Decreasing the frequency and rate of wet brewers grains supplementation did not impact growth but reduced humoral immune response of preconditioning beef heifers[1]

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ABSTRACT: This study evaluated growth and measurements of innate and humoral immunity of preconditioning beef heifers supplemented with wet brewers grains (WBG) at 2 supplementation rates and frequencies. At 14 d after weaning (d 0), Angus heifers ($n = 36$; 213 ± 2 kg BW and 254 ± 7 d of age) were stratified by BW and age and randomly assigned to 1 of 12 drylot pens (3 heifers/pen). Treatments were randomly assigned to pens, in a 2 × 2 factorial design, and consisted of heifers provided ground tall fescue hay ad libitum (55% TDN and 12% CP of DM) and supplemented with WBG (75% TDN and 36% CP of DM) either daily (7X) or 3 times weekly (3X; Monday, Wednesday, and Friday) at 0.5 or 1.0% of BW (DM basis) for 42 d. Heifers were vaccinated against infectious bovine rhinotracheitis (IBR), bovine viral diarrhea virus (BVDV), Mannheimia haemolytica, and Clostridium on d 14 and 28. Individual BW was measured before feeding on d 0 and 42 following 12 h of feed and water withdrawal. Blood samples were collected via jugular venipuncture 4 h after WBG supplementation on d 14, 15, 16, 17, 21, 28, 29, 30, 31, 35, and 42. Heifers fed WBG 3X had less hay DMI (2.6 ± 0.16 vs. 3.2 ± 0.16 kg/d; $P < 0.0001$) but greater total DMI (5.6 ± 0.16 vs. 3.8 ± 0.16 kg/d; $P < 0.0001$) than 7X heifers on days that all heifers received WBG supplementation. However, overall hay and total DMI was not affected ($P \geq 0.40$) by supplementation frequency. Therefore, ADG, BW, and G:F from d 0 to 42 did not differ among treatments ($P \geq 0.29$). Plasma concentrations of haptoglobin on d 15 and cortisol on d 14 were greater for 3X heifers vs. 7X heifers ($P \leq 0.04$). Heifers fed WBG at 0.5% of BW tended to have greater plasma cortisol concentrations on d 15, 17, and 35 ($P \leq 0.09$) than heifers fed at 1.0% of BW. Serum BVDV-1a titers were greater ($P = 0.04$) for 7X heifers vs. 3X heifers on d 42 (4.2 ± 0.28 vs. 3.3 ± 0.28 $\log_2$), whereas serum titers against BVDV-2 and IBR were greater for heifers fed WBG at 1.0% of BW vs. heifers fed WBG at 0.5% of BW (7.6 vs. 6.7 and 3.3 vs. 2.8 ± 0.19 $\log_2$, respectively). In summary, decreasing WBG supplementation frequency (7 vs. 3 times weekly) or rate (1.0 vs. 0.5% of BW) for recently weaned beef heifers did not affect growth but decreased vaccine-induced antibody production against pathogens associated with bovine respiratory disease during a 42-d preconditioning period.

Key words: heifers, immune, preconditioning, supplementation frequency, vaccination

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INTRODUCTION

Wet brewers grains (**WBG**) are byproducts of barley brewing. Due to the removal of sugar and starch after malting and mashing processes, WBG contains greater concentrations of trace minerals than the foundation grains (Westendorf and Wohlt, 2002; Homm et al., 2008) and was an effective substitute for ground corn in supplements for preconditioning beef calves (Moriel et al., 2015b). Preconditioning

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beef calves typically experience processes, such as weaning, vaccination, and feedlot entry, that induce an acute-phase protein response (**APR**) and impair immunity and growth (Arthington et al., 2013). Recently, we demonstrated that decreasing the frequency of concentrate supplementation from daily to 3 times weekly reduced feeding costs but impaired growth and humoral immunity of preconditioning beef steers (Artioli et al., 2015). Hence, it is crucial to identify nutritional strategies that would enable the reduction on frequency of supplementation to decrease feed costs without impairing growth and immunity of calves.

Growth and immunity of calves described above were likely impaired due to a greater reduction on hay DMI and fluctuations in nutrient intake of steers offered concentrate 3 vs. 7 times weekly (Artioli et al., 2015), which was expected because supplements often decrease forage DMI when supplemental TDN intake is greater than 0.7% of BW (Moore et al., 1999). We hypothesized that reducing the frequency of WBG supplementation would not impact growth and immunity of beef heifers if supplementation rate was also decreased, leading to less reduction on hay DMI and variation in nutrient consumption. Hence, our objectives were to evaluate the growth, trace mineral status, and vaccine-induced innate and humoral immunity of preconditioning beef heifers supplemented WBG at 2 supplementation rates (0.5 and 1.0% of BW) and frequencies (3 vs. 7 times weekly).

MATERIALS AND METHODS

The Institutional Animal Care and Use Committee of North Carolina State University (protocol number 15-090-A) approved all procedures for the experiment conducted at the Mountain Research Station (Waynesville, NC; 35.48° N, 82.99° W, and 659 m elevation) from July to August 2015.

Animals, Diets, and Sample Collection

Angus heifers ($n = 36$; 213 ± 2 kg BW and 254 ± 7 d of age) were weaned on d −14, immediately allocated into a single 22-ha tall fescue pasture (Lolium arundinaceum; 16% CP and 59% TDN, DM basis), and provided concentrate DM at 0.5% of BW (50:50 soy hulls and corn gluten pellets; 17% CP and 72% TDN, DM basis) and free-choice access to white salt without trace mineral fortification for 14 d. On d 0, heifers were stratified by BW and age and randomly assigned to 1 of 12 concrete floor pens (3 heifers/pen; 18 by 4 m; 24 m^2/heifer) in a half-covered drylot feeding facility. Treatments were randomly assigned to pens (3 pens/treatment), in a 2 × 2 factorial design, and consisted of heifers provided daily free-choice access to ground tall fescue hay and supplemented with WBG at 0.5 or 1.0% of BW (DM basis). Within each supplementation rate, heifers were then assigned to receive a similar weekly concentrate amount (weekly WBG DMI of 0.5 or 1% of BW multiplied by 7 d) that was offered either daily (**7X**) or 3 times weekly (**3X**; Monday, Wednesday and Friday) from d 0 to 42.

