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Decreasing the frequency of energy supplementation from daily to three times weekly impairs growth and humoral immune response of preconditioning beef steers[1]

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ABSTRACT: We evaluated the effects of frequency of energy supplementation on growth and measurements of innate and humoral immune responses of preconditioning beef steers following vaccination. Angus steers (n = 24; 221 ± 6.3 kg; 177 ± 4 d of age) were weaned on d –7 and kept in a single drylot pen with free access to tall fescue hay and concentrate DMI at 0.5% of BW (50:50 mix of soyhulls and corn gluten pellets; DM basis) from d –7 to 0. On d 0, steers were stratified by BW and age and randomly assigned to 1 of 8 feedlot pens (3 steers/pen). Treatments were randomly assigned to pens (4 pens/treatment) and consisted of steers provided daily free access to ground tall fescue hay and similar weekly concentrate DMI (1% of BW times 7 d), which was divided and offered either daily (S7) or 3 times weekly (S3; Monday, Wednesday, and Friday) from d 0 to 42. Individual BW was measured before feeding on d 0 and 42, after 12 h of feed and water withdrawal. Steers were vaccinated against infectious bovine rhinotracheitis, bovine viral diarrhea virus (BVDV), *Mannheimia haemolytica,* and clostridium on d 7 and 21. Blood samples were collected from the jugular vein on d –7 and 4 h after concentrate supplementation on d 0, 7, 8, 9, 10, 14, 21, 22, 23, 24, 28, 35, and 42. Steers offered concentrate daily had greater ($P \leq 0.02$) BW on d 42, overall ADG, and total DMI, but similar ($P = 0.14$) G:F, than S3 steers. On days that S7 and S3 steers were offered concentrate, total DMI was greater and hay DMI was less for S3 vs. S7 steers ($P \leq 0.05$). On days that only S7 steers were supplemented, hay DMI was greater, but total DMI was less for S3 vs. S7 steers ($P \leq 0.05$). Mean CP and NEg intake were greater ($P \leq 0.03$) for S7 vs. S3 steers. Plasma cortisol concentrations on d 7 and 28, and mean plasma haptoglobin concentrations, but not liver mRNA expression of haptoglobin ($P = 0.75$), were greater for S3 vs. S7 steers ($P \leq 0.03$). Plasma IGF-1 concentrations on d 0 and urea nitrogen on d 1 and 3, relative to vaccination, were greater for S7 vs. S3 steers ($P \leq 0.008$). Positive seroconversion to BVDV-1b on d 42 and mean serum BVDV-1b titers were greater for S7 vs. S3 steers ($P \leq 0.05$). In summary, decreasing the frequency of concentrate supplementation from daily to three times weekly, during a 42-d preconditioning period, decreased growth performance, increased plasma concentrations of haptoglobin and cortisol, and decreased vaccine-induced antibody production against BVDV-1b of beef steers.

Key Words: humoral immune, innate, preconditioning, steers, supplementation frequency, vaccination

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INTRODUCTION

Decreasing the frequency of energy supplementation from daily to 3 times weekly or alternate days decreases costs associated with labor and feeding, but it has either decreased (Cooke et al., 2007, 2008) or not affected growth performance of beef cattle (Moriel et al., 2012; Drewnoski et al., 2014). Discrepancies among those studies were attributed to differences in breed, supplement type, and forage

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quality, but one similarity among them is the use of beef cattle around 10–12 mo of age. Limited studies investigated the effects of decreasing the frequency of energy supplementation on growth of beef calves at different production stages, such as immediately after weaning and preconditioning (Drewnoski et al., 2011).

Preconditioning calves typically experience multiple processes, including weaning, vaccination, and feedlot entry, that elicit an acute phase protein response (**APR**) and impair immunity and growth performance (Arthington et al., 2008, 2013). Fluctuations in nutrient intake due to decreased frequency of energy supplementation impacted metabolic parameters and decreased age at puberty of beef heifers (Cooke et al., 2008; Moriel et al., 2012); however, it is not known if fluctuations in nutrient intake caused by decreased frequency of concentrate supplementation impair the immunity of stressed calves during preconditioning. Our hypothesis was that decreasing the frequency of energy supplementation would further enhance the physiological stress and APR following vaccination leading to detrimental impacts on growth performance and immunity of preconditioning beef calves. Thus, this study evaluated the effects of decreasing the frequency of energy supplementation from daily to 3 times weekly during a 42-d preconditioning period on growth performance and measurements of innate and humoral immune response following vaccination of beef calves.

MATERIALS AND METHODS

The Institutional Animal Care and Use Committee of NC State University (14-054-A) approved all procedures for the experiment conducted at the Mountain Research Station (Waynesville, NC; 35.48°N, 82.99°W; elevation = 659 m) from October to November 2014.

Animals, Diets, and Sample Collection

Angus steers (n = 24; 221 ± 6.3 kg; 177 ± 4 d of age) were weaned on d –7 and immediately transferred to a single drylot pen with daily free-choice access to long-stem tall fescue hay (*Lolium arundinaceum*; 17% CP and 58% TDN; DM basis) and concentrate DMI at 0.5% of BW from d –7 to 0 (50% soyhulls pellets:50% corn gluten pellets; DM basis). On d 0, steers were stratified by BW and age and randomly allocated into 1 of 8 concrete floor pens (3 steers/pen; 18 × 4 m; 24 m^2/steer) in a half-covered feedlot facility. Treatments were randomly assigned to pens (4 pens/treatment) and consisted of steers provided daily free-choice access to ground tall fescue hay and similar weekly concentrate amount offered either daily (**S7**) or 3 times weekly (**S3**; Monday, Wednesday, and Friday) during a 42-d preconditioning period (d 0 to 42). Hence, days when all S7 and S3 steers received concentrate supplementation were defined as SUPPALL days (Monday, Wednesday, and Friday), whereas days that only S7 steers received concentrate supplementation were defined as S7ONLY days (Tuesday, Thursday, Saturday, and Sunday). Concentrate and hay were offered separately in the same feed bunk at 0800 h. Nutritional composition of hay and concentrate used from d –7 to 42 are shown in Table 1. Concentrate offered was completely consumed within 1 h by S7 steers and within 6 h by S3 steers. Individual BW was measured before feeding on d 0 and 42, following 12 h of feed and water withdrawal. Weekly concentrate DM offered (concentrate DMI = 1% of BW multiplied by 7 d) was estimated based on average shrunk BW of each pen on d 0 and readjusted on d 21 using average full BW of each pen obtained before feeding. Shrunk BW was not obtained on d 21 to not disturb feeding behavior and avoid an unnecessary physiological stress response due to shrink, which could affect plasma measurements and vaccine response (Marques et al., 2012). All steers were provided daily free-choice access to a complete mineral mix (RU-MIN 1600; Southern States, Richmond, VA; DM basis: 18.2% Ca, 0.72% K, 0.88% Mg, 0.76% S, 7.0% Na, 10.8% Cl, 2.9% P, 29 mg/kg Co, 1220 mg/kg

