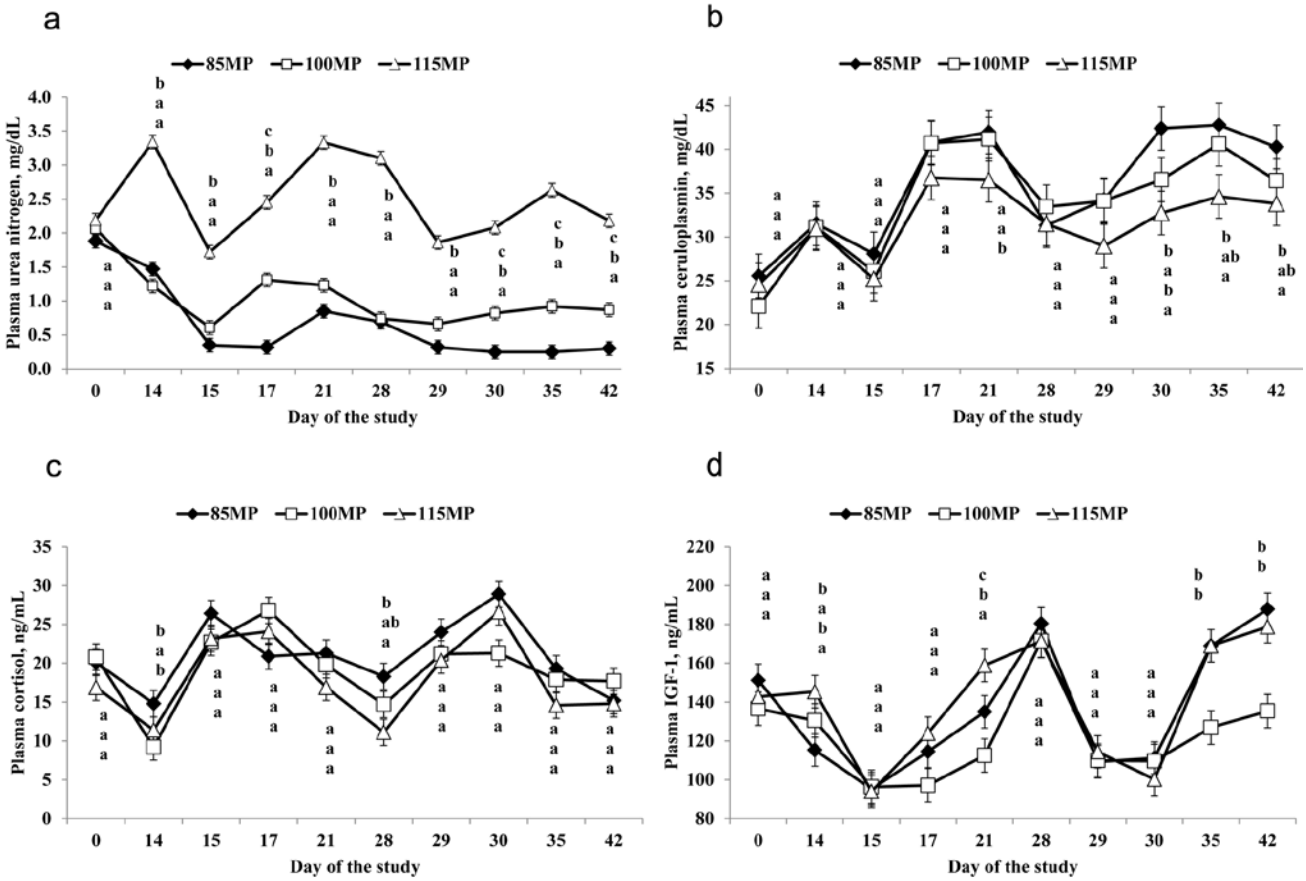

Figure 2. Plasma concentrations of (a) PUN, (b) ceruloplasmin, (c) cortisol, and (d) IGF-1 of beef steers provided 85%, 100%, and 115% of MP requirements of a beef steer gaining 1.1 kg of BW daily (NRC, 2000). On d 14, steers were vaccinated subcutaneously with 2 mL of Bovi Shield One Shot and Ultrabac 7 (Zoetis Inc., Kalamazoo, MI). On d 28, steers received a 2-mL subcutaneous booster of Bovi Shield Gold 5 and Ultrabac 7 (Zoetis Inc.). Effects of treatment × day were detected for plasma concentrations of PUN ($P < 0.0001$), ceruloplasmin ($P = 0.01$), cortisol ($P = 0.07$), and IGF-1 ($P = 0.001$). [a–c]Within day, means without a common superscript differ ($P \leq 0.05$).

