

# Frequency of wet brewers grains supplementation during late gestation of beef cows and its effects on offspring postnatal growth and immunity<sup>1</sup>

P. Moriel,\*<sup>†2</sup> L. F. A. Artioli,\* M. B. Piccolo,\* R. S. Marques,‡ M. H. Poore,\* and R. F. Cooke‡

\*Department of Animal Science, North Carolina State University, Raleigh 27695;

†Mountain Research Station, North Carolina Department of Agriculture and Consumer Services, Waynesville, NC 28786; and ‡Eastern Oregon Agricultural Research Center, Oregon State University, Burns 97720

**ABSTRACT:** Our objectives were to evaluate postnatal growth and measurements of innate and humoral immunity of beef calves born to dams fed wet brewers grains (WBG) daily or 3 times weekly during late gestation. On d 0 (approximately 60 d before calving), 28 multiparous, spring-calving Angus cows (BW = 578 ± 19 kg; age = 4.7 ± 0.65 yr; BCS = 7.0 ± 0.18) were stratified by sire, age, BW, and BCS and then randomly allocated into 1 of 14 drylot pens (2 cows/pen; 18 by 3 m; 27 m<sup>2</sup>/cow). Cows were offered ground tall fescue hay ad libitum and received similar weekly WBG supplementation (DMI = 0.5% of BW multiplied by 7 d). Treatments were randomly assigned to pens (7 pens/treatment) and consisted of cows receiving WBG supplementation daily (S7; weekly DMI of WBG divided by 7 d) or 3 times weekly (S3; weekly DMI of WBG divided by 3 d; Mondays, Wednesdays, and Fridays) from d 0 until calving. Cow–calf pairs were managed as a single group on tall fescue pastures from calving to weaning (d 226). Calves were immediately submitted to a preconditioning period from d 226 to 266 and vaccinated against infectious bovine rhinotracheitis, bovine viral diarrhea virus, *Mannheimia haemolytica*, and *Clostridium* on d 231

and 245. Decreasing the frequency of WBG supplementation did not impact ( $P \geq 0.21$ ) precalving intake of total DM, CP, and TDN; BW and BCS change; overall plasma cortisol concentrations; and post-calving growth and pregnancy rate of cows. Overall plasma concentrations of glucose and insulin did not differ ( $P \geq 0.28$ ) between S3 and S7 cows, whereas S3 cows had greater ( $P = 0.002$ ) plasma glucose concentrations and tended ( $P = 0.06$ ) to have greater plasma insulin concentrations on days they were not fed WBG vs. days of WBG supplementation. Calf plasma concentrations of haptoglobin and cortisol at birth but not serum IgG ( $P = 0.63$ ) tended ( $P = 0.10$ ) to be greater for S3 vs. S7 calves. However, additional calf growth and immunity variables obtained during pre- and postweaning phases did not differ between S3 and S7 calves ( $P \geq 0.21$ ). Hence, decreasing the frequency of WBG supplementation during late gestation caused oscillations on precalving plasma glucose and insulin concentrations but did not affect plasma cortisol concentrations, growth, and pregnancy rate of cows. Also, reduced frequency of WBG supplementation during late gestation did not have carryover effects on postnatal calf growth and immunity.

**Key words:** fetal programming, immune, preconditioning, supplementation frequency, vaccination, wet brewers grains

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## INTRODUCTION

Nutritional insult of late-gestating beef cows can be detrimental for placental environment and calf development (Funston et al., 2012) and how individuals will respond to their environment throughout life (Arnott et al., 2012). Multiple scenarios can create a negative nutritional environment for placental development (Funston et al., 2012), and those might include the altered metabolic status of cows induced by

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<sup>2</sup>Corresponding author: pmoriel@ncsu.edu; pmoriel@ufl.edu  
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infrequent concentrate supplementation. Decreasing the supplementation frequency reduces labor and feeding costs of beef cattle, but it also modulates blood concentrations of hormones and metabolites, such as glucose and insulin (Cooke et al., 2008). For instance, beef cattle infrequently supplemented experienced oscillations on release of glucose and insulin between days that cattle received supplementation and days they did not (Moriel et al., 2012; Artioli et al., 2015).

In addition, decreasing the frequency of concentrate supplementation from daily to 3 times weekly enhanced plasma cortisol concentrations and impaired humoral immunity of preconditioning beef steers (Artioli et al., 2015). Glucocorticoids are thought to mediate the effects of maternal stress on the fetus (Harris and Seckl, 2011) by passing through the placenta and affecting the maturation of the fetal hypothalamic–pituitary–adrenal (HPA) axis, which might suppress postnatal offspring health (Wu et al., 2006). Hence, we hypothesized that beef cows infrequently supplemented during late gestation would experience oscillations in plasma concentrations of glucose and insulin as well as increased plasma cortisol concentrations, which would impact calf postnatal health and growth. Our objectives were to evaluate postnatal growth and measurements of innate and humoral immunity of beef calves born to cows supplemented with wet brewers grains (WBG) daily or 3 times weekly during late gestation.