Table 1. Average chemical composition of ground tall fescue hay and concentrate[1]

Item	Tall fescue hay	Concentrate[2]
DM, %	91.2	89.6
	DM basis	
CP, %	17.4	17.0
ADF, %	34.4	26.5
NDF, %	57.7	47.4
TDN[3], %	58.0	72.0
NE_m[4], Mcal/kg	1.14	1.67
NE_g[4], Mcal/kg	0.60	1.10
Ca, %	0.51	0.41
K, %	2.53	1.48
Mg, %	0.32	0.32
Na, %	0.05	0.20
P, %	0.38	0.58
Cu, mg/kg	8	6
Fe, mg/kg	358	252
Mn, mg/kg	68	24
Mo, mg/kg	0.40	1.4
Zn, mg/kg	31	58

[1]Hay and concentrate samples were collected weekly and sent in duplicate to a commercial laboratory for wet chemistry analysis (Dairy One Laboratory, Ithaca, NY).

[2]Same concentrate was used from weaning to end of study (d –7 to 42) and consisted of 50% soybean hull pellets and 50% corn gluten feed pellets (DM basis).

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

[4]Calculated using the equations proposed by the NRC (2000).

Cu, 2130 mg/kg Mn, 29 mg/kg Se, and 2530 mg/kg Zn) and water from d 0 to 42.

Hay DM offered and refused were obtained daily for each pen by drying samples of hay offered and refused in a forced-air oven at 56°C for 48 h. Daily DMI was determined by subtracting the daily hay DM refused from the daily hay DM offered. Samples of hay, concentrate and mineral mix offered were collected weekly and sent in duplicate to a commercial laboratory (Dairy One Laboratory, Ithaca, NY) for wet chemistry analysis of all nutrients (Table 1). Samples were analyzed for concentrations of CP (method 984.13; AOAC, 2006), ADF (method 973.18 modified for use in an Ankom 200 fiber analyzer; Ankom Technology Corp., Fairport, NY; AOAC, 2006), and NDF (Van Soest et al., 1991; modified for use in an Ankom 200 fiber analyzer; Ankom Technology Corp.). Concentrations of TDN were calculated as proposed by Weiss et al. (1992), whereas NEm and NEg were calculated using equations from NRC (2000).

On d 7, all steers were treated with doramectin for internal and external parasites (5 mL subcutaneous; Dectomax injectable; Zoetis Inc., Kalamazoo, MI), and vaccinated against infectious bovine rhinotracheitis, bovine viral diarrhea Types 1 and 2 viruses, *Mannheimia haemolytica* (2 mL subcutaneous; Bovi Shield Gold One Shot; Zoetis Inc., New York, NY) and *clostridium* (2 mL subcutaneous; Ultrabac 7; Zoetis Inc., New York, NY). On d 21, steers received 2-mL subcutaneous boosters of Bovi Shield Gold 5 (Zoetis Inc., New York, NY) and Ultrabac 7. The vaccination and parasite control protocol described above was chosen to replicate the protocol utilized by the local preconditioning alliance (Mountain Cattle Alliance, Canton, NC; Moriel et al., 2015). The vaccination protocol was initiated 14 d after weaning and 7 d after feedlot entry to avoid the weaning- and feedlot-entry-induced inflammatory response that could interfere with vaccine response.

Blood samples (10 mL) were collected via jugular venipuncture into sodium-heparin (158 USP) containing tubes (Vacutainer; Becton Dickinson, Franklin Lakes, NJ) for plasma harvest 4 h after concentrate supplementation for 4 consecutive days in 2 wk (d 7, 8, 9, and 10 in wk 2 and d 21, 22, 23, and 24 in wk 4 of the study). The approach of collecting blood samples 4 h after feeding was utilized previously to correspond to the peak of ruminal fermentation and end-product release after concentrate consumption (Cooke et al., 2008; Moriel et al., 2012). Days for blood collection were chosen to evaluate the postvaccination energy and protein metabolism of S7 and S3 steers on days that all S7 and S3 steers received concentrate supplementation (SUPPALL; d 7, 9, 21, and 23) and days that only S7 steers received concentrate supplementation (S7ONLY; d 8, 10, 22, and 24). Additional blood samples (10 mL) were collected via jugular venipuncture into sodium-heparin (158 USP) containing tubes (Vacutainer; Becton Dickinson, Franklin Lakes, NJ) for plasma harvest immediately after weaning (d –7) and 4 h after concentrate supplementation on d 0, 14, 28, and 35 to complement the blood collection schedule described previously and to characterize the APR of S7 and S3 steers. Blood samples (10 mL) from jugular vein were collected into tube containing no additives (Vacutainer; Becton Dickinson, Franklin Lakes, NJ) for serum harvest on d –7, 21, and 42 to evaluate serum antibody titers against BVDV-1b. Blood samples were immediately placed on ice following collection and then centrifuged at 1,200 × *g* for 25 min at 4°C. Plasma and serum samples were stored frozen at -20°C until later laboratory analysis.

On d 0, steers were randomly selected from each pen for liver biopsy (2 steers/pen) on d 10 and 24, which corresponds to the vaccination-induced peak of inflammatory response based on plasma concentrations of haptoglobin (Moriel and Arthington, 2013; Arthington et al., 2013; Moriel et al., 2015). Liver samples were collected via needle biopsy, following the procedure described by Arthington and Corah (1995). Immediately following collection, 100 mg of wet tissue was stored in 1.5 mL of RNA stabilization solution (RNAlater; Ambion Inc., Austin, TX), kept on ice for 8 h, and stored at –80°C.