of treatment ($P = 0.05$), but not treatment × day ($P = 0.40$) and day ($P = 0.22$), were detected for covariately adjusted mean serum *M. haemolytica* leukotoxin titers, which was greater ($P \leq 0.05$) for 85% vs. 100% and 115% MP steers (Table 5). Although serum leukotoxin titers were included as covariate ($P = 0.03$), they did not differ among treatments on d 0 ($P \geq 0.34$).

Postpreconditioning Phase. Body weight and DMI from d 43 to 262 and carcass characteristics at slaughter did not differ ($P \geq 0.11$) among treatments (Table 6). Average daily gain and G:F from d 43 to 70, but not from d 71 to 262 ($P \geq 0.26$), were least for 115% MP steers and greatest for 85% MP steers ($P \leq 0.04$) and intermediate for 100% MP steers ($P \geq 0.19$; Table 6).

DISCUSSION

Weaning, feedlot entry, and vaccination may elicit APR, leading to increased concentrations of APP and depressed feed intake, feed efficiency, and growth performance of beef cattle (Cooke et al., 2011; Arthington et al., 2013; Moriel and Arthington, 2013). During an

Table 4. Mean mRNA expression of genes associated with hepatic acute-phase response (ceruloplasmin and haptoglobin) and muscle protein degradation (m-calpain and ubiquitin) and synthesis (mammalian target of rapamycin [mTOR]) of steers provided increasing MP supply ($n = 6$ pens/treatment) during a 42-d preconditioning period[1]

	Treatment				*P*-value	
Item	85% MP	100% MP	115% MP	SEM	Treatment	Treatment × day
Hepatic genes[2]						
Ceruloplasmin	10.0	16.0	12.4	3.86	0.56	0.56
Haptoglobin	3.94	4.47	4.82	0.63	0.62	0.20
Muscle genes[2]						
m-calpain	6.79	9.88	9.34	1.23	0.17	0.96
mTOR	5.39	5.68	6.91	1.37	0.68	0.55
Ubiquitin[2]	3.01	4.17	3.84	0.52	0.28	0.39

[1]Steers were randomly selected within each pen for a liver or LM biopsy (1 steer/pen; 1 liver or muscle tissue/steer) on d 17 and 30. Each steer remained in its respective tissue assignment on d 17 and 30.

[2]Responses were quantified on the basis of the threshold cycle (CT) and were normalized to cyclophilin CT (ΔCT) examined in the same sample and assessed at the same time as the targets. Within each target gene, results are expressed as relative fold change ($2^{-\Delta\Delta CT}$) using the sample with the greatest ΔCT as reference, as described by Ocón-Grove et al. (2008).

Table 5. Seroconversion and serum concentrations of antibody titers against bovine viral diarrhea viral type 1b (BVDV1b), infectious bovine rhinotracheitis (IBR), and *Mannheimia haemolytica* leukotoxin in steers provided increasing MP supply (85%, 100%, or 115% MP; n = 6 pens/treatment) during a 42-d preconditioning period (d 0 to 42)[1]

	Treatment				*P*-value	
Item	85% MP	100% MP	115% MP	SEM	Treatment	Treatment × day
BVDV1b						
Seroconversion, %	41.7	50.0	44.4	7.48	0.75	0.61
Titers,[2] $\log_2$						
d 0	1.26[a]	1.47[a]	2.01[a]	0.597	0.002	0.03
d 28	0.63[a]	0.20[a]	0.48[a]			
d 42	3.73[a]	2.96[a]	5.79[b]			
IBR						
Seroconversion, %	25.8	36.1	25.8	8.55	0.61	0.42
Titers, $\log_2$	0.99	0.99	0.91	0.224	0.95	0.88
Mannheimia haemolytica leukotoxin						
Titers,[2,3] ng/antibody bound	3.12[a]	2.39[b]	2.49[b]	0.21	0.05	0.40

[a,b]Within a row, means without a common superscript differ ($P \leq 0.05$).