## MATERIALS AND METHODS

The Institutional Animal Care and Use Committee of North Carolina State University (protocol number 15-014-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 January to October 2015.

### *Animals, Diets, and Sample Collection*

**Pre-calving Cow Management.** On d 0 (approximately 60 d before the expected calving date, assuming a 283-d gestation length after embryo transfer), 28 multiparous, spring-calving Angus cows (BW = 578 ± 19 kg; age = 4.7 ± 0.65 yr; BCS = 7.0 ± 0.18 according to Wagner et al., 1988) were randomly selected from a herd of cows pregnant to embryo transfer. Using nonsurgical embryo collection techniques, embryos were collected from mature donor cows that were cohorts of the selected herd and sired by 2 Angus sires. Embryos were kept frozen until the moment of embryo transfer. On d 0, cows were also stratified by sire, age, BW, and BCS on d 0 and randomly allocated into 1 of 14 concrete floor pens in a half-covered drylot feeding facility ( $n = 2$  cows/pen; 18 ×

3 m; 27 m<sup>2</sup>/cow). Cows were offered free choice access to ground tall fescue (*Lolium arundinaceum*) hay daily and received similar weekly WBG supplementation (weekly WBG DMI = 0.5% of BW on d 0 multiplied by 7 d) to meet the daily nutrient requirements for maintenance of a 580-kg beef cow at 8 mo of gestation (NRC, 2000). Treatments were randomly assigned to pens ( $n = 7$  pens/treatment) and consisted of cows receiving WBG supplementation daily (S7; weekly DMI of WBG divided by 7 d) or 3 times weekly (S3; weekly DMI of WBG divided by 3 d; Mondays, Wednesdays, and Fridays) from d 0 until calving. Days when all S7 and S3 cows received WBG supplementation were defined as SUPPALL days (Monday, Wednesday, and Friday), whereas days that only S7 cows received WBG supplementation were defined as S7ONLY days (Tuesday, Thursday, Saturday, and Sunday). A complete mineral mix (Tennessee Farmers Cooperative, La Vergne, TN; average composition, DM basis, was 14.1% Ca, 0.72% K, 11.5% Mg, 0.76% S, 7.0% Na, 10.8% Cl, 2.0% P, 29 mg/kg Co, 900 mg/kg Cu, 2,130 mg/kg Mn, 20 mg/kg Se, and 1,800 mg/kg Zn) was top-dressed daily over supplemental WBG at a rate of 0.150 kg/cow from d 0 to calving. Hay was ground through a 2.54-cm screen weekly before feeding.

Hay and WBG were offered separately in the same concrete, fence-line bunk at 0800 h. Hay and WBG daily offer amounts were adjusted daily to alterations on DM concentration of each ingredient. Dry matter of hay and WBG offered and refused were obtained daily for each pen by drying samples of hay and WBG offered and refused 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 to cows were collected daily from d 0 to calving, pooled within each week, 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 from the NRC (2000).

Individual cow BW and BCS were obtained on d 0 after 12 h of feed and water withdrawal and also immediately after calving and complete placental expulsion. Blood samples (10 mL) were collected from all cows via jugular venipuncture into sodium heparin (158 United States Pharmacopeia [USP] units)–containing tubes

**Table 1.** Average weekly chemical composition of ground tall fescue hay and wet brewers grains (WBG) provided to cows from d 0 to calving (cow precalving phase) and of ground tall fescue hay and concentrate offered to calves from d 226 to 266 (calf preconditioning phase)<sup>1</sup>

Item	Cow precalving phase		Calf preconditioning phase	
	Tall fescue hay	WBG	Tall fescue hay	Concentrate <sup>2</sup>
DM, %	92.6	21.2	91.8	89.0
	DM basis			
CP, %	12.0	32.4	11.9	15.9
ADF, %	41.1	25.0	40.1	29.0
NDF, %	64.1	50.6	67.8	50.0
TDN, <sup>3</sup> %	56.0	72.5	55.0	72.0
NEm, <sup>4</sup> Mcal/kg	1.07	1.71	1.04	1.65
NEg, <sup>4</sup> Mcal/kg	0.52	1.09	0.48	1.04
Ca, %	0.40	0.24	0.33	0.35
K, %	1.98	0.08	1.95	1.53
Mg, %	0.23	0.18	0.29	0.34
Na, %	0.01	0.003	0.02	0.12
P, %	0.27	0.62	0.24	0.58
Cu, mg/kg	6.0	21.0	6.0	6.0
Fe, mg/kg	426	165	609	292
Mn, mg/kg	100	47	75	20
Mo, mg/kg	0.40	2.65	0.80	1.30
Zn, mg/kg	26	86	25	59

<sup>1</sup>Samples of hay, WBG, and concentrate offered to cows (precalving phase) and calves (postweaning phase) were collected daily from d 0 to calving and d 266 to 266, respectively, pooled within each week, and then sent in duplicate to a commercial laboratory for wet chemistry analysis of all nutrients.