Hay and WBG were offered separately in the same feed bunk at 0800 h. Daily WBG offered was adjusted daily to account for alterations on DM concentration, whereas weekly WBG offered 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. Individual BW was measured before feeding on d 0 and 42, following 12 h of feed and water withdrawal. Shrunk BW was not obtained on d 21 to not disturb feeding behavior and avoid an unnecessary physiological stress response due to shrink that could interfere with plasma measurements and vaccine response (Marques et al., 2012). A complete mineral mix (RU-MIN 1600; Southern States, Richmond, VA; average composition, 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, 1,220 mg/kg Cu, 2,130 mg/kg Mn, 29 mg/kg Se, and 2,530 mg/kg Zn) was top-dressed daily over the supplement at a rate of 0.114 g/heifer from d 0 to 42.

Hay and WBG DM offered and refused were obtained daily for each pen by drying samples of hay and WBG offered and refusal in a forced-air oven at 56°C for 48 (hay) or 72 h (WBG). Daily DMI was determined by subtracting the daily hay and WBG DM refused from the daily hay and WBG DM offered. Samples of hay, WBG, and mineral mix offered were collected daily and pooled within each week (1 to 6) and then sent in duplicate to a commercial laboratory (Dairy One Forage 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 proposed by the NRC (2000).

On d 0, all heifers were treated with doramectin for internal and external parasites (5 mL subcutaneous; Dectomax injectable; Zoetis Inc., Kalamazoo, MI). On d 14, heifers were vaccinated against infectious bovine rhinotracheitis (**IBR**), bovine viral diarrhea virus (**BVDV**) types 1a and 2, *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 28, heifers received 2-mL subcutaneous boosters of Bovi-Shield

Table 1. Average weekly chemical composition of ground tall fescue hay and wet brewers grains (WBG) provided to heifers from d 0 to 42[1]

Item	Tall fescue hay	WBG
DM, %	92.6	21.2
	——— DM basis ———	
CP, %	12.0	32.4
ADF, %	41.1	25.0
NDF, %	64.1	50.6
TDN,[2] %	56.0	72.5
NEm,[3] Mcal/kg	1.07	1.71
NEg,[3] Mcal/kg	0.52	1.09
Ca, %	0.40	0.24
K, %	1.98	0.08
Mg, %	0.23	0.18
Na, %	0.01	0.003
P, %	0.27	0.62
Co, mg/kg	0.37	0.17
Cu, mg/kg	6.0	21.0
Fe, mg/kg	426	165
Mn, mg/kg	100	47
Mo, mg/kg	0.40	2.65
S, %	0.19	0.39
Se, mg/kg	0.03	0.65
Zn, mg/kg	26	86

[1] Hay and WBG samples were collected daily, pooled within each week, and sent in duplicate to a commercial laboratory for wet chemistry analysis (Dairy One Forage Laboratory, Ithaca, NY).

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

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

Gold 5 (Zoetis Inc., New York, NY) and Ultrabac 7. The vaccination protocol described above was chosen to replicate the protocol used by the local preconditioning alliance (Mountain Cattle Alliance, Canton, NC; Moriel et al., 2015a; Artioli et al., 2015). The vaccination protocol was initiated 14 d after feedlot entry to avoid the feedlot entry–induced inflammatory response that could interfere with vaccine response (Richeson et al., 2008).

Blood samples (10 mL) were collected via jugular venipuncture into sodium heparin (158 United States Pharmacopeia units)–containing tubes (Vacutainer; Becton, Dickinson and Company, Franklin Lakes, NJ) for plasma harvest 4 h after WBG supplementation on d 14, 15, 16, 17, 21, 28, 29, 30, 31, 35, and 42. The approach of collecting blood samples 4 h after feeding was previously used to correspond to the peak of ruminal fermentation and end products release after concentrate consumption (Moriel et al., 2012, 2015a; Artioli et al., 2015) and to correspond with days that all heifers received WBG supplementation (d 14, 16, 28, and 30) and days that only 7X heifers received WBG supplementation (d 15, 17, 29, and 31). Additional blood samples (10 mL) from the jugular vein were collected into a tube containing no additives (Vacutainer; Becton, Dickinson and Company) for serum harvest on d 0 and 42 to evaluate serum antibody titers against IBR, BVDV-1a, and BVDV-2. 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.

Liver samples (100 mg of tissue wet weight) were collected via needle biopsy from all heifers on d 0 and 42, following the procedure described by Arthington and Corah (1995), and then stored at −80°C until later laboratory analyses. Samples were then assessed for trace mineral concentrations at Michigan State University Diagnostic Center for Population & Animal Health (Lansing, MI). Liver trace mineral concentrations on d 0 were included to covariately adjust liver trace mineral concentrations on d 42. Liver samples were collected only on d 0 and 42 1) because our goal was to evaluate the final liver trace mineral concentrations of heifers after receiving WBG supplementation for 42 d at different rates and frequencies and 2) to avoid a surgery-induced inflammatory response in the middle term of the study that could interfere with vaccine response. Hence, the analyses of trace mineral consumption were calculated by multiplying the mean weekly DMI of hay, WBG, and mineral mix by the respective weekly mean concentration of each trace mineral present in hay, WBG, and mineral mix.

Laboratory Analyses

Plasma concentrations of haptoglobin were determined in duplicate samples using a biochemical assay assessing haptoglobin–hemoglobin complex by the estimation of differences in peroxidase activity (Cooke and Arthington, 2013). Plasma concentrations of cortisol were determined using a single chemiluminescent enzyme immunoassay (Immulite 1000; Siemens Medical Solutions Diagnostics, Los Angeles, CA). Intra- and interassay CV for assays of haptoglobin were 2.0 and 8.0%, respectively, and for cortisol were 2.9 and 2.7%, respectively.