[1]On d 14, steers were vaccinated subcutaneously with 2 mL of Bovi Shield One Shot and Ultrabac 7 (Zoetis Inc., Kalamazoo, MI). On d 28, steers received a 2-mL subcutaneous booster of Bovi Shield Gold 5 and Ultrabac 7 (Zoetis Inc.).

[2]Least squares means covariate adjusted ($P \leq 0.03$) to results obtained on d 0.

[3]Measured as nanograms of secondary antibody that bound to sample.

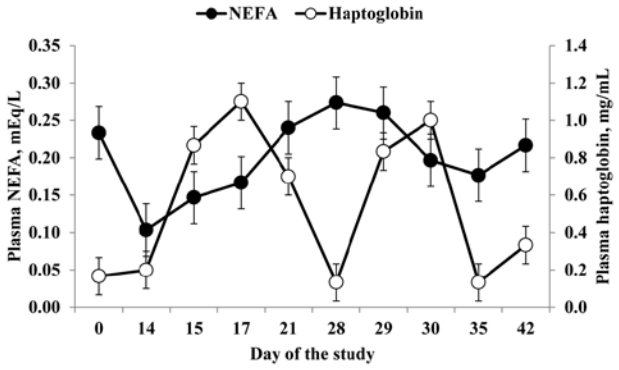

Figure 3. Plasma concentrations of haptoglobin and NEFA of beef steers provided 85%, 100%, and 115% of MP requirements of a beef steer gaining 1.1 kg of BW daily (NRC, 2000). On d 14, steers were vaccinated subcutaneously with 2 mL of Bovi Shield One Shot and Ultrabac 7 (Zoetis Inc., Kalamazoo, MI). On d 28, steers received a 2-mL subcutaneous booster of Bovi Shield Gold 5 and Ultrabac 7 (Zoetis Inc.). Effects of time were detected for plasma concentrations of haptoglobin and NEFA ($P < 0.0001$).

Table 6. Postpreconditioning growth performance during feedlot receiving (d 43 to 70), growing (d 71 to 206), and finishing (d 207 to 262) phases and carcass characteristics at slaughter (d 263) of steers provided increasing MP supply (85%, 100%, or 115% MP) during a 42-d preconditioning period (d 0 to 42)[1]

	Treatment				
Item[2]	85% MP	100% MP	115% MP	SEM	*P*-value
BW, kg					
d 43	274	290	296	7.6	0.11
d 70	308	317	320	7.3	0.47
d 206	527	536	534	12.6	0.87
d 262	561	572	563	14.8	0.84
ADG, kg/d					
d 43 to 70	1.21[b]	0.99[a,b]	0.85[a]	0.076	0.006
d 71 to 206	1.49	1.49	1.46	0.057	0.89
d 207 to 262	1.21	1.30	1.05	0.137	0.42
d 43 to 262	1.42	1.39	1.32	0.052	0.40
DMI,[3] kg/d					
d 53 to 70	7.18	7.35	7.33	0.113	0.51
d 71 to 206	10.42	10.68	10.79	0.275	0.61
d 207 to 262	8.51	9.02	8.73	0.422	0.69
d 53 to 262	9.43	9.67	9.73	0.217	0.58
G:F[4]					
d 43 to 70	0.17[b]	0.14[a,b]	0.12[a]	0.011	0.004
d 71 to 206	0.16	0.15	0.15	0.004	0.42
d 207 to 262	0.07	0.07	0.06	0.007	0.26
d 43 to 262	0.13	0.13	0.13	0.003	0.11
HCW, kg	354	352	350	11.4	0.97
Back fat thickness, cm	1.73	1.70	1.88	0.175	0.71
LM area,[5] cm^2	80	81	80	2.0	0.83
KPH, %	1.85	2.04	2.00	0.179	0.72
Yield grade	3.37	3.52	3.69	0.244	0.77
Marbling[6]	613	607	567	36.7	0.60

[a,b]Within a row, means without a common superscript differ ($P \leq 0.05$).