<sup>2</sup>Concentrate consisted of 50% soy hulls pellets and 50% corn gluten pellets (DM basis).

<sup>3</sup>Calculated as described by Weiss et al. (1992).

<sup>4</sup>Calculated using the equations proposed by the NRC (2000).

(Vacutainer; Becton, Dickinson and Company, Franklin Lakes, NJ) for plasma harvest 4 h after WBG supplementation on d 13, 14, 15, 16, 27, 28, 29, and 30 to determine the plasma concentrations of cortisol, glucose, and insulin. Blood samples were collected 4 h after feeding to correspond to the peak of ruminal fermentation and end products release after concentrate consumption (Moriel et al., 2012, 2015; Artioli et al., 2015, 2016) and to correspond with SUPPALL days (d 13, 15, 27, and 29) and S7ONLY days (d 14, 16, 28, and 30).

**Preweaning Phase.** Immediately after calving, all cow-calf pairs were transferred to 1 of 6 tall fescue pastures (22 ha/pasture) with free choice access to water and a complete mineral mix (the same as previously described). Hand-plucked pasture samples were collected monthly, pooled by month, and then sent in duplicate to a commercial laboratory (Dairy One Forage Laboratory) to assess the average nutritional value of pasture (16% CP and 59% TDN, DM basis). Cows and calves were managed as a single group and

rotated among pastures monthly from calving until weaning (d 226; approximately 165 d of age). Calf BW was obtained within 12 h after birth. All male calves were castrated by banding immediately after birth. Cows were placed with 2 Angus bulls from d 121 to 189, and pregnancy rate was determined by rectal palpation on d 226 and confirmed at calving. Individual cow BW and BCS as well as calf BW were obtained at weaning after 12 h of feed and water withdrawal.

**Postweaning Phase.** Immediately after weaning, all steers and heifers were assigned to a 40-d preconditioning period from d 226 to 266. Calves were stratified by treatment and pen distribution that was previously assigned to their dams on d 0 and then randomly allocated into 1 of 14 concrete floor pens in a half-covered drylot feeding facility (1 to 2 calves/pen; 18 by 3 m; 27 to 54 m<sup>2</sup>/calf). This approach was selected because 1) calf gender was not known at the time of treatment assignment, and hence, calf gender was not controlled in the experimental design, which made the test of treatment × gender interaction not possible, and 2) percentage of live male calves did not differ between treatments at weaning ( $P \geq 0.33$ ). In addition, calf gender was a nonsignificant covariate in the analyses of pre- and postweaning calf BW and ADG ( $P \geq 0.28$ ).

From d 226 to 266, all calves were limit-fed ground tall fescue hay at 1.2% of BW (DM basis) and concentrate DMI at 1.0% of BW obtained on d 226. Concentrate consisted of 50% soy hulls pellets and 50% corn gluten pellets (DM basis; Table 1). Hay and concentrate were offered separately in the same concrete, fence-line bunk once daily at 0800 h. Calves consumed the concentrate offered within 30 min of supplementation. Daily hay DM was 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 hay DMI was determined by subtracting the daily hay DM refused from the daily hay DM offered. A complete mineral mix (RU-MIN 1600; Southern States, Richmond, VA; average composition, DM basis, was 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 kg/calf from d 226 to 266. Samples of hay, concentrate, and mineral mix offered were collected daily, pooled within each week, and then sent in duplicate to a commercial laboratory (Dairy One Forage Laboratory) for wet chemistry analysis of all nutrients (Table 1).

On d 226, all calves were treated with doramectin for internal and external parasites (5 mL subcutaneous; Dectomax injectable; Zoetis Inc., Kalamazoo, MI). On d 231, calves 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 245, calves received 2-mL subcutaneous boosters of Bovi Shield 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., 2015; Artioli et al., 2015, 2016). The vaccination protocol was initiated 5 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 from all calves via jugular venipuncture into sodium heparin (158 USP units)-containing tubes (Vacutainer; Becton, Dickinson and Company) within 12 h of birth and also on d 226, 231, 232, 234, 238, 245, 246, 248, 251, and 266 to evaluate the plasma concentrations of haptoglobin and cortisol. Additional blood samples (10 mL) from jugular vein were collected from all calves into tubes containing no additives (Vacutainer; Becton, Dickinson and Company) for serum harvest within 12 h of birth to evaluate serum concentrations of IgG and on d 226 and 266 to evaluate the vaccination-induced serum antibody titers against IBR and BVDV-1a and -2. Blood samples were immediately placed on ice following collection and then centrifuged at  $1,200 \times g$  for 25 min at 4°C. Plasma and serum samples were stored frozen at -20°C until later laboratory analysis.