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). Plasma ceruloplasmin oxidase activity was measured in duplicate samples by using the colorimetric procedures (Demetriou et al., 1974). Commercial quantitative colorimetric kits were used to determine the plasma concentrations of PUN (B7551; Pointe Scientific, Inc., Canton, MI) and glucose (G7521; Pointe Scientific, Inc., Canton, MI). Inter- and intra-assay CV for assays of haptoglobin, ceruloplasmin, PUN, and glucose were 3.4% and 9.7%, 7.1% and 4.3%, 3.1% and 6.9%, and 2.3% and 3.2%, respectively.

Plasma concentrations of cortisol and insulin were determined using a single chemiluminescent enzyme immunoassay (Immulite 1000; Siemens Medical Solutions Diagnostics, Los Angeles, CA). Plasma IGF-1 concentrations were determined using commercial enzyme-linked immunosorbent assay kits (SG100; R&D Systems, Inc., Minneapolis, MN) that were previously validated for bovine samples (Moriel et al., 2012). Intra-assay CV for assays of cortisol, insulin, glucose, and

IGF-1 were 3.3%, 2.2%, 3.2%, and 4.5%, respectively. Inter-assay for IGF-1 assay was 4.3%.

Serum antibody titers against bovine viral diarrhea virus 1b (**BVDV-1b**) were determined by the Oklahoma Animal Disease and Diagnostic Laboratory using a virus neutralization test (Rosenbaum et al., 1970). Serum titers against BVDV-1b were reported as the log base 2 of the greatest dilution of serum that provided complete protection of the cells (lowest and greatest tested dilution = 1:4 and 1:256, respectively). For the seroconversion analysis, samples with serum neutralization value of < 4 were considered negative and assigned a value of 0, whereas samples with 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 (% of steers with positive serum neutralization) to BVDV-1b (Richeson et al., 2008; Moriel et al., 2015)

Detailed description of procedures for mRNA isolation and tissue gene expression are described 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 mg) 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 FAST SYBR Green PCR Master Mix (Applied Biosystems) and gene-specific primers (20 pM each; Table 2) with the StepOne Real-time PCR system (Applied Biosystems). At the end of each RT-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 were purified with the QIAquick PCR purification kit (Qiagen Inc., Valencia, CA) and sequenced at the Oregon State University Center for Genome Research and Biocomputing to verify the specificity of amplification. All amplified products represented only the genes of interest. Responses were quantified based on the threshold cycle (**CT**) and normalized to cyclophilin CT examined in the same sample and assessed at the same time as the targets. Results are expressed as relative fold change (2–ΔΔCT), as described by Ocón-Grove et al. (2008).

Table 2. Nucleotide sequence of bovine-specific primers used in the quantitative real-time reverse transcription PCR to determine the hepatic mRNA expression of haptoglobin, IGF-1, pyruvate carboxylase, and cyclophilin

Target gene	Primer sequence[1]	Accession number
Haptoglobin		
Forward	GTC TCC CAG CAT AAC CTC ATC TC	AJ_271156
Reverse	AAC CAC CTT CTC CAC CTC TAC AA	
IGF-1		
Forward	CTC CTC GCA TCT CTT CTA TCT	NM_001077828
Reverse	ACT CAT CCA CGA TTC CTG TCT	
Pyruvate carboxylase		
Forward	CCA ACG GGT TTC AGA GAC AT	NM_177946.3
Reverse	TGA AGC TGT GGG CAA CAT AG	
Cyclophilin		
Forward	GGT ACT GGT GGC AAG TCC AT	NM_178320.2
Reverse	GCC ATC CAA CCA CTC AGT CT	

[1]Primer sequences obtained for haptoglobin (Hiss et al., 2004), IGF-1 and pyruvate carboxylase (Cooke et al., 2008), and cyclophilin (Cappellozza et al. 2014).

Statistical Analyses

Except for seroconversion, all data were analyzed as a completely randomized design using the MIXED procedure of SAS (SAS Inst. Inc., Cary, NC; version 9.3) with Satterthwaite approximation to determine the denominator degrees of freedom for the test of fixed effects. Pen was the experimental unit, and steer(pen) and pen(treatment) were included as random effects in all analysis, except for analyses of DM and nutrient intake that included only pen(treatment) as the random effect. All measurements (growth performance, intake, plasma, and serum measurement) obtained on d 0 were included as covariate only when $P \leq 0.05$ and will be described below. Feed efficiency, ADG, and mean total intake of DM, mean daily intake of CP and NEg were tested for fixed effects of treatment. Within each wk of the study, daily DMI data were pooled by SUPPALL and S7ONLY days to simplify data analyses, interpretation, and report. Daily intake of DM (concentrate, hay, and total), CP and NEg data were analyzed as repeated measures and tested for fixed effects of treatment, day (SUPPALL and S7ONLY), wk of the study, and resulting interactions, using pen(treatment) as the subject. Body weight, plasma measurements, serum BVDV-1b titers, and tissue mRNA expression were analyzed as repeated measures and tested for fixed effects of treatment, day, wk of the study, and resulting interactions. The covariance structures were chosen using the lowest Akaike information criterion. The unstructured covariance structure was used for all analysis, except for liver mRNA expression of haptoglobin (Toeplitz structure), BW from d 0 to 42, plasma IGF-1 concentrations (Compound symmetry structure), and plasma concentrations of cortisol, haptoglobin, and ceruloplasmin (Autoregressive structure). Positive seroconversion to BVDV-1b were analyzed as repeated measures using the GLIMMIX

Table 3. Growth performance of steers provided daily free-choice access to ground tall fescue hay and similar weekly concentrate amount offered daily (S7) or 3 times weekly (S3) during a 42-d preconditioning period (n = 4 pens/treatment; 3 steers/pen)

	Treatments[1]			
Item	S7	S3	SEM	P-value
Body weight[2], kg				Treatment × day
d 0	218[a]	218[a]	2.31	0.01
d 42	273[b]	262[a]		
				Treatment
ADG (d 0 to 42), kg/d	1.30	1.03	0.040	0.02
Total DMI (d 0 to 42), kg	252	222	3.9	0.01
G:F[3] (d 0 to 42)	0.22	0.20	0.010	0.14

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

[1]From d 0 to 42, steers were provided a similar weekly concentrate amount (DMI = 1% of BW multiplied by 7 d) offered either daily (S7) or 3 times weekly (S3; Mondays, Wednesdays, and Fridays) at 0800 h.