[1]Diets were formulated to provide 85%, 100%, and 115% of daily MP requirement of a beef steer gaining 1.1 kg of BW daily and were limit fed at 2.01%, 2.14%, and 2.24% of BW (DM basis), respectively, to ensure similar TDN intake from d 0 to 42.

[2]On d 43, steers were transferred to a feedlot facility and individually fed a common corn silage–based diet in Calan feeders (American Calan Inc., Northwood, NH). Full BW was obtained monthly before feeding (0800 h) on d 43, 70, 206, and 262.

[3]Steers were trained to eat from Calan-gate feeders for 10 d, and hence, DMI from d 43 to 52 was not measured.

[4]Estimated by dividing total BW gain by total DMI of the respective period.

[5]Actual measurement obtained after slaughter measured between the 12th and 13th ribs.

[6]Marbling score: small = 500 to 590; modest = 600 to 690.

immunological challenge, the metabolic demand for APP synthesis is increased (Reeds and Jahoor, 2001). Consequently, the APR induced by weaning, feedlot entry, and vaccination of preconditioning calves substantially increases nutrient demand, which, coupled

with suppressed feed intake, may also decrease growth performance. In our study, ADG from d 0 to 14, d 28 to 42, and d 0 to 42 increased as MP supply increased. Reasons for the better growth performance as MP was increased may be several and will be discussed below. Plasma urea N is an indicator of CP intake and urea synthesis in the liver (Huntington et al., 2001). Hence, the differences detected for plasma PUN concentrations signify that despite observed DMI differences, we successfully achieved our goals of inducing different MP supply to beef steers. However, the fact that G:F was approximately 21% and 26% greater for 100% and 115% vs. 85% MP steers, respectively, indicates that factors beyond DMI are also influencing growth performance.

Synthesis of several APP may consume a large proportion of available amino acids (Kushner, 1982; Reeds et al., 1994). Besides increasing nutrient demand, the APR causes nutrients absorbed from diet digestion or mobilized from muscle and fat reserves to be partitioned toward the support of the immune response rather than growth (Johnson, 1997; Elsasser et al., 2008). In support, ADG from d 14 to 28, which corresponds to the first vaccination-induced APR, did not differ among treatments. Also, PUN concentrations decreased abruptly after vaccination on d 14 and 28, suggesting greater N usage rather than N excretion via urea synthesis, whereas plasma NEFA concentrations increased from d 14 to 28, suggesting that steers were mobilizing fat reserves to support the APR, even though steers were gaining weight.

We hypothesized that the substrate availability and hepatic synthesis of APP, such as haptoglobin and ceruloplasmin, would increase as additional MP was provided. Haptoglobin prevents Fe utilization for bacterial growth (Wassell, 2000) and may be used as an indicator of inflammatory conditions in cattle when plasma concentrations are ≥0.11 mg/mL (Tourlomoussis et al., 2004). In our study, plasma haptoglobin concentrations were not affected by MP supply, which is in agreement with the results of Waggoner et al. (2009), who reported no effects of dietary CP concentrations (14% vs. 16% of DM) on serum concentrations of haptoglobin within 12 h after lypopolysaccharide (**LPS**) infusion in beef steers. In contrast, we previously showed that plasma haptoglobin concentrations, but not ADG, were enhanced by increasing MP supply from 85% to 115% of daily requirements of early weaned Brangus crossbred steers (Moriel and Arthington, 2013). Reasons for this inconsistency may be associated with breed differences and the interaction between breed and source of stress affecting the APR magnitude and calf age at vaccination. In support, peak haptoglobin concentration of steers provided 100% of MP requirements was 38% greater in the present study than previously reported (Moriel and Arthington, 2013). Hence, the extent of amino acid repartitioning from growth to the immune system was likely greater in the present study, leading to differences in growth performance, whereas previously, the magnitude of APR was relatively less and no effects on growth performance occurred (Moriel and Arthington, 2013).