### Laboratory Analyses

Plasma concentrations of haptoglobin were determined in duplicate samples using a biochemical assay assessing the haptoglobin-hemoglobin complex by the estimation of differences in peroxidase activity (Cooke and Arthington, 2013). Plasma concentrations of cortisol and insulin were determined using a single chemiluminescent enzyme immunoassay (Immulite 1000; Siemens Medical Solutions Diagnostics, Los Angeles, CA). Intra- and interassay CV for haptoglobin assays were 5.3 and 2.2, respectively, whereas intra-assay CV for the analyses of cortisol and insulin were 1.9 and 0.8%, respectively.

Commercial quantitative colorimetric kits were used to determine the plasma concentrations of glucose (G7521; Pointe Scientific, Inc., Canton, MI) and serum concentrations of bovine IgG (E11-118; Bethyl Laboratories, Montgomery, TX; Shoshani et al., 2014). Intra- and interassay CV for glucose assays were 2.5 and 7.7, respectively, whereas intra-assay CV for IgG analysis was 3.4%.

Serum antibody titers against IBR and BVDV-1a and -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 = 1:4 and 1:256, respectively; Richeson et al., 2008; Moriel et al., 2015; Artioli et al., 2015).

### Statistical Analyses

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, whereas animal(pen) and pen(treatment) were included as random effects in all analyses, except for intake data and feed efficiency, which included only pen(treatment) as a random effect. Initially, bull source was included as block effect, whereas cow age was included as covariate in all analyses. However, both factors were removed from the model as effects of treatment  $\times$  block, block, and covariate were not detected ( $P \geq 0.19$ ) for any variable analyzed. Cow BW and BCS change, calf plasma data at birth, calf pre- and postweaning ADG, and calf age at weaning were tested for fixed effects of supplementation frequency. Cow daily intake data was pooled by SUPPALL and S7ONLY days to simplify data analyses, interpretation, and reporting. Cow daily intake of DM (WBG, hay, and total), CP, and TDN were analyzed as repeated measures and tested for fixed effects of supplementation frequency, day (SUPPALL and S7ONLY), and resulting interactions, using pen(treatment) as the subject. Pre- and postcalving BW and BCS of cows, pre- and postweaning calf BW, postweaning calf intake (hay, concentrate, and total DM), and blood measurements of cows (precalving phase) and calves (postweaning phase) were also analyzed as repeated measures and tested for fixed effects of supplementation frequency, day of the study, and resulting interactions. Compound symmetry covariance structure was used for the analyses of calf postweaning intake and BW of cows and calves, whereas autoregressive 1 was used for the analyses of blood measurements of cows and calves, as these covariance structures generated the lowest Akaike information criterion. Calf gender was included as covariate in all analyses of calf growth and blood measurements but was removed from the model when  $P \geq 0.10$ . Percentage of calves that were live and male at birth and weaning were tested for fixed effects of supplementation frequency, using the GLIMMIX procedure of SAS. All results are reported as least squares means. Data were separated using PDIF if a significant preliminary F-test was detected. Significance was set at  $P \leq 0.05$  and tendencies if  $P > 0.05$  and  $P \leq 0.10$ .

**Table 2.** Growth and reproductive performance of mature cows that received wet brewers grains (WBG) supplementation daily (S7) or 3 times weekly (S3; Mondays, Wednesdays, and Fridays) from d 0 until calving ( $n = 2$  cows/pen; 7 pens/treatment)

Item	Supplementation frequency <sup>1</sup>		SEM	P-value	
	S3	S7		Treatment	Treatment × day
BCS					
d 0	7.1	7.1	0.32	0.72	0.43
Calving	6.9	6.6			
Weaning (d 226)	6.8	6.8			
BW, <sup>2</sup> kg					
d 0	598	557	29.5	0.55	0.65
Calving	601	551			–
Weaning (d 226)	604	556			–
BCS change					
d 0 to calving	–0.28	–0.52	0.231	0.38	–
Calving to weaning	–0.08	0.31	0.433	0.59	–
BW change, kg/d					
d 0 to calving	–0.05	–0.16	0.325	0.82	–
Calving to weaning	0.02	0.03	0.144	0.52	–
Days on treatment <sup>3</sup>	69	63	9.9	0.65	–
Overall pregnancy rate, <sup>4</sup> %	87.5	100.0	8.05	0.30	–

<sup>1</sup>Cows were offered free choice access to ground tall fescue hay and limit-fed similar weekly amounts of WBG supplementation (weekly DMI = 0.5% of BW on d 0 multiplied by 7 d) from d 0 to calving.