Serum antibody titers against IBR, BVDV-1a, and BVDV-2 were determined by the Oklahoma Animal Disease and Diagnostic Laboratory (Stillwater, OK) using a virus neutralization test (Rosenbaum et al., 1970). Serum titers 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 were 1:4 and 1:256, respectively). For the seroconversion analyses, 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

Table 2. Growth performance of heifers provided, in a 2 × 2 factorial design, wet brewers grains supplementation rate at 0.5 or 1.0% of BW (DM basis) that was offered either daily (7X) or 3 times weekly (3X; Monday, Wednesday, and Friday) from d 0 to 42 (n = 3 pens/treatment; 3 heifers/pen)[1]

	Treatment[2]					
Item	3X0.5	7X0.5	3X1.0	7X1.0	SEM	*P*-value
BW,[3] kg						Frequency × rate × day
d 0	212	213	214	213	2.0	0.43
d 42	255	253	258	262	3.4	
d 0 to 42						Frequency × rate
ADG, kg/d	1.01	0.96	1.04	1.15	0.104	0.43
Total DMI, kg	185	174	192	196	10.2	0.48
G:F[4]	0.23	0.23	0.23	0.25	0.011	0.58

[1] From d 0 to 42, heifers were provided daily free-choice access to ground tall fescue hay.

[2] 3X0.5 = WBG supplementation at 0.5% of BW (DM basis) and offered 3 times weekly (Mondays, Wednesdays, and Fridays); 7X0.5 = WBG supplementation at 0.5% of BW (DM basis) and offered 7 times weekly (daily); 3X1.0 = WBG supplementation at 1.0% of BW (DM basis) and offered 3 times weekly (Mondays, Wednesdays, and Fridays); 7X1.0 = WBG supplementation at 1.0% of BW (DM basis) and offered 7 times weekly (daily).

[3] Body weight obtained after 12 h of feed and water withdrawal.

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

calculate the positive seroconversion (% of steers with positive serum neutralization; Richeson et al., 2008; Moriel et al., 2015a; Artioli et al., 2015).

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 heifer(pen) and pen(treatment) were included as random effects in all analyses, except for analyses of hay, WBG, and total and trace mineral consumption, which included only pen(treatment) as random effect. Feed efficiency, ADG, and mean total DMI were tested for fixed effects of supplementation frequency, rate, and frequency × rate. Within each week of the study, daily DMI data was pooled by days that all 3X and 7X heifers were fed WBG (Monday, Wednesday, and Friday) and days that only 7X heifers were fed WBG (Tuesday, Thursday, Saturday, and Sunday) to simplify data analyses, interpretation, and reporting. Daily intake of DM (WBG, hay, and total), CP, and NEg data were analyzed as repeated measures and tested for fixed effects of supplementation frequency, rate, week of the study, day of supplementation, and all resulting interactions using pen(treatment) as the subject. Body weight, plasma, and serum measurements were analyzed as repeated measures and tested for fixed effects of frequency, rate, day of supplementation, week of the study (except for BW analyses), and resulting interactions. Compound symmetry covariance structure was used for the analyses of BW and serum titers whereas autoregressive 1 was used for the analyses of plasma concentrations of cortisol and haptoglobin, as these covariance structures generated the lowest Akaike information criterion. Positive seroconversion to IBR, BVDV-1a, and BVDV-2 were analyzed as repeated measures using the GLIMMIX procedure of SAS with pen(treatment) and heifer(pen) as random effects. 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 if $P > 0.05$ and $P \leq 0.10$.

RESULTS

Effects of frequency × rate × day of study, frequency × rate, and frequency were not detected ($P \geq 0.21$) for BW, ADG, total DMI, and G:F from d 0 to 42 (Table 2). A tendency for rate of supplementation effect was detected ($P = 0.07$) for mean BW, which was greater for heifers supplemented with WBG at 1.0 vs. 0.5% of BW (237 vs. 233 ± 1.3 kg, respectively).

Effect of frequency × rate × day of supplementation was detected ($P < 0.0001$) for WBG DMI (% relative to initial offer; Table 3). Heifers supplemented with WBG at 0.5% of BW daily consumed 100% of the initial WBG DM offered, whereas 7X1.0 heifers consumed in average 99.6% of daily WBG DM offered, resulting in a loss of 0.4% of WBG DM offered (Table 3). On days of supplementation (Monday, Wednesday, and Friday), 3X0.5 heifers consumed 99.8% of initial WBG DM offered and did not consume the remaining 0.2% of WBG DM on the next day, and therefore, WBG refused had to be discarded before the next WBG supplementation event. On days of supplementation (Monday, Wednesday, and

Table 3. Supplemental wet brewers grains (WBG) DMI of heifers provided, in a 2 × 2 factorial design, WBG supplementation rate at 0.5 or 1.0% of BW (DM basis) that was offered either daily (7X) or 3 times weekly (3X) from d 0 to 42 ($n = 3$ pens/treatment; 3 heifers/pen)

	Treatment[2]					*P*-value[3]
Item[1]	3X0.5	7X0.5	3X1.0	7X1.0	SEM	Frequency × rate × day
WBG DMI,[4] % of initial DM offered						
Monday, Wednesday, and Friday	99.8^{b}	100.0^{b}	73.1^{a}	99.9^{b}	0.90	<0.0001
Tuesday, Thursday, Saturday, and Sunday	0.0^{a}	100.0^{c}	19.5^{b}	99.4^{c}	0.90	
P-value[5]	<0.0001	1.00	<0.0001	0.68		

[a–c]Within supplementation day, means without a common superscript differ ($P \le 0.05$).

[1] Monday, Wednesday, and Friday: days when all 3X and 7X heifers received WBG supplementation; Tuesday, Thursday, Saturday, and Sunday: days that only 7X heifers received WBG supplementation.

[2] 3X0.5 = WBG supplementation at 0.5% of BW (DM basis) and offered 3 times weekly (Mondays, Wednesdays, and Fridays); 7X0.5 = WBG supplementation at 0.5% of BW (DM basis) and offered 7 times weekly (daily); 3X1.0 = WBG supplementation at 1.0% of BW (DM basis) and offered 3 times weekly (Mondays, Wednesdays, and Fridays); 7X1.0 = WBG supplementation at 1.0% of BW (DM basis) and offered 7 times weekly (daily).

[3] *P*-value for effect of frequency × rate × day of supplementation.

[4] Dry matter intake of WBG relative to initial WBG DM offered.

[5] Treatment comparison within each day.