Contrary to our hypothesis, but in agreement with Moriel and Arthington (2013), plasma concentrations of ceruloplasmin decreased as the MP supply was increased. Ceruloplasmin transports 90% to 95% of serum Cu (Cousins, 1985) but may decrease during periods of Cu deficiency (Mulhern and Koller, 1988) caused by S-induced (Suttle, 1974) and Mo-induced (Mason, 1990) decreased absorption of Cu. In the present study, dietary concentrations of Mo and S were numerically greater for 100% and 115% vs. 85% MP diets. Thus, plasma concentrations of ceruloplasmin in our study are likely a reflection of the differences on dietary concentrations of S and Mo rather than a direct effect of MP supply.

The amino acid requirement to support hepatic APP synthesis is met by dietary protein digestion (Waggoner et al., 2009) and muscle protein mobilization (Reeds et al., 1994; Reeds and Jahoor, 2001). However, because of differences in amino acid profile between muscle and APP (Reeds and Jahoor, 2001), the amount of amino acids mobilized from muscle is more than twice the amount needed to accommodate the APR (Reeds et al., 1994). To further test the hypothesis that greater MP supply provides greater substrate availability for APP synthesis but simultaneously alleviates muscle mobilization, liver and muscle samples were collected at the peak of APR and were analyzed for mRNA expression of haptoglobin and ceruloplasmin in the liver and genes associated with muscle protein synthesis and degradation.

Although we did not detect statistically significant differences, it is interesting to note that liver mRNA expression of ceruloplasmin was numerically greater for 100% and 115% vs. 85% MP steers, whereas liver mRNA expression of haptoglobin numerically increased as MP supply was increased, which partially supports our hypothesis of a greater substrate-induced synthesis of APP as MP was provided. Muscle protein metabolism is regulated by several physiological, genetic, and environmental factors (Métayer et al., 2008) but is also directly regulated by amino acids via mTOR-induced protein translation (Bodine et al., 2001). The mTOR signaling pathway controls nutrient-stimulated muscle protein synthesis and is downregulated by amino acid deficiency (Bodine et al., 2001), whereas calpain and ubiquitin-proteasome systems control muscle protein degradation (Goll et al., 2008) and are also downregulated by nutrient re-

striction (Du et al., 2004). In agreement with our hypothesis, 85% MP steers had numerically less mRNA expression of mTOR and greater mRNA expression of m-calpain and ubiquitin than steers provided 100% and 115% MP diets. Thus, we may suggest that steers fed 85% MP were likely more dependent on muscle mobilization to support the amino acids requirements for the APR, whereas the additional MP supply in steers fed 100% and 115% MP contributed to the APR and alleviated muscle tissue mobilization, which may explain the lack of treatment effects on plasma haptoglobin but the greater growth performance as MP supply was increased. The main reason for the failure to detect treatment effects on liver and muscle mRNA expression was likely the relatively small number of observations, once mRNA expression of m-calpain and ubiquitin nearly approached significance levels.

Insulin-like growth factor-1 was positively correlated with LM deposition rate in growing cattle (Vestergaard et al., 2003), and depending on ME intake, supplementary protein may be a mechanism to enhance IGF-1 synthesis and growth in ruminants (Elsasser et al., 1989). Increasing dietary CP concentrations (8%, 11%, and 14% of DM) linearly increased plasma IGF-1 concentrations when steers consumed 17 Mcal of ME daily but not when steers consumed 12.5 Mcal of ME (Elsasser et al., 1989). In agreement, steers in our study consumed between 15.5 and 16.5 Mcal of ME daily and had plasma IGF-1 concentrations increased by MP supply on d 14 and 21. Unexpectedly, plasma IGF-1 was greater for 85% and 115% vs. 100% MP steers on d 35 and 42. Reasons for that response are unknown since DMI during the receiving period did not differ among treatments. Although DMI of steers was not reduced in response to vaccination, plasma IGF-1 concentrations decreased immediately following vaccination on d 14 and 28 and required approximately 3 d to return to baseline concentrations. Elsasser et al. (1995) observed that plasma IGF-1 in Angus × Hereford steers decreased for 3 d following an endotoxin challenge and that plasma IGF-1 was not altered in pair-fed steers receiving no endotoxin challenge. This indicates that plasma IGF-1 may decrease by intake-independent mechanisms (Elsasser et al., 1995). For instance, proinflammatory cytokines released after an immunological challenge may induce a state of IGF-1 resistance, which inhibits the anabolic effects of IGF-1 and facilitates energy and protein mobilization from body stores (O'Connor et al., 2008), which may explain the reduction on plasma IGF-1 following vaccination in our study. However, IGF-1 may antagonize the actions of proinflammatory cytokines and induce a state of proinflammatory cytokine resistance (O'Connor et al., 2008). Hence, the greater plasma IGF-1 immediately before vaccination on d 14 in steers fed 115% MP may have alleviated the proinflammatory cytokine-induced catabolic effects on muscle protein. Also, it likely indicates that the metabolism to support the immune system of 115% MP steers was less dependent on energy stores from the body, which may have contributed to the greater growth performance of these steers compared with protein deficient steers.