<sup>2</sup>Body weight on d 0 and 226 were obtained after 12 h of feed and water withdrawal, whereas BW at calving were obtained after complete placental expulsion (within 12 h of calving).

<sup>3</sup>Days on respective treatment (d 0 to calving).

<sup>4</sup>Pregnancy rate was determined by rectal palpation at weaning (d 226) and confirmed at calving.

## RESULTS AND DISCUSSION

### Precalving Phase

Cows remained on their respective treatment for an average of 66 d before calving (Table 2). However, decreasing the frequency of WBG supplementation during late gestation did not impact ( $P = 0.65$ ) the number of days that cows remained on their respective treatment (Table 2), suggesting that decreasing the WBG supplementation frequency did not impact gestational length. Also, BW and BCS change from d 0 to calving did not differ between S3 and S7 cows ( $P \geq 0.38$ ; Table 2). Likewise, Klein et al. (2014) observed similar BW and BCS change of mid- to late-gestating cows offered free choice access to bromegrass hay and supplemented with dried distillers' grains daily or 3 times weekly (daily supplementation rate = 0.4% of BW, DM basis). Our results agree with others (Drewnoski et al., 2011; Moriel et al., 2012) and also support our previous study that demonstrated that reducing the frequency of WBG supplementation (0.5 or 1.0% of BW, DM basis) from daily to 3 times weekly did not reduce growth of recently weaned beef heifers (Artioli et al., 2016). The lack of differences on BW and BCS change of cows from d 0 to calving was likely a reflection of S7 and S3 cows having similar ( $P \geq 0.21$ ) overall intake of hay DM (6.9 vs.

6.7 ± 0.53 kg/d, respectively), total DM (9.8 vs. 10.3 ± 0.59 kg/d, respectively), CP (2.1 vs. 2.3 ± 0.10 kg/d, respectively), and TDN (6.0 vs. 6.4 ± 0.34 kg/d, respectively). Likewise, Artioli et al. (2016) reported similar overall intake of hay DM, total DM, CP, and NEg between recently weaned beef heifers supplemented with WBG (0.5 or 1.0% of BW, DM basis) daily or 3 times weekly. Also, Klein et al. (2014) observed similar total and hay DMI of mid- to late-gestating cows offered free choice access to bromegrass hay and supplemented with dried distillers' grains daily or 3 times weekly.

Although overall intake of forage and nutrients did not differ between treatments, effects of supplementation frequency × day of supplementation were detected ( $P \leq 0.002$ ) for intake of hay DM, WBG DM, total DM, CP, and TDN of cows from d 0 to calving (Table 3). Cows supplemented with WBG 3 times weekly had less hay DMI ( $P < 0.0001$ ) but greater intake of total DM, CP, and TDN ( $P < 0.0001$ ) on SUPPALL days than on S7ONLY days, whereas S7 cows had similar ( $P \geq 0.48$ ) intake of hay DM, total DM, WBG DM, CP, and TDN on SUPPALL and S7ONLY days. Except for hay DMI ( $P \geq 0.36$ ), intake of total DM, WBG DM, CP, and TDN were greater for S3 cows than for S7 cows on SUPPALL days ( $P \leq 0.004$ ) but less for S3 cows than for S7 cows on S7ONLY days ( $P \leq 0.02$ ). These responses on daily intake of hay DM, CP, and

**Table 3.** Ingredient and nutrient intake of mature cows that received wet brewers grains (WBG) supplementation daily (S7) or 3 times weekly (S3; Mondays, Wednesdays, and Fridays) from d 0 until calving ( $n = 2$  cows/pen; 7 pens/treatment)

Item <sup>1</sup>	Supplementation frequency <sup>2</sup>		<i>P</i> -value <sup>3</sup>	SEM	<i>P</i> -value Treatment × day
	S3	S7			
Hay DMI, kg/d					
SUPPALL	6.1	6.9	0.36	0.54	0.002
S7ONLY	7.3	6.9	0.58		
<i>P</i> -value <sup>4</sup>	<0.0001	0.94			
WBG DMI, kg/d					
SUPPALL	7.1	3.0	<0.0001	0.12	<0.0001
S7ONLY	0.0	2.9	<0.0001		
<i>P</i> -value <sup>4</sup>	<0.0001	0.48			
Total DMI, kg/d					
SUPPALL	13.2	9.9	0.004	0.61	<0.0001
S7ONLY	7.3	9.8	0.02		
<i>P</i> -value <sup>4</sup>	<0.0001	0.70			
CP intake, kg/d					
SUPPALL	3.5	2.1	<0.0001	0.10	<0.0001
S7ONLY	1.0	2.0	<0.0001		
<i>P</i> -value <sup>4</sup>	<0.0001	0.59			
TDN intake, kg/d					
SUPPALL	8.7	6.0	0.0004	0.35	<0.0001
S7ONLY	4.0	5.9	0.003		
<i>P</i> -value <sup>4</sup>	<0.0001	0.64			

<sup>1</sup>SUPPALL = days when all S7 and S3 cows received WBG supplementation; S7ONLY = days that only S7 cows received WBG supplementation.