Friday), 3X1.0 heifers consumed 73.1% of initial WBG DM offered. On the following days (Tuesday, Thursday, Saturday, and Sunday), 3X1.0 heifers consumed only 19.5 of the 26.9% of WBG DM remaining from the previous day, resulting on a loss of 7.4% of WBG DM offered that was discarded (Table 3).

Effects of frequency × day of supplementation (Table 4) and rate of supplementation, but not frequency × rate × day of supplementation and rate × day of supplementation ($P \ge 0.42$), were detected for hay DMI ($P \le 0.02$). Regardless of supplementation rate, 7X heifers had similar daily hay DMI throughout the week, whereas 3X heifers had greater hay DMI on days that they did not receive WBG supplementation compared with days that WBG was provided ($P < 00001$). However, hay DMI of 7X and 3X heifers did not differ regardless of the day of the week ($P \ge 0.18$; Table 4). Mean hay DMI was greater for heifers supplemented with WBG at 0.5% of BW vs. heifers supplemented with WBG at 1.0% of BW (3.21 vs. 2.58 ± 0.156 kg/d, respectively).

Effects of frequency × day of supplementation, but not frequency × rate × day of supplementation, rate × day of supplementation, frequency × rate, frequency, and rate of supplementation ($P \ge 0.16$), were detected for intake of total DM, CP, and NEg ($P < 0.0001$; Table 4). Regardless of supplementation rate, 7X heifers had a similar daily intake of total DM, CP, and NEg throughout the week ($P \ge 0.12$), whereas 3X heifers had greater intake of total DM, CP, and NEg on days that they received WBG supplementation compared with days that WBG was not provided ($P < 0.0001$). On days of supplementation, however, intake of total DM, CP, and NEg were greater for 3X heifers vs. 7X heifers ($P \le 0.001$), whereas intake of total DM, CP, and NEg were less for 3X heifers vs. 7X heifers on days that only 7X heifers were fed WBG ($P \le 0.01$; Table 4). Effects of supplementation rate were detected ($P \le 0.02$) for intake of total CP and NEg, which were less for heifers supplemented with WBG at 0.5 vs. 1.0% of BW (0.77 vs. 1.02 ± 0.030 kg/d of CP, and 2.97 vs. 3.74 ± 0.116 Mcal/d of NEg, respectively).

Liver trace mineral concentrations on d 0 were included to covariately adjust liver trace mineral concentrations on d 42. Effects of frequency × rate and frequency were not detected ($P \ge 0.18$) for daily intake and liver concentrations of trace minerals. Effects of rate of supplementation were detected for daily intake of Cu, Mo, Se, and Zn ($P < 0.0001$) and liver concentrations of Co and Se ($P \le 0.02$) but not for the remaining trace minerals ($P \ge 0.31$; Table 5). Heifers supplemented with WBG at 1.0% of BW had greater intake of Cu, Mo, Se, and Zn and less liver Co concentrations but greater liver Se concentrations than heifers fed WBG at 0.5% of BW. Effects of supplementation rate, but not frequency and frequency × rate ($P \ge 0.59$), were detected for mean daily S intake, which was greater ($P = 0.001$) for heifers fed WBG at 1.0 vs. 0.5% of BW (13.7 vs. 11.1 ± 0.33 g/d, respectively).

Plasma concentrations of haptoglobin and cortisol on d 0 did not differ among treatments ($P \le 0.29$) but were included as covariates ($P \le 0.04$). Effects of frequency of supplementation × day of the study ($P = 0.05$; Fig. 1), but not frequency × rate × day of the study and rate of supplementation × day of the study ($P \ge 0.61$), were detected for plasma haptoglobin concentrations. Plasma haptoglobin concentrations were greater for 3X heifers vs. 7X heifers on d 14 ($P = 0.006$) but greater for 7X heifers vs. 3X heifers on d 16 ($P = 0.03$; Fig. 1). Effects of frequency of supplementation × day of the study ($P = 0.05$; Fig. 2a) and rate of supplementation × day of the study ($P = 0.05$; Fig. 2b), but not frequency ×

Table 4. Ingredient and nutrient intake of heifers provided, in a 2 × 2 factorial design, wet brewers grains (WBG) supplementation rate at 0.5 or 1.0% of BW (DM basis) that was offered either daily (7X) or 3 times weekly (3X) from d 0 to 42 (*n* = 3 pens/treatment; 3 heifers/pen)

	Supplementation frequency				*P*-value
Item[1]	3X	7X	*P*-value[2]	SEM	Frequency × day[3]
Hay DMI, kg/d					
Monday, Wednesday, and Friday	2.56	2.89	0.18	0.160	<0.0001
Tuesday, Thursday, Saturday, and Sunday	3.22	2.91	0.20	0.160	
P-value[4]	<0.0001	0.74			
Total DMI, kg/d					
Monday, Wednesday, and Friday	5.58	4.40	0.001	0.166	<0.0001
Tuesday, Thursday, Saturday, and Sunday	3.81	4.59	0.01	0.166	
P-value[4]	<0.0001	0.55			
CP intake,[5] kg/d					
Monday, Wednesday, and Friday	1.30	0.85	<0.0001	0.031	<0.0001
Tuesday, Thursday, Saturday, and Sunday	0.55	0.88	<0.0001	0.031	
P-value[4]	<0.0001	0.21			
NEg intake,[5] Mcal/d					
Monday, Wednesday, and Friday	4.66	3.20	<0.0001	0.120	<0.0001
Tuesday, Thursday, Saturday, and Sunday	2.25	3.30	0.0001	0.120	
P-value[4]	<0.0001	0.12			

[1] Monday, Wednesday, and Friday: days when all 3X and 7X heifers received WBG supplementation; Tuesday, Thursday, Saturday, and Sunday: days that only 7X heifers received WBG supplementation.

[2] Comparison of frequency of WBG supplementation within each day.

[3] *P*-value for effects of supplementation frequency × day of supplementation.

[4] Comparison of day within each WBG supplementation frequency.