[2]Body weights obtained after 12 h of feed and water withdrawal. Body weight at weaning (d –7) did not differ between treatments ($P = 0.75$), but was included as a covariate ($P < 0.0001$) for BW statistical analysis.

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

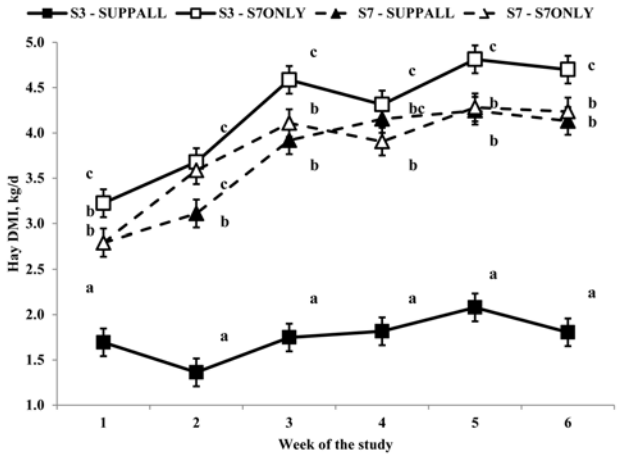

Figure 1. Daily hay DMI (kg/d) of steers provided daily free-choice access to ground tall fescue hay and similar weekly concentrate amount offered daily (S7) or 3 times weekly (S3) during a 42-d preconditioning period. A treatment × day × wk was detected for hay DMI ($P < 0.0001$). When all S7 and S3 steers received concentrate supplementation (SUPPALL), hay DMI was greater for S7 vs. S3 steers from wk 1 to 6 ($P < 0.0001$). When only S7 steers received supplementation (S7ONLY), hay DMI was greater for S3 vs. S7 steers from wk 1 to 6 ($P \leq 0.05$), except for wk 2 in which hay DMI did not differ ($P = 0.68$) between treatments. Hay DMI of S3 steers was less on SUPPALL vs. S7ONLY days, whereas hay DMI of S7 steers was similar between SUPPALL vs. S7ONLY days, except for wk 2 in which hay DMI was less on SUPALL vs. S7ONLY days (Fig. 1). [a–c]Within wk, means without a common superscript differ ($P \leq 0.05$).

procedure of SAS with pen(treatment) and steer(pen) as random effects. All results are reported as least squares means. Data were separated using LSD if a significant preliminary F-test was detected. Significance was set at $P \leq 0.05$ and tendencies if $P > 0.05$ and ≤ 0.10.

RESULTS

Body weight at weaning (d –7) did not differ between treatments ($P = 0.75$), but was included as a covariate ($P < 0.0001$) for the statistical analysis of BW. Treatment × day effect was detected for BW from d 0 to 42 ($P \leq 0.01$; Table 3), as S7 steers had greater BW than S3 steers on d 42 ($P = 0.001$). Overall ADG and total DMI were greater for S7 vs. S3 steers ($P \leq 0.02$), whereas overall G:F did not differ between treatments ($P = 0.14$; Table 3). A treatment × day × wk was detected for hay DMI ($P < 0.0001$; Fig. 1). On SUPPALL days, hay DMI was greater for S7 vs. S3 steers from wk 1 to 6 ($P < 0.0001$). On S7ONLY days, hay DMI was greater for S3 vs. S7 steers from wk 1 to 6 ($P \leq 0.05$), except for wk 2 in which hay DMI did not differ between treatments ($P = 0.68$; Fig. 1). Hay DMI of S3 steers was less on SUPPALL vs. S7ONLY days ($P < 0.0001$), whereas hay DMI of S7 steers did not differ on SUPPALL vs. S7ONLY days ($P \geq 0.13$), except for wk 2 in which hay DMI of S7 steers was less on SUPPALL vs. S7ONLY days ($P = 0.004$; Fig. 1). Total hay DMI (d 0 to 42) was less for S3 vs. S7 steers ($P = 0.002$; Table 4).

Effects of treatment × day ($P < 0.0001$), but not treatment × wk × day and treatment × wk ($P \geq 0.30$), were detected for daily intake of concentrate DM, total DM, CP, and NEg (Table 4). Steers offered concentrate 3 times weekly had greater daily total DMI on SUPPALL days ($P = 0.05$), but less on S7ONLY days ($P = 0.002$) compared to S7 steers. On SUPPALL days, daily CP intake tended to be greater ($P = 0.07$), whereas daily NEg intake was greater ($P = 0.001$) for S3 vs. S7 heifers. On S7ONLY days, daily CP and NEg intake were greater for S7 vs. S3 steers ($P \leq 0.002$). Hence, mean daily CP and NEg intake were greater ($P \leq 0.03$) for S7 vs. S3 steers.

Plasma concentrations of cortisol, haptoglobin, and ceruloplasmin on d 0 did not differ between treatments ($P \geq 0.23$), but were included as covariates ($P \leq 0.03$) for the statistical analyses of plasma concentrations of cortisol, haptoglobin, and ceruloplasmin, respectively. A tendency for treatment × day effect was detected ($P = 0.07$) for plasma concentrations of cortisol, which did not differ between treatments from d 9 to 23 and 28 to 35 ($P \geq 0.22$), but were greater for S3 vs. S7 steers on d 7, 8, and 28 ($P \leq 0.03$; Fig. 2A). Effects of time ($P < 0.0001$), but not treatment × day of study ($P \geq 0.17$), were detected for plasma concentrations of ceruloplasmin and haptoglobin (Fig. 2B). However, treatment effects were detected for mean plasma concentrations of haptoglobin ($P = 0.004$), but not for mean plasma concentrations of ceruloplasmin ($P = 0.48$). Mean plasma concentration of haptoglobin was greater for S3 vs. S7 steers (0.91 vs. 0.69 ± 0.048 mg/mL, respectively).