<sup>2</sup>Cows were offered free choice access to ground tall fescue hay and limit-fed similar weekly amounts of WBG supplementation (weekly DMI = 0.5% of BW on d 0 multiplied by 7 d) from d 0 to calving.

<sup>3</sup>Comparison of treatments within each day.

<sup>4</sup>Comparison of day within each treatment.

TDN were 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., 1999a). Likewise, other studies reported that feeding a low-starch energy supplement daily vs. 3 times weekly reduced the daily oscillation in intake of forage DM, total DM, and nutrients (Cooke et al., 2007a; Moriel et al., 2012; Artioli et al., 2015).

Supplementation frequency can affect performance of beef cattle by multiple mechanisms, including the modulation of blood concentrations of hormones and metabolites (Cooke et al., 2008). Increasing the frequency of low-starch supplements from 3 times weekly to daily reduced the daily oscillation in the release of glucose and insulin (Moriel et al., 2012; Artioli et al., 2015). In the current study, effects of supplementation frequency × day of supplementation were detected ( $P \leq 0.002$ ) for plasma concentrations of glucose and insulin of cows from d 0 to calving (Table 4). Cows

**Table 4.** Plasma concentrations of glucose, insulin and cortisol of mature cows that received wet brewers grains (WBG) supplementation daily (S7) or 3 times weekly (S3; Mondays, Wednesdays, and Fridays) from d 0 until calving ( $n = 2$  cows/pen; 7 pens/treatment)

Item <sup>1</sup>	Supplementation frequency <sup>2</sup>		<i>P</i> -value <sup>3</sup>	SEM	<i>P</i> -value Treatment × day
	S3	S7			
Plasma glucose, mg/dL					
SUPPALL	67.0	65.7	0.70	2.19	0.03
S7ONLY	71.1	68.8	0.10		
<i>P</i> -value <sup>4</sup>	0.002	0.98			
Plasma insulin, $\mu$ IU/mL					
SUPPALL	2.8	3.2	0.76	1.02	0.01
S7ONLY	3.6	2.5	0.47		
<i>P</i> -value <sup>4</sup>	0.06	0.11			
Plasma cortisol, ng/mL					
SUPPALL	14.7	14.7	0.99	3.07	0.49
S7ONLY	15.0	13.3	0.70		
<i>P</i> -value <sup>4</sup>	0.86	0.43			

<sup>1</sup>SUPPALL = days when all S7 and S3 cows received WBG supplementation; S7ONLY = days that only S7 cows received WBG supplementation.

<sup>2</sup>Cows were offered free choice access to ground tall fescue hay and limit-fed similar weekly amounts of WBG supplementation (weekly DMI = 0.5% of BW on d 0 multiplied by 7 d) from d 0 to calving.

<sup>3</sup>Comparison of treatments within each day.

<sup>4</sup>Comparison of day within each treatment.

supplemented with WBG 3 times weekly had greater ( $P = 0.002$ ) plasma glucose concentrations and tended ( $P = 0.06$ ) to have greater plasma insulin concentrations on S7ONLY days vs. SUPPALL days, whereas plasma concentrations of glucose and insulin of S7 cows did not differ between SUPPALL and S7ONLY days ( $P \geq 0.11$ ). However, overall plasma concentrations of glucose and insulin did not differ ( $P \geq 0.28$ ) between S3 and S7 cows ( $69.0$  vs.  $65.8 \pm 2.08$  mg/dL and  $3.2$  vs.  $2.9 \pm 0.99$   $\mu$ IU/mL, respectively). Similarly, other studies (Cooke et al., 2007b, 2008; Moriel et al., 2012) reported that plasma concentrations of glucose and insulin in heifers fed low-starch energy byproducts 3 times weekly were increased on days that supplementation was not provided (28 vs. 4 h after supplementation). This outcome on glucose concentration was associated with the time required for synthesis and activation of gluconeogenic enzymes to substantially change the magnitude of glucose synthesis and release by the liver (Cooke et al., 2008), whereas the outcome on insulin concentrations was expected as insulin is directly influenced by nutrient intake and circulating glucose concentrations (Vizcarra et al., 1998).