Neutralizing antibody titers provide an indication of immune protection (Bolin and Ridpath, 1990) and vaccine efficacy in calves (Richeson et al., 2008) and have been correlated positively with disease prevention (Howard et al., 1989). The ability of an animal to respond to vaccination varies from animal to animal and depends on environmental and genetic factors, maternal antibody concentrations (Downey et al., 2013), and timing of vaccination relative to feedlot entry (Richeson et al., 2008). As emphasized by Duff and Galyean (2007), little research has been conducted to evaluate the interaction between vaccination and nutrition. Hence, we also investigated the MP supply–induced effects on antibody production of vaccinated preconditioning steers. Although positive seroconversion to BVDV-1b did not differ among treatments, steers provided 115% of MP requirements had greater serum BVDV-1b titers on d 42 than 85% and 100% MP steers. The majority of bovine respiratory disease cases occur within 30 d postweaning or 14 d relative to feedlot entry (Kirkpatrick et al., 2008). Also, calves with serum BVDV-890 neutralizing titers >4 in a $\log_2$ scale did not develop severe clinical signs of fever, leukopenia, and diarrhea (Bolin and Ridpath, 1990). Hence, our data indicate that steers provided 115% MP might have greater immune protection against BVDV-1b and less chance of developing bovine respiratory disease following feedlot entry. Downey et al. (2013) reported that BVDV antibody titers increased by 0.068 titer units ($\log_2$) for every 1 kg increase on ADG during the first 21 d after vaccination. Thus, the greater overall ADG of 115% MP steers likely contributed to, but do not fully explain, the differences observed for BVDV-1b titers since the magnitude of the increase on serum BVDV-1b titers was greater than the magnitude that could be explained by the findings of Downey et al. (2013).

Additional factors contributing to the observed differences on serum BVDV-1b titers may be the immune modulatory effects of protein deprivation (Dai and McMurray, 1998) and cortisol immune suppression effects (Salak-Johnson and McGlone, 2007). Dietary protein malnutrition impairs macrophage function, including tumor necrosis factor α (proinflammatory cytokine) production and cooperation with T lymphocytes; decreases T-lymphocyte function (such as production of

anti-inflammatory interferon γ cytokine); and potentiates the secretion of immunosuppressive transforming growth factor β cytokine (Dai and McMurray, 1998), whereas cortisol blocks the cytokine secretion by T helper cells with Cluster of differentiation 4 **($CD4^+T$)**, which are involved in antibody production (Salak-Johnson and McGlone, 2007). In agreement, plasma cortisol concentrations tended to decrease as MP supply increased on d 14 and 28. Hence, a MP deficiency of 15% led to greater plasma cortisol concentrations and may have decreased the communication between innate and humoral immune response, leading to decreased antibody production against BVDV-1b. In contrast, treatment effects were not detected for positive seroconversion and serum titers against IBR. The positive seroconversion percentage and serum titer units were similar in magnitude to those reported by Richeson et al. (2008) and numerically lower for IBR vs. BVDV-1b in the present study. Hence, the lower magnitude of vaccine response to IBR vs. BVDV-1b likely made difficult the detection of treatment effects. Furthermore, it was reported that the pattern of cytokine release from gastric and intestinal epithelial cells varied with site of infection and type of pathogen (Jung et al., 1995). Hence, it is plausible that the cytokine profile induced by vaccination against IBR was not affected by MP supply. However, further research is needed to investigate this hypothesis.