[5] Calculated as daily DMI of hay and WBG multiplied by respective CP and NEg concentrations.

rate × day of the study and frequency × rate of supplementation ($P \geq 0.25$), were detected for plasma concentrations of cortisol. Heifers fed WBG 3 times weekly had greater ($P = 0.04$) plasma cortisol concentrations on d 15 compared with 7X heifers. Heifers fed WBG at 1.0% of BW (DM basis) had greater plasma concentrations of cortisol on d 17 ($P = 0.05$) and tended to have greater plasma cortisol concentrations on d 15 ($P = 0.06$) and 35 ($P = 0.09$) compared with heifers fed WBG at 0.5% of BW (DM basis).

Effects of frequency × rate × day of supplementation, frequency × day of supplementation, rate × day of supplementation, frequency × rate, rate, and frequency were not detected ($P \geq 0.30$) for positive seroconversion for IBR (44.4, 44.4, 44.4, and 50.0 ± 4.81% for 3X0.5, 7X0.5, 3X1.0, and 7X1.0 heifers, respectively), BVDV-1a (44.4, 50.0, 38.9, and 44.4 ± 5.30% for 3X0.5, 7X0.5, 3X1.0, and 7X1.0 heifers, respectively), and BVDV-2 titers (50.0, 55.6, 50.0, and 50.0 ± 2.78% for 3X0.5, 7X0.5, 3X1.0, and 7X1.0 heifers, respectively). Effects of frequency × rate × day of supplementation, frequency × rate, rate, and frequency were not detected ($P \geq 0.15$) for serum titers against IBR, BVDV-1a, and BVDV-2. However, a tendency for effects of frequency × day of study was detected ($P = 0.06$) for serum BVDV-1a titers (Table 6), which was greater for 7X heifers vs. 3X heifers on d 42 ($P = 0.04$). Effects of rate × day of study were detected for serum titers against BVDV-2 and IBR ($P \leq 0.05$), both of which were greater ($P \leq 0.04$) for heifers supplemented with WBG at 1.0% of BW vs. heifers supplemented with WBG at 0.5% of BW on d 42 (Table 6).

DISCUSSION

Previously, decreasing the frequency of energy supplementation from daily to 3 times weekly reduced ADG of beef steers and heifers by 10 to 21% (Cooke et al., 2008; Loy et al., 2008; Artioli et al., 2015). In the current study, decreasing the frequency of WBG supplementation from daily to 3 times weekly did not decrease ADG of heifers (regardless of supplementation rate), which is partially in agreement with our hypothesis and in accordance with others (Moriel et al., 2012; Drewnoski et al., 2011, 2014a), but it is in contrast with Artioli et al. (2015), even though these authors used the same preconditioning and vaccination protocols used in the current study. Discrepancies among our results and those from Artioli et al. (2015) are probably related to differences on supplement composition (high-moisture WBG vs. grain pellet–based concentrate), although breed, gender, location, forage species and quality, and potential resulting interactions among these factors may have also played a role.

Table 5. Intake and liver concentrations of trace minerals of heifers provided wet brewers grains (WBG) supplementation rate at 0.5 or 1.0% of BW (DM basis) from d 0 to 42

	Supplementation rate			
Item	0.5%	1.0%	SEM	*P*-value
Intake[1]	—mg/d (DM basis)—			
Co	4.68	4.61	0.063	0.45
Cu	180	197	1.12	<0.0001
Fe	1,549	1,436	72.4	0.31
Mn	615	597	17.0	0.48
Mo	4.04	6.40	0.089	<0.0001
Se	4.08	4.7	0.014	<0.0001
Zn	462	530	4.8	<0.0001
Liver concentration[2]	—mg/kg (DM basis)—			
Co	0.29	0.24	0.012	0.01
Cu	321	356	24	0.31
Fe	517	543	138	0.87
Mn	13.3	15.5	2.08	0.44
Mo	3.73	3.87	0.126	0.39
Se	2.2	2.54	0.098	0.02
Zn	195	181	10.4	0.34

[1] Trace mineral consumption was calculated by multiplying the mean weekly DMI of hay, WBG, and mineral mix by the respective weekly mean concentration of each trace mineral present in hay, WBG, and mineral mix.

[2] Covariate-adjusted for liver concentration of the respective trace mineral on d 0 ($P \leq 0.05$), except for Fe and Mn ($P \geq 0.27$).

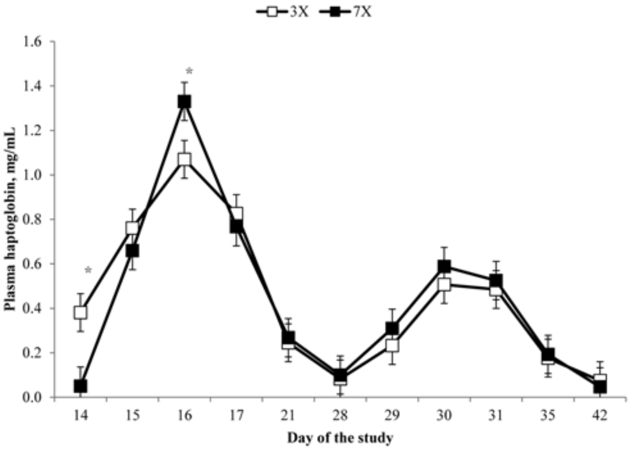

Figure 1. Plasma haptoglobin concentrations of heifers provided, in a 2 × 2 factorial design, wet brewers grains supplementation rate at 0.5 or 1.0% of BW (DM basis) that was offered either daily (7X) or 3 times weekly (3X) from d 0 to 42 ($n = 3$ pens/treatment; 3 heifers/pen). Means were covariate adjusted to plasma concentrations of haptoglobin on d 0 ($P = 0.04$). Effects of frequency of supplementation × day ($P = 0.05$), but not rate of supplementation × day ($P = 0.61$), were detected for plasma haptoglobin concentrations. Plasma haptoglobin concentrations were greater for 3X heifers vs. 7X heifers on d 14 ($P = 0.006$) but greater for 7X heifers vs. 3X heifers on d 16 ($P = 0.03$). *Within day, means without a common superscript differ ($P \leq 0.05$).