Based on previous studies (Artioli et al., 2015, 2016), we hypothesized that plasma cortisol concentrations of late-gestating cows would be enhanced by

reducing the frequency of supplementation from daily to 3 times weekly. 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 (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). Contrary to our hypothesis, effects of supplementation frequency and supplementation frequency  $\times$  day of supplementation were not detected ( $P \geq 0.49$ ) for precalving plasma cortisol concentrations of cows (Table 4). Overall plasma cortisol concentrations were 14.9 and  $14.0 \pm 2.95$  ng/mL for S3 and S7 cows, respectively. In contrast, plasma concentrations of cortisol were greater in recently weaned steers supplemented with a soybean hull-based concentrate 3 vs. 7 times weekly (Artioli et al., 2015) and also when preconditioning heifers were offered WBG supplementation 3 times weekly vs. daily (Artioli et al., 2016). However, in both studies, steers and heifers were vaccinated against pathogens associated with bovine respiratory disease and received concentrate supplementation at 1.0% of BW (DM basis), whereas cows in the current study were not vaccinated and were supplemented with WBG at 0.5% of BW (DM basis). Although we did not measure endotoxin levels in the ruminal fluid, it is possible that the relatively low supplementation rate used in the current study reduced the load of fermentable carbohydrates, leading to potentially fewer differences on microbial endotoxin concentration in the ruminal fluid of S3 and S7 cows. In support of this rationale, plasma cortisol concentrations were greater for heifers supplemented with WBG at 1.0 vs. 0.5% of BW (Artioli et al., 2016). Therefore, the lack of differences on plasma concentrations of cortisol between S3 and S7 cows might be associated with the lower supplementation rate (0.5 vs. 1.0% of BW) and that cows were not immunologically challenged to elicit an inflammatory response greater than the observed or simply because reducing the frequency of supplementation to mature beef cows does not elicit a physiological stress response in mature beef cows.

Glucose and insulin are associated with BW gain and reproductive function of cattle (Schillo et al., 1992; Spicer and Echternkamp, 1995). Infrequent supplemental energy intake was detrimental to puberty achievement of heifers (Cooke et al., 2008; Moriel et al., 2012) and growth of preconditioning steers (Artioli et al., 2016). In the current study, S7 and S3 cows had similar overall pregnancy rates ( $P = 0.30$ ) and BW and BCS change from calving to weaning ( $P \geq 0.52$ ; Table 2). Hence, reducing the frequency of WBG supplementation from daily to 3 times weekly caused daily alterations in plasma concentrations of insulin and glucose

during the last 60 d of gestation, but it did not have carryover effects on postcalving growth and reproductive performance of these cows. However, it is important to mention that measuring the impact of frequency of WBG supplementation on subsequent pregnancy rates was not the primary goal of the study and that a greater number of cows per treatment would be required to detect differences on pregnancy rates.

### ***Preweaning Phase***

As previously indicated, calf gender was not known at the time of treatment assignment. Therefore, calf gender was not controlled in the experimental design, which prevented the test of treatment  $\times$  gender interaction for all calf variables. However, calf gender was a nonsignificant covariate ( $P \geq 0.28$ ) for the analyses of calf birth BW, weaning BW, plasma concentrations of cortisol and haptoglobin at birth, and serum IgG concentrations at birth. In addition, percentage of live male calves at birth and weaning did not differ between treatments ( $P \geq 0.33$ ; Table 2).

Glucose is also essential for fetal growth (Bell et al., 2005), and its supply to the fetus is modulated by maternal glucose concentration (Baumann et al., 2002), whereas stress on the dam can result in fetal growth restriction and birth BW, leading to greater risk of neonatal mortality and morbidity in livestock (Vonnahme et al., 2013). For instance, Lay et al. (1997) observed that exposing pregnant Brahman cows to repeated transport on d 60, 80, 100, 120, and 140 of gestation increased the offspring stress-induced cortisol response to restraint at 10 and 150 d of age, which could affect their ability to cope with challenges throughout life. Hence, we hypothesized that supplementation frequency to beef cows during late gestation would impact calf postnatal health and growth. Although plasma cortisol concentrations of cows were not affected by frequency of WBG supplementation during late gestation, calf plasma concentrations of haptoglobin and cortisol at birth tended ( $P = 0.10$ ) to be greater for calves born to S3 cows than for calves born to S7 cows (Table 5), which is partially in agreement with our hypothesis. Haptoglobin and cortisol are released after an acute-phase response (Arthington et al., 2013; Moriel and Arthington, 2013). Haptoglobin prevents Fe utilization for bacterial growth (Wassell, 2000) and may be used as an indicator of inflammatory conditions in cattle when plasma concentrations are  $\geq 0.11$  mg/mL (Tourolmoussis et al., 2004). Cortisol is released by the adrenal cortex and may also stimulate an acute-phase response (Cooke and Bohnert, 2011), cause immune suppression effects (Salak-Johnson and McGlone, 2007), weaken the innate immune response (Dai and McMurray, 1998), and impair