Mannheimia haemolytica is an opportunistic inhabitant of the upper respiratory tract of healthy cattle (Prado et al., 2006) that contains multiple virulence factors, such as leukotoxin and LPS, to promote lung colonization (Singh et al., 2011) and is also the major pathogen detected in cases of bovine respiratory disease (Duff and Galyean, 2007). A leukotoxin is an exotoxin that causes transmembrane pores in bovine lymphocytes, neutrophils, macrophages, mast cells, and platelets, which eventually leads to oncotic cell cytotoxicity (Clinkenbeard et al., 1989). Contrary to our hypothesis, serum *M. haemolytica* leukotoxin titers were greater for 85% vs. 100% and 115% MP steers. Reasons for this response are unknown at this moment. However, under stressful conditions, *M. haemolyt*ica present in the respiratory tract may multiply and induce an immune response (Prado et al., 2006), whereas cortisol has been shown to weaken the innate immune response (Dai and McMurray, 1998), decreaseT helper cells 1 and 2 (**Th1** and **Th2**, respectively) cytokine secretion, and suppress cellular immunity, causing a shift toward a Th2-mediated humoral immunity (Elenkov, 2002). Hence, it is possible that the stress of weaning, feedlot entry, vaccination, and protein deficiency experienced by 85% MP steers may have induced greater multiplication of *M. haemolytica* in the respiratory tract and greater antibody production against *M. haemolytica* leukotoxin to compensate for the potential cortisol-induced suppression in innate immunity. It is important to highlight that this rationale is speculative and further research needs to be conducted to examine the effects of physiological stress on *M. haemolytica* multiplication in the respiratory tract.

As expected, steers provided 85% MP supply during the preconditioning phase experienced compensatory growth during the feedlot receiving period (d 43 to 70) and had 30% greater ADG and 31% greater G:F compared with the average ADG and G:F of 100% and 115% MP steers. We are unaware of other studies evaluating the effects of MP supply during preconditioning on subsequent performance and carcass characteristics. Hence, we compared our results with those observed in beef steers fed increasing protein levels during feedlot growing phase. Neville et al. (1977) observed that ADG in steers and heifers fed diets with 18.9% or 23.7% CP was 0.19 kg greater during the first 12 wk postweaning compared with that of steers and heifers fed a diet with 14.5% CP. However, during the subsequent 6 wk, steers and heifers consuming a diet with 14.5% CP had ADG that were 0.02 and 0.04 kg greater than those fed diets with 18.9% and 23.7% CP, respectively. Perry et al. (1983) reported a greater ADG of Angus × Hereford steers by increasing dietary CP concentrations (8%, 11%, and 13% of DM) during a 114-d growing phase but reduced subsequent finishing ADG and no effect on carcass weight, fat thickness, and yield grade at slaughter. Segers et al. (2014) observed that CP concentration (16% vs. 20% of DM) used in growing diets for early weaned beef steers enhanced ADG during the experimental feeding period (d 0 to 112) but had no subsequent effect on DMI and ADG when a common diet was provided to all steers (d 113 to 224) or on carcass characteristics at slaughter.

In summary, increasing the MP supply to stressed, preconditioning beef steers did not affect the plasma concentrations of haptoglobin, decreased plasma ceruloplasmin, increased plasma IGF-1, and corrected the imbalances between amino acid supplied and required by the immune system, which consequently may have alleviated muscle protein mobilization, leading to greater growth performance during a 42-d preconditioning period. Also, the greater MP supply provided during the vaccination protocol did not affect antibody production against IBR but significantly increased antibody production against BVDV-1b, which might enhance the immune protection and decrease future risk of developing bovine respiratory disease. Increasing the MP supply during the preconditioning phase decreased ADG in the first 30 d after preconditioning

but had no effect on finishing growth performance and carcass characteristics of beef steers.

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