High-moisture supplements cause different feeding behavior compared with high-DM, grain-based supplements, leading to different outcomes on forage intake (Arthington et al., 2004; Cooke et al., 2007). For instance, Arthington et al. (2004) reported that beef heifers had complete consumption of dry range cube-based supplement within 1 h after feeding, whereas heifers fed isocaloric, isonitrogenous, high-moisture molasses-based supplement had a longer period for total supplement consumption (approximately 48 h). In agreement, Artioli et al. (2015) reported that steers supplemented 3 times weekly with grain-based concentrate (50:50 soy hulls corn and gluten feed pellets) at 1% of BW consumed 100% of the supplement offered within 6 h after supplementation, whereas in the current study, 3X1.0 heifers consumed 73.1% of supplement DM on days of supplementation and only 19.5% on the next day. Consequently, hay DMI of 3X1.0 heifers was 20.5% less on days of WBG supplementation, whereas in the study of Artioli et al. (2015), hay DMI of steers fed concentrate at 1% of BW 3 times weekly was 57.1% less on days of WBG supplementation vs. days that WBG was not provided. Despite the low starch concentration of WBG, this reduction on daily hay DMI was expected because supplements often decrease forage DMI when the TDN:CP ratio is less than 7 and supplemental TDN is greater than 0.7% of BW (Moore et al., 1999). Therefore, the high-moisture characteristics of WBG likely caused rumen fill effects and limited supplemental DM intake of 3X heifers on days of supplementation, whereas the relatively high spoilage rate of WBG (Moriel et al., 2015c) resulted in an offensive odor (and likely taste) that reduced intake of WBG left from the previous day. This combination of high moisture and high spoilage rate of WBG caused smaller daily variation on daily supplemental DMI throughout the week and decreased the magnitude of supplement-induced reduction on hay DMI, leading to similar total DMI and ADG compared with 7X heifers. It is also important to highlight that due to the slower consumption and high spoilage rate of WBG, 0.2 and 7.4% of initial WBG DM offered were not consumed by 3X0.5 and 3X1.0 heifers, respectively, and had to be discarded. This supplement wastage needs to be accounted for when evaluating the economic feasibility of using WBG supplementation.

Due to the removal of sugar and starch after malting and mashing processes, WBG contains greater concentrations of minerals than the unprocessed grains (Westendorf and Wohlt, 2002). Dietary concentrations of Cu, Fe, Mn, Mo, and Zn linearly increased as WBG was gradually added from 0 to 45% of diet DM provided to feedlot beef heifers (Homm et al., 2008), whereas serum Se concentration was greater for feedlot beef heifers fed WBG at 34% of diet DM vs. heifers fed WBG at 0% of diet DM (Crickenberger and Johnson, 1982). In the current study, the concentrations of Mo, Cu, Se, and Zn were greater whereas Co concentrations were less for WBG vs. tall fescue hay (Table 1), which explains the

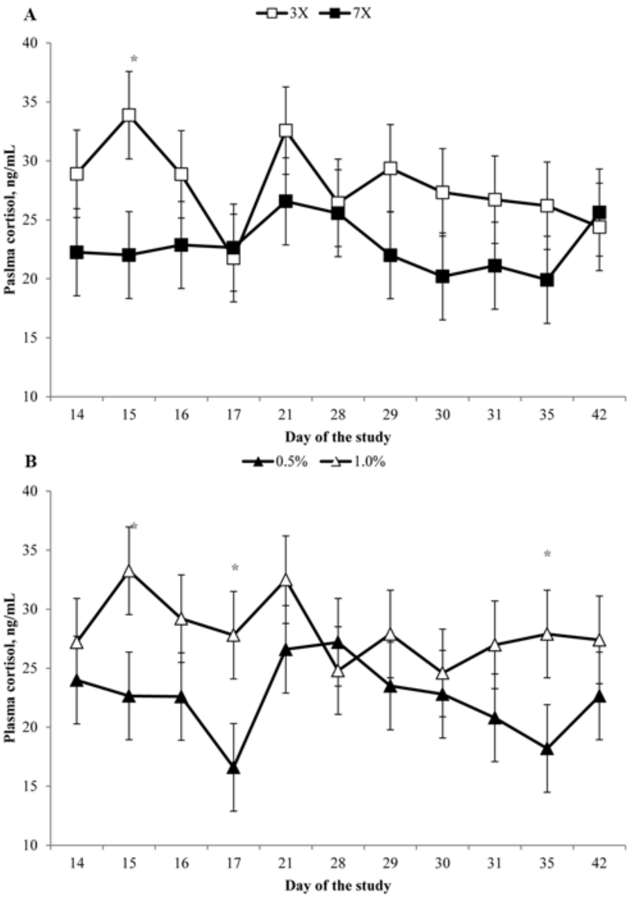

Figure 2. Plasma cortisol concentrations of heifers provided, in a 2 × 2 factorial design, wet brewers grains (WBG) supplementation rate at 0.5 or 1.0% of BW (DM basis) that was offered either daily (7X) or 3 times weekly (3X) from d 0 to 42 (*n* = 3 pens/treatment; 3 heifers/pen). Means were covariate adjusted to plasma concentrations of cortisol on d 0 (P = 0.04). Effects of frequency of supplementation × day ($P = 0.05$; Fig. 2a) and rate of supplementation × day ($P = 0.05$; Fig. 2b) were detected for plasma concentrations of cortisol. Heifers fed WBG 3 times weekly had greater ($P = 0.04$) plasma cortisol concentrations on d 15 than heifers supplemented daily. Heifers fed WBG at 0.5% of BW (DM basis) had greater plasma concentrations of cortisol on d 17 ($P = 0.05$) and tended to have greater plasma cortisol concentrations on d 15 ($P = 0.06$) and 19 ($P = 0.09$) compared with heifers fed WBG at 1.0% of BW (DM basis). *Within day, means without a common superscript differ ($P \leq 0.05$).