Effects of treatment × day × wk and treatment × wk were not detected for plasma concentrations of glucose,

Table 4. Ingredient and nutrient intake of steers provided daily free-choice access to ground tall fescue hay and similar weekly concentrate amount offered either daily (S7) or 3 times weekly (S3) during a 42-d preconditioning period (n = 4 pens/treatment; 3 steers/pen)

	Treatments[1]			
Item[2]	S7	S3	SEM	P-value[3]
Concentrate DMI, kg/d				
SUPPALL	2.31	5.18	0.184	<0.0001
S7ONLY	2.31	0	0.184	<0.0001
P-value[4]	1.00	<0.0001		
Total DMI, kg/d				
SUPPALL	6.03	6.93	0.270	0.05
S7ONLY	6.12	4.22	0.270	0.002
P-value[4]	0.33	<0.0001		
CP intake, kg/d				
SUPPALL	1.04	1.18	0.046	0.07
S7ONLY	1.05	0.69	0.046	0.002
P-value[4]	0.88	<0.0001		
NEg intake, Mcal/d				
SUPPALL	4.71	6.63	0.247	0.001
S7ONLY	4.76	2.51	0.247	0.0005
P-value[4]	0.51	<0.0001		
				Treatment
Total hay DMI, kg	156	129	2.0	0.002
Mean CP intake,[5] kg/d	1.02	0.90	0.016	0.01
Mean NEg intake,[5] Mcal/d	4.69	4.22	0.085	0.03

[1]From d 0 to 42, steers were provided daily free-choice access to ground tall fescue hay and a similar weekly concentrate amount (DMI = 1% of BW multiplied by 7 d) that was offered either daily (S7) or 3 times weekly (S3; Monday, Wednesday, and Friday) at 0800 h.

[2]SUPPALL = days when all S3 and S7 steers were offered concentrate; S7ONLY = days that only S7 steers received concentrate.

[3]Comparison of treatments within each day.

[4]Comparison of day within each treatment.

[5]Determined by multiplying the total DMI of hay and concentrate by their respective concentration of CP or NEg, and then divided by 42 d.

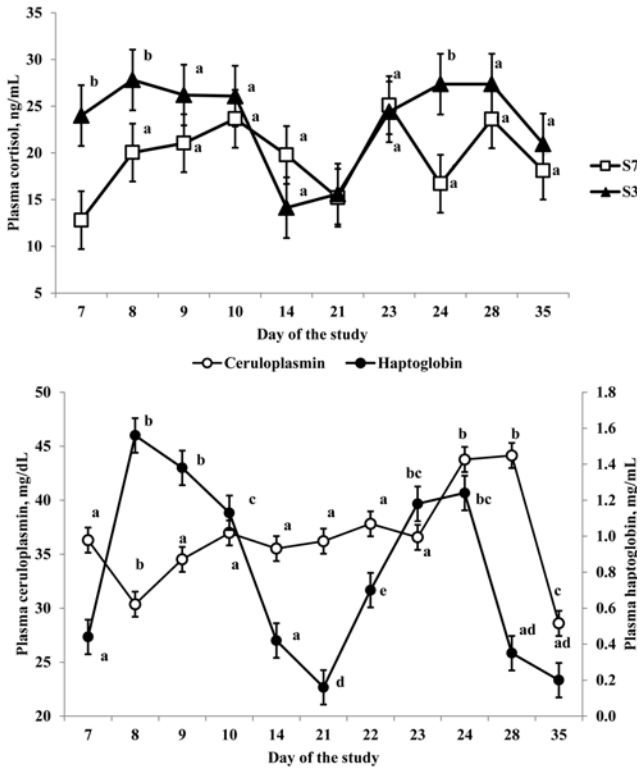

Figure 2. Plasma concentrations of cortisol (A) and haptoglobin and ceruloplasmin (B) of steers provided daily free-choice access to ground tall fescue hay and similar weekly concentrate amount offered daily (S7) or 3 times weekly (S3) during a 42-d preconditioning period. A tendency for treatment × day effect was detected (P = 0.07) for plasma concentrations of cortisol, which were similar between from d 9 to 23 and 28 to 35 ($P \geq 0.22$), but were greater on d 7, 8, and 28 ($P \leq 0.03$) for S3 vs. S7 steers. Effects of time ($P < 0.0001$), but not treatment × day ($P \geq 0.17$), were detected for plasma concentrations of ceruloplasmin and haptoglobin. [a–d]Within day (Fig. 2A) or across days (Fig. 2B), means without a common superscript differ ($P \leq 0.05$).

IGF-1, PUN, and insulin ($P \geq 0.19$). However, effects of treatment × day were detected for plasma concentrations of glucose, IGF-1, and PUN ($P \leq 0.01$), but not for insulin (P = 0.12). Therefore, data for plasma concentrations of glucose, IGF-1, and PUN were presented as plasma concentrations of each measurement immediately before (d 0) and 1, 2, and 3 d after vaccination, which represent the average plasma results of d 7 and 21 (first and second rounds of vaccination, respectively), 8 and 22, 9 and 23, and 10 and 24 of the study, respectively. Regardless of treatment, plasma concentrations of glucose decreased on d 2 and 3 vs. 0 and 1 ($P \leq 0.006$), relative to vaccination, whereas plasma concentrations of IGF-1 and PUN decreased ($P < 0.0001$) on d 1, 2, and 3 vs. 0, relative to vaccination. Steers provided concentrate supplementation daily tended (P = 0.07) to have greater plasma concentrations of glucose on d 2 and had greater ($P \leq 0.002$) plasma concentrations of IGF- 1 on d 0 and plasma concentrations of PUN on d 1 and 3, relative to vaccination, compared to S3 steers (Table 5). Effect of time ($P < 0.0001$), but not treatment (P = 0.71), was detected for plasma concentrations of insulin, which did not differ on d 1 and 3 (P = 0.14), was greatest on d 2, and was least on d 4 ($P \leq 0.05$), relative to vaccination (Table 5).