observed differences on intake of these minerals and on liver concentrations of Co and Se as WBG supplementation rate increased (Table 6). Liver concentrations of Co was less for heifers supplemented with WBG at 1.0% of BW vs. heifers supplemented with WBG at 0.5% of BW, which reflects the numerically less intake of Co caused by the reduction on hay DMI as WBG supplementation rate increased. Liver Se concentrations were greater for heifers supplemented with 1.0 vs. 0.5% of BW, which is in agreement Crickenberger and Johnson (1982), who observed that adding WBG (34 or 62% of diet DM) to a corn silage–based diet increased serum Se concentrations of growing beef heifers. However, liver concentrations of the remaining trace minerals were not affected by WBG supplementation rate. Wet brewers grains contain greater S concentrations than corn and fescue hay (Moriel et al., 2015b). Dietary S concentrations above 0.30% of DM may reduce Cu and Se bioavailability by associating with Mo in the rumen (Suttle, 1974; Mason, 1990; NRC, 2005). Consequently, the impact of WBG supplementation rate on dietary S-induced absorption of Cu and Se needs to be addressed. In the current study, estimated dietary S concentrations (including contributions from mineral mix) were 0.25 and 0.29% of diet DM for heifers supplemented with WBG at 0.5 and 1.0% of BW, respectively, which is close to the low end range of maximum tolerable limit for S in beef cattle diets (NRC, 2005; Drewnoski et al., 2014b). Therefore, dietary S intake might have impacted Cu and Se as consistently reported in the literature, but it was not sufficient to prevent an increase on liver Se concentrations and decrease liver Cu concentrations of heifers supplemented with WBG at 0.5 or 1.0% of BW. Further studies evaluating the impact of greater WBG supplementation rates on growth and trace mineral status are warranted, but based on optimal dietary S concentrations to avoid reduction of intestinal Cu and Se absorption, WBG supplementation rate above 1.0% might not be recommended.

In addition, frequency of WBG supplementation used in the current study did not affect liver concentrations of all trace minerals. Hence, the liver capacity to store trace minerals was not impacted by providing WBG supplementation 3 vs. 7 times weekly. Plausible explanations for the lack of differences on liver concentrations of most trace minerals, due to WBG supplementation rate and frequency, may be an adequate initial liver concentration of trace minerals and metabolic homeostasis (Miller, 1975). All heifers were nursing their dams and had free-choice access to a commercial trace mineral-fortified salt-based supplement from birth to weaning. Liver trace mineral concentrations on d 0 imply that heifers were in adequate trace mineral status at the start of the study. For instance, liver Mo concentrations of all heifers were slightly above the normal range of 2 to 3 mg of Mo/kg of tissue DM (Anke et al., 1985). Also, animals metabolically compensate for mineral imbalances to maintain cellular concentrations of trace minerals within narrow limits (McDowell, 1989, 1992; Underwood and Suttle, 1999). Therefore, the adequate liver trace minerals status and homeostatic control mechanisms of mineral absorption likely prevented further absorption and storage of trace minerals of heifers fed WBG at different supplementation rates (0.5 or 1.0% of BW) and frequencies (3 vs. 7 times weekly). Further studies evaluating the impact of decreasing the frequency of WBG supplementation on liver capacity to store trace minerals, in heifers with trace mineral deficiency, are warranted.

Weaning, feedlot entry, and vaccination stimulate an APR leading to increased hepatic synthesis of acute-

Table 6. Serum antibody titers against infectious bovine rhinotracheitis, bovine viral diarrhea viral types 1a and 2 of heifers provided, in a 2 × 2 factorial design, wet brewers grains supplementation rate at 0.5 or 1.0% of BW (DM basis) that was offered either daily (7X) or 3 times weekly (3X) from d 0 to 42 ($n = 3$ pens/treatment; 3 heifers/pen)[1]

	Supplementation frequency			*P*-value	Supplementation rate			*P*-value
Item	3X	7X	SEM	Frequency × day[2]	0.5%	1.0%	SEM	Rate × day[3]
Bovine viral diarrhea virus type-1a titers, log$_2$								
d 0	0.00^a	0.00^a	0.270	0.06	0.00	0.00	0.270	0.82
d 42	3.26^a	4.17^b	0.280		3.78	3.66	0.280	
Bovine viral diarrhea virus type-2 titers, log$_2$								
d 0	0.00	0.08	0.224	0.93	0.08^a	0.00^a	0.224	0.04
d 42	7.13	7.18	0.224		6.74^a	7.58^b	0.224	
Infectious bovine rhinotracheitis titers, log$_2$								
d 0	0.00	0.00	0.186	0.38	0.00^a	0.00^a	0.186	0.05
d 42	3.20	2.87	0.186		2.76^a	3.31^a	0.186	

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

[1] Heifers were vaccinated with Bovi-Shield Gold One Shot and Ultrabac 7 (Zoetis Inc., New York, NY) on d 14 and Bovi-Shield Gold 5 (Zoetis Inc.) and Ultrabac 7 on d 28.

[2] *P*-value for effects of supplementation frequency × day of the study.

[3] *P*-value for effects of supplementation rate × day of the study.

phase proteins (i.e., haptoglobin; Moriel et al., 2015a). Although cortisol can directly reduce synthesis of proinflammatory cytokines by leukocytes (Kelley, 1988), acute increases in circulating cortisol, such as during a stress or corticotropin releasing hormone challenge, can indirectly stimulate an inflammatory response (Higuchi et al., 1994; Cooke and Bohnert, 2011) and increase plasma haptoglobin concentrations (Cooke and Bohnert, 2011), which may be used as an indicator of inflammation when plasma concentrations are ≥0.11 mg/mL (Tourlomoussis et al., 2004). Therefore, the greater plasma haptoglobin concentrations of 3X heifers vs. 7X heifers on d 15 may be partially associated with the acute rise on prevaccination plasma cortisol concentrations. This response was previously reported by our research group (Artioli et al., 2015) and occurred regardless of supplementation rate (0.5 or 1.0% of BW on a DM basis), which partially agrees with our hypothesis and confirms that decreasing the frequency of supplementation exacerbates the physiological stress (as indicated by greater plasma concentrations of cortisol and haptoglobin before vaccination). 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 and increased synthesis of acute-phase proteins (Zebeli et al., 2010). For instance, plasma haptoglobin concentrations of beef steers peaked after 3 to 9 wk of feeding starch-based diets containing (DM basis) 45 or 95% barley grain (Ametaj et al., 2009). Although WBG has relatively low starch concentrations, the greater supplement DMI of 3X heifers vs. 7X heifers on days that WBG was provided to all heifers likely induced an accumulation of endotoxins in the ruminal fluid and increased hepatic synthesis of haptoglobin. This rationale might also explain the greater plasma cortisol concentrations of heifers fed WBG at 1.0% of BW vs. heifers fed WBG at 0.5% of BW (DM basis), although plasma concentrations of haptoglobin were not impacted by WBG supplementation rate. Further studies need to be conducted to evaluate the rationale of reduced frequency of supplementation causing accumulation of ruminal endotoxin.