Effects of treatment × day and day were not detected ($P \geq 0.12$) for mRNA expression of haptoglobin, IGF-1, and pyruvate carboxylase. Treatment effect was detected for mRNA expression of pyruvate carboxylase (P = 0.03), but not for haptoglobin and IGF-1 ($P \geq 0.42$). Mean hepatic mRNA expression of pyruvate carboxylase was greater for S7 vs. S3 steers (Table 6). Positive seroconversion and serum BVDV-1b titers on d –7 did not differ between treatments ($P \geq 0.23$), but were included as covariates ($P \leq 0.003$) for the seroconversion and serum BVDV-1 titers statistical analyses, respectively. A tendency for treatment × time effect was detected (P = 0.06) for positive seroconversion to BVDV-1b, which did not differ between treatments on d 28 (P = 0.55), but was greater for S7

Table 5. Plasma concentrations of glucose, insulin, and IGF-1 of steers provided daily free-choice access to ground tall fescue hay and similar weekly concentrate amount offered daily (S7) or 3 times weekly (S3) during a 42-d preconditioning period[1] (n = 4 pens/treatment; 3 steers/pen)

	Day relative to vaccination[2]					
Item	0	1	2	3	SEM	P-value
Glucose, mg/dL						Treatment × day
S7	77.7[a]	80.3[a]	75.1[b]	73.8[b]	2.89	0.01
S3	81.1[a]	81.8[a]	70.4[b]	73.7[b]	2.89	
P-value[3]	0.41	0.63	0.07	0.94		
IGF-1, ng/mL						
S7	106.5[a]	75.8[b]	65.5[c]	63.1[c]	4.76	0.007
S3	87.8[a]	68.7[b]	55.7[c]	63.9[b]	4.76	
P-value[3]	0.008	0.30	0.16	0.91		
PUN, mg/dL						
S7	12.1[a]	8.9[b]	9.2[b]	9.3[b]	0.54	< 0.0001
S3	13.1[a]	6.1[c]	9.4[b]	5.4[c]	0.54	
P-value[3]	0.23	0.002	0.78	<0.0001		
						Day
Insulin, pmol/L	13.9[a]	20.3[b]	12.2[a]	10.3[c]	1.98	<0.0001

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

[1]Steers were vaccinated with Bovi Shield Gold One Shot and Ultrabac 7 (Zoetis Inc., New York, NY) on d 7, and Bovi Shield Gold 5 (Zoetis Inc., New York, NY) and Ultrabac 7 on d 21.

[2]Data presented as plasma concentrations of each measurement immediately before (d 0) and 1, 2, and 3 d after vaccination, which corresponds to the mean plasma results of d 7 and 21 (first and second rounds of vaccination, respectively), 8 and 22, 9 and 23, and 10 and 24 of the study, respectively.

[3]Comparison of treatments within each day (S7 vs. S3 steers).

vs. S3 steers on d 42 (P = 0.05; Table 7). Effect of treatment (P = 0.03), but not treatment × time (P = 0.14), was detected for serum BVDV-1b titers, which was greater for S7 compared to S3 steers (Table 7).

DISCUSSION

Published data on frequency of energy supplementation observed variable responses in ADG of beef steers and heifers. In the current study, decreasing the frequency of energy supplementation from 3 to 7 times weekly decreased overall ADG of beef steers by 21%, which is in agreement with others (Cooke et al., 2008; Loy et al., 2008). Decreasing the frequency of energy supplementation from daily to 3 times weekly decreased ADG by 18% of beef heifers fed low-quality warm-season forages and low-starch, wheat middling-based energy supplements (Cooke et al., 2008) and decreased ADG by 10% of beef steers fed low-quality native grasses and dry rolled corn- or dry distillers grains plus soluble-based supplements (Loy et al., 2008). In contrast, decreasing the frequency of low-starch concentrate supplementation from daily to 3 times weekly (Moriel et al., 2012; Drewnoski et al., 2011) or alternate days (Drewnoski et al., 2014) did not affect ADG of beef heifers and steers fed medium-quality forages. Discrepancies among results are likely due to differences in supplement composition, breed, gender, location, forage species, and quality, and potentially resulting interactions among these factors. Discussing each of those factors is beyond the scope of this manuscript, and hence our discussion will focus on comparing the differences between our results and those from Drewnoski et al. (2011), who used similar supplement composition (corn gluten feed and soyhulls mix), supplementation frequency (7 or 3 times weekly), production stage (immediately after weaning), and beef steers of the same breed as in our study.

Table 6. Mean hepatic mRNA expression of haptoglobin, IGF-1, and pyruvate carboxylase of steers provided daily free-choice access to ground tall fescue hay and similar weekly concentrate amount offered daily (S7) or 3 times weekly (S3) during a 42-d preconditioning period[1]

	Treatments			
Item	S7	S3	SEM	P-value
Hepatic genes	— Relative fold change[2]—			
Haptoglobin	3.12	3.38	0.552	0.75
IGF-1	1.86	2.06	0.172	0.42
Pyruvate carboxylase	2.05	1.53	0.128	0.03

[1]Steers were randomly selected within each pen (2 steers/pen) for a liver biopsy on d 10 and 24, which corresponds the vaccination-induced peak of inflammatory response base on plasma haptoglobin concentrations.

[2]Responses were quantified based on the threshold cycle (CT) and normalized to cyclophilin CT examined in the same sample and assessed at the same time as the targets. Results are expressed as relative fold change ($2^{-\Delta\Delta CT}$), as described by Ocón-Grove et al. (2008).

Table 7. Positive seroconversion and serum antibody titers against bovine viral diarrhea viral type 1b of steers provided daily free-choice access to ground tall fescue hay and similar weekly concentrate amount offered daily (S7) or 3 times weekly (S3) during a 42-d preconditioning period (n = 4 pens/treatment; 3 steers/pen)

	Treatments			
Item	S7	S3	SEM	P-value
Bovine viral diarrhea virus type 1b				
Seroconversion,[2] %				Treatment × day
d 21	12.8[a]	20.5[a]	8.81	0.06
d 42	100.0[b]	78.8[a]		
				Treatment
Mean titers,[2] log base 2	2.51	1.46	0.306	0.03

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

[1]Steers were vaccinated with Bovi Shield Gold One Shot and Ultrabac 7 (Zoetis Inc., New York, NY) on d 7 and Bovi Shield Gold 5 (Zoetis Inc., New York, NY) and Ultrabac 7 on d 21.

[2]Positive seroconversion and serum BVDV-1b titers on d –7 did not differ between treatments ($P \geq 0.23$), but were included as a covariate ($P \leq 0.003$) for the seroconversion and serum BVDV-1 titers analyses, respectively.