It is interesting to notice that although 3X heifers had greater plasma haptoglobin concentrations before vaccination, the postvaccination plasma haptoglobin concentrations were greater for 7X heifers vs. 3X heifers on d 16 and did not differ between these treatments for the remainder of the study. Previously, our laboratory showed that reducing the frequency of energy supplementation (7 vs. 3 times weekly) increased pre- and postvaccination plasma concentrations of haptoglobin (Artioli et al., 2015). Reasons for the discrepancy on vaccination-induced plasma concentrations of haptoglobin between the current and previous studies (Artioli et al., 2015) might be associated with the differences on supplement DM concentration (21.2 vs. 90% of DM, respectively) and time to consume the entire supplemental DM offered. Steers in the study of Artioli et al. (2015) consumed the grain pellet–based supplement within 6 h of feeding, whereas in the current study, heifers consumed the WBG offered within 24 to 48 h of feeding, which may have slowed the ruminal fermentation process. Hence, it is possible that the accumulation of endotoxins in the ruminal fluid and, consequently, the APR-induced synthesis of acute-phase proteins was lessened in the current study. In support of this rationale, mean plasma concentrations of haptoglobin reported by Artioli et al. (2015) and the current study were 0.80 and 0.44 mg/mL, respectively, even though the vaccination and preconditioning protocols were similar.

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). Vaccination response differs 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), MP supply (Moriel et al., 2015a), energy concentration of maternal diet offered during late gestation (Moriel et al., 2016), and frequency of supplementation (Artioli et al., 2015). In the present study, serum titers against BVDV-1a, but not BVDV-2 and IBR, were less for heifers fed WBG 3 times weekly vs. heifers fed WBG 7 times weekly, which is in agreement with our previous study (Artioli et al., 2015) and confirms that decreasing the supplementation frequency lessened the vaccine response. More importantly, the current study also demonstrated that decreasing the frequency of WBG supplementation reduced vaccine-induced BVDV-1a titers regardless of supplementation rate (0.5 or 1.0% of BW; DM basis). The lessened vaccine response of heifers fed 3 times weekly vs. heifers fed 7 times weekly may have resulted in less immune protection against BVDV-1a and greater chances of developing bovine respiratory diseases. For instance, the majority of bovine respiratory disease cases occur within 30 d after weaning or 14 d relative to feedlot entry (Kirkpatrick et al., 2008), whereas calves with serum BVDV-890 neutralizing titers >4 ($\log_2$ scale) did not develop severe clinical signs of fever, leucopenia, and diarrhea (Bolin and Ridpath, 1990). Similar to our previous study (Artioli et al., 2015), the lessened vaccine response may be associated with the greater prevaccination plasma cortisol concentrations of heifers fed WBG 3 times weekly vs. heifers fed WBG 7 times weekly. 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 involved on antibody production (Salak-Johnson and McGlone, 2007). Hence, the exacerbated physiological stress experienced by reducing the frequency of WBG supplementation may have decreased the communication between innate and humoral immune response causing a decreased antibody production against serum BVDV-1a. The exact reasons for the lack of treatment effects on serum BVDV-2 and IBR titers are not known, and further research is needed to elucidate this lack of response.

Although heifers fed WBG at 1.0% of BW (DM basis) had greater plasma cortisol concentrations (and, consequently, greater likelihood for causing immune suppression effects), serum titers against BVDV-2 and IBR were greater for heifers fed WBG at 1.0% of BW vs. heifers fed WBG at 0.5% of BW (DM basis). Previously, we demonstrated that serum titers against BVDV-1a were boosted by increasing dietary concentrations of MP offered to beef steers (115 vs. 85% of daily requirements; Moriel et al., 2015a). Hence, the greater total CP intake of heifers fed WBG at 1.0% of BW vs. heifers fed WBG at 0.5% of BW (1.02 vs. 0.77 kg of CP/d, respectively) might have contributed to the greater postvaccination serum BVDV-2 and IBR titers. In addition, adequate dietary Se concentration is vital for immune function (Arthur et al., 2003) and might affect the resistance to infection in ruminants (Suttle and Jones, 1989). Although the contribution of other trace minerals cannot be disregarded, the greater dietary intake and liver concentrations of Se of heifers supplemented with WBG at 1.0% of BW may further explain the greater serum titers against BVDV-2 and IBR compared with heifers supplemented with WBG at 0.5% of BW. In agreement, beef heifers administered a single injection of trace minerals (60, 10, and 15 mg/mL of Zn, Mn, and Cu, respectively, as disodium EDTA chelates, and 5 mg/mL of Se as sodium selenite) had greater liver Se concentrations and serum titers against porcine red blood cells compared with heifers injected with 0.9% saline solution (Arthington et al., 2014). Also, serum titers against *M. haemolytica* and *Escherichia coli* were increased by intramuscular injections of Se in beef calves (0, 25, and 50 mg of Se; Droke and Loerch, 1989) and dairy cows, respectively (0 or 0.1 mg of Se/kg of BW; Panousis et al., 2001).

In summary, this experiment demonstrated that regardless of supplementation rate, decreasing the frequency of WBG supplementation from daily to 3 times weekly did not affect growth but increased prevaccination plasma cortisol concentrations and postvaccination plasma haptoglobin concentrations and decreased vaccine-induced antibody production against BVDV-1a of beef heifers during a 42-d preconditioning period. Collectively, our results suggest that decreasing the frequency of energy supplementation during preconditioning and vaccination are not recommended as it might lead to less immune protection against pathogens associated with bovine respiratory disease. Furthermore, these results indicate that increasing the WBG supplementation rate from 0.5 to 1.0% of BW increased pre- and postvaccination plasma concentrations of cortisol but did not affect growth or contribute to immune suppression.

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