The observed difference on daily hay intake between S7 and S3 steers was the primary factor impacting differences in growth performance. Daily hay DMI of S3 steers decreased by 53% on SUPPALL days and increased by 10% on S7ONLY days compared to S7 steers, leading to an overall reduction on intake of total hay DM, CP, and NEg. Drewnoski et al. (2011) reported that hay DMI was decreased by approximately 32% when all steers received concentrate supplementation, but similar hay DMI on days that only S7 steers received concentrate supplementation. Despite the low-starch supplements used in this study, this response on daily hay DMI was expected because supplements often decrease forage DMI when TDN:CP ratio is less than 7 and supplemental TDN is greater than 0.7% of BW (Moore et al., 1999). However, concentrate supplementation-induced depression in forage intake is greater as forage quality increases (Horn and McCollum, 1987). Hence, differences observed in the magnitude of hay DMI reduction due to concentrate supplementation between our study and Drewnoski et al. (2011) may be attributed to differences on average forage quality (17% vs. 8.4% CP of DM, respectively). In addition, as part of our experimental design, all S7 and S3 steers received 2 rounds of vaccinations against pathogens associated with bovine respiratory disease that have been shown to elicit APR and decrease growth performance (Arthington et al., 2013; Moriel and Arthington, 2013; Moriel et al., 2015). However, Drewnoski et al. (2011) did not report if vaccinations were provided and only reported that steers were implanted with 36 mg of Zeranol and treated for internal and external parasites before starting the study. Hence, our results are in agreement with our hypothesis that growth performance of beef calves is impaired by decreasing the frequency of energy supplementation from daily to 3 times weekly during a preconditioning period including rounds of vaccination.

Weaning, feedlot entry, and vaccination elicit an APR leading to increased hepatic synthesis of acute-phase proteins (i.e., haptoglobin and ceruloplasmin), depressed feed intake, enhanced muscle and fat mobilization (Johnson, 1997), and decreased feed efficiency of beef cattle (Arthington et al., 2013). Also, an increase in plasma concentrations of cortisol reduced DMI (Allen et al., 2009), induced an APR, and increased plasma haptoglobin concentrations (Cooke and Bohnert, 2011), whereas plasma concentrations of haptoglobin increased following vaccination if beef steers against *M. haemolytica* (Arthington et al., 2013; Moriel et al., 2015) and may be used as an indicator of inflammation when plasma concentrations are ≥ 0.11 mg/mL (Tourlomoussis et al., 2004). In the present study, S3 steers had greater plasma concentrations of cortisol on d 7, 8, and 24 of the study and had mean plasma concentrations of haptoglobin 31.8% greater than S7 steers. These responses agree with our hypothesis and indicate that decreasing the frequency of energy supplementation exacerbated the physiological stress (as indicated by greater plasma concentrations of cortisol), leading to a heightened vaccine-induced APR (as indicated by the greater plasma concentrations of haptoglobin) and less growth performance compared to daily energy supplementation of preconditioning beef calves. In addition, the fact that only treatment effects, and not treatment × time effect, was detected for plasma concentrations of haptoglobin indicates that S3 steers had greater plasma concentrations of haptoglobin even before vaccine administration. An increasing number of studies indicated that feeding beef cattle high grain-based diets led to an accumulation of microbial endotoxins in the ruminal fluid that induced a general nonspecific inflammatory response (Berry et al., 2004; Jafari et al., 2006). For instance, plasma haptoglobin concentrations of beef steers peaked after 3 to 9 wk of feeding backgrounding and finishing starch-based diets containing (DM basis) 76% wheat (Gozho et al., 2006) and 45% or 95% barley grain (Ametaj et al., 2009). Although the concentrate utilized in the present study had low-starch concentrations, it is possible that the greater concentrate consumption of S3 vs. S7 steers on SUPPALL days resulted in the accumulation of endotoxins in the ruminal fluid and increased plasma concentrations of haptoglobin. Further studies need to be conducted to support this rationale.

To further explore the effects of frequency of energy supplementation on APR, liver biopsies were collected to evaluate the hepatic mRNA expression of haptoglobin. We are unaware of published studies describing the hepatic mRNA expression of haptoglobin following vaccination of beef cattle. Hence, we decided to collect liver samples 3 d after vaccination, which corresponds to the postvaccination peak of plasma haptoglobin concentrations as indicated previously (Arthington et al., 2013; Moriel and Arthington, 2013; Moriel et al., 2015). Hepatic mRNA expression of haptoglobin did not differ in our study, suggesting that decreasing the frequency of energy supplementation does not affect hepatic mRNA expression of haptoglobin 3 d after vaccination. However, the greater mean plasma haptoglobin concentrations of S3 vs. S7 steers indicates that the hepatic synthesis of haptoglobin was affected and that the increase on mRNA synthesis of haptoglobin occurred within 3 d of vaccination. Further studies are warranted to characterize and identify the peak of hepatic haptoglobin mRNA expression of beef cattle following an inflammatory challenge.

Although APR is essential for early defense mechanism in response to cellular injury (Eckersall and Conner, 1988), nutrient demand is increased to ac-

commodate the synthesis of acute-phase proteins, immune cells, and gluconeogenic precursors (Reeds and Jahoor, 2001). To support an immunological response, muscle protein and fat reserves are mobilized (Jahoor et al., 1999), and absorbed AA are shifted from growth toward hepatic uptake (Reeds et al., 1994) leading to a negative correlation with growth performance (Qiu et al., 2007). Indeed, circulating cortisol stimulates degradation of hepatic, adipose, and muscle cells (Nelson and Cox, 2005). Thus, the greater plasma concentrations of haptoglobin and cortisol of S3 vs. S7 steers may indicate that nutrient mobilization from body energy and protein reserves to support the immune system were increased by decreasing the frequency of energy supplementation and might further explain the greater growth performance of S7 vs. S3 steers.

Insulin-like growth factor 1 is an essential constituent of multiple systems controlling growth (Le Roith et al., 2001), with liver as the primary source of circulating IGF-1 (Yakar et al., 1999). Circulating concentrations of IGF-1 increases with increasing nutrient intake and growth rate (Elsasser et al., 1989; Moriel et al., 2012, 2015). Also, PUN concentration is an indicator of protein intake (Hammond, 1997), with optimal levels for growing cattle varying between 11 and 15 mg/dL (Byers and Moxon, 1980). In the current study, plasma concentrations of IGF-1 were greater for S7 vs. S3 steers immediately before vaccination, whereas PUN concentrations were greater for S7 vs. S3 steers on d 1 and 3, relative to vaccination, which likely reflect the differences on nutrient (CP and NEg) intake and further explain the greater growth performance of S7 vs. S3 steers. However, plasma concentrations of IGF-1 and PUN decreased for 3 d following vaccination, regardless of treatment, as previously reported by others (Moriel and Arthington, 2013; Moriel et al., 2015). Also, hepatic mRNA expression of IGF-1 did not differ between treatments on d 3, relative to vaccination, which explains the similar plasma concentrations of IGF-1 on that respective day. Proinflammatory cytokines released during APR 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). Hence, this decrease on plasma concentrations of IGF-1 and PUN following vaccination further supports that nutrients were being partitioned to support the immune system rather than growth and that S7 steers likely had less mobilization of body energy and protein reserves than S3 steers.

Drewnoski et al. (2014) investigated the variation on the area under the curve (**AUC**) of plasma concentrations of insulin, glucose, and PUN for 48 h after steers were offered low-starch concentrate supplementation daily or on alternate days. Only plasma insulin AUC was affected, and steers offered daily supplementation had decreased plasma insulin AUC on days that both treatments received supplementation, but increased plasma insulin AUC when only those steers received supplementation. In contrast, Cooke et al. (2008) and Moriel et al. (2012) reported that plasma concentrations of glucose and insulin were greater for steers receiving concentrate supplementation 3 times weekly (S3) vs. daily supplementation (S7) on days that only S7 received supplementation, but similar plasma concentrations of glucose and insulin on days that both received concentrate supplementation. Those authors attributed the differences in plasma concentrations of glucose and insulin to the pattern of nutrient intake of each treatment, as both insulin and glucose are influenced positively by rate of nutrient intake (Vizcarra et al., 1998) and to the time required for synthesis and activation of gluconeogenic enzymes to substantially increase the magnitude of hepatic synthesis and release of glucose. In support of this rationale, hepatic mRNA expression of pyruvate carboxylase (one of the key gluconeogenic enzymes; Jitrapakdee et al., 2008) in the current study was greater for S7 vs. S3 steers on d 10 and 24 of the study, which correspond to days that only S7 received concentrate supplementation. However, plasma concentrations of glucose differed only on previous days (d 9 and 23 of the study), which correspond to days when both treatments received supplementation.

Reasons for not detecting differences in plasma insulin concentrations and additional differences in plasma glucose concentrations may be attributed to the inflammatory state and APR induced by vaccination. Acute phase response alters carbohydrate metabolism by inhibiting de novo synthesis of glucose (Gifford et al., 2012) and increasing insulin synthesis (Andersson et al., 2001) likely to decrease hepatic utilization of carbohydrates to meet the demand of peripheral tissues. For instance, hyper metabolic states that occur during the acute phase of illness impact carbohydrate and lipid metabolism (Chioléro et al., 1997), whereas LPS infusions depleted carbohydrate stores, caused hypoglycemic states for 10 h in cattle (Kushibiki et al., 2002), and inhibited in vitro hepatic expression of gluconeogenic enzymes (Jones and Titheradge, 1993). In the current study, plasma concentrations of insulin increased on d 1 and gradually decreased below baseline levels on d 2 and 3, relative to vaccination, whereas plasma concentrations of glucose decreased on d 2 and 3, relative to vaccination, regardless of treatment assignment. These effects on plasma concentrations of glucose and insulin, in combination with the decrease in plasma concentrations of IGF-1 and PUN after vaccination, support the rationale that nutrients were partitioned to support immunity rather than growth. In addition, the release of glucocorticoids stimulates gluconeogenesis (Carroll and Forsberg, 2007), and hence S3 steers may have increased glucose synthesis due to greater plasma

cortisol concentrations, which may have prevented the detection of differences in plasma concentrations of glucose and insulin compared to S7 steers.

Neutralizing serum antibody titers may be used as an indicator of immune protection, disease prevention, and vaccine efficacy in calves (Howard et al., 1989; Bolin and Ridpath, 1990; Richeson et al., 2008). 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), timing of vaccination after feedlot entry (Richeson et al., 2008), and MP supply (Moriel et al., 2015). Duff and Galyean (2007) highlighted that few studies focused on the interaction between vaccination and nutrition. Thus, we explored the potential effects of decreasing the frequency of energy supplementation on postvaccination antibody production of preconditioning steers. In the present study, positive seroconversion and serum titers against BVDV-1b were less for S3 vs. S7 steers indicating that decreasing the frequency of energy supplementation lessened the vaccine response, which might lead to less immune protection against BVDV-1b and greater chances of developing bovine respiratory diseases. For instance, the majority of bovine respiratory disease cases occur within 30 d postweaning or 14 d relative to feedlot entry (Kirkpatrick et al., 2008), whereas calves with serum BVDV-890 neutralizing titers > 4 (log base 2 scale) did not develop severe clinical signs of fever, leukopenia, and diarrhea (Bolin and Ridpath, 1990). Downey et al. (2013) reported that BVDV antibody titers increased by 0.068 titer units (log base 2) for every 1 kg increase on ADG during the first 21 d after vaccination. Therefore, the greater mean daily CP intake (Moriel et al., 2015) and overall ADG (Downey et al., 2013) of S7 vs. S3 steers likely contributed to the differences observed on serum BVDV-1b titers. In addition, cortisol may induce immune suppression effects (Salak-Johnson and McGlone, 2007), weaken the innate immune response (Dai and McMurray, 1998), and block the cytokine secretion by $CD4^{+T}$ helpers 1 and 2 that are involved in antibody production (Salak-Johnson and McGlone, 2007). Hence, the exacerbated physiological stress experienced by S3 steers led to greater plasma concentrations of cortisol than S7 steers and may have decreased the communication between innate and humoral immune response causing a decreased antibody production against BVDV-1b.

In summary, decreasing the frequency of energy-based, low-starch concentrate supplementation from daily to 3 times weekly decreased growth performance, increased plasma concentrations of haptoglobin and cortisol, and decreased vaccine-induced antibody production against BVDV-1b of recently weaned beef steers during a 42-d preconditioning period. Taken together, our results suggest that decreasing the frequency of energy supplementation during preconditioning and vaccination are not recommended because it might decrease growth performance, vaccine response, and potentially immune protection against pathogens associated with bovine respiratory disease.

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