**Table 5.** Preweaning growth (birth to d 226) and blood measurements at birth of calves born to mature cows that received wet brewers grains (WBG) supplementation daily (S7) or 3 times weekly (S3; Mondays, Wednesdays, and Fridays) from d 0 until calving

Item	Supplementation frequency <sup>1</sup>		SEM	<i>P</i> -value	
	S3	S7		Treatment	Treatment × day
BW, <sup>2</sup> kg					
Birth	35	35	9.9	0.88	0.61
Weaning (d 226)	190	199			
Birth					
Live calves at birth, %	100.0	100.0	—	—	—
Male calves at birth, %	55.6	33.3	17.1	0.37	—
Serum IgG, <sup>3</sup> mg/dL	3,509	4,103	866.1	0.63	—
Plasma cortisol, <sup>3</sup> ng/mL	59.1	37.7	8.51	0.10	—
Plasma haptoglobin, <sup>3</sup> mg/mL	0.21	0.11	0.04	0.10	—
Weaning (d 226)					
Live calves at weaning, %	88.9	100.0	7.89	0.33	—
Male calves at weaning, %	50.0	33.3	18.1	0.52	—
Age at weaning, d	162	169	9.9	0.65	—
205 d adjusted BW, <sup>4,5</sup> kg	242	250	10.6	0.59	—
ADG birth to weaning, kg/d	1.01	1.04	0.048	0.61	—

<sup>1</sup>Cows were offered free choice access to ground tall fescue hay and limit-fed similar weekly amounts of WBG supplementation (weekly DMI = 0.5% of BW on d 0 multiplied by 7 d) from d 0 to calving. Cows and calves were managed as a single group and rotated among tall fescue pastures from calving until weaning (d 226).

<sup>2</sup>Individual calf BW was obtained immediately after birth and also on d 226 after 12 h of feed and water withdrawal.

<sup>3</sup>Blood samples were collected from all calves after first suckling event but within 12 h of birth.

<sup>4</sup>Calculated according to the Beef Improvement Federation (2010).

<sup>5</sup>Covariate adjusted to calf gender ( $P = 0.04$ ).

antibody production (Salak-Johnson and McGlone, 2007). Therefore, although cows were not physiologically stressed during late gestation (as indicated by similar plasma concentrations of cortisol), the reduction on cow precalving supplementation frequency induced a physiological stress and inflammatory response on calves at birth, which tended to be greater for S3 calves than for S7 calves (Table 5).

Transfer of immunoglobulins from maternal serum to colostrum in cattle typically begins 4 wk before parturition and reaches a maximum rate a few days before parturition (Olson et al., 1981). Also, there is a linear relationship between calf colostrum IgG intake and serum IgG concentrations (Hopkins and Quigley, 1997). Hough et al. (1990) reported similar IgG concentrations in the colostrum of beef cows fed diets at 100 or 57% of NRC requirements just before parturition. Likewise, serum IgG concentration of calves within 12 h after birth did not differ between S3 calves and S7 calves ( $P = 0.63$ ; Table 5) and were above the minimum threshold considered as adequate passive immunity transfer ( $>1,600$  mg/dL; Wittum and Perino, 1995).

Effects of supplementation frequency and supplementation frequency × day of the study were not detected ( $P \geq 0.61$ ) for preweaning calf growth. Calf BW at birth and weaning did not differ ( $P \geq 0.50$ ) between calves born to S3 cows and calves born to S7 cows (Table 5), which might indicate that the maternal ability

to support glucose to the fetus was not altered by reducing the frequency of supplementation during late gestation. In addition, effects of supplementation frequency were not detected ( $P \geq 0.59$ ) for calf age at weaning, calf 205 d adjusted BW, and calf ADG from birth to weaning (Table 5). Likewise, Klein also reported similar birth BW of calves born to mid- to late-gestating cows supplemented with dried distillers' grains daily or 3 times weekly. It is plausible that the lack of treatment effects on calf birth BW and postnatal growth in the current study was due to the similar plasma cortisol concentrations of S3 and S7 cows during late gestation. In addition, Long et al. (2009, 2010a) showed that the negative effects of early- to mid-gestational nutrient restriction on fetal growth was ameliorated when beef cows were provided nutrients to meet their nutritional requirements during late gestation. Using the same rationale, it is possible that any potential detrimental effects caused by reducing the supplementation frequency during late gestation of cows on calf development and subsequent performance could have been masked by the relatively high nutritional status of cows (as indicated by the high BCS throughout the study).

### Postweaning Phase

Effects of supplementation frequency and supplementation frequency × day of the study were not detected



**Table 6.** Postweaning growth performance, plasma concentrations of cortisol and haptoglobin, and serum titers against infectious bovine rhinotracheitis (IBR), bovine viral diarrhea virus (BVDV) types 1a and 2 of calves during a 40-d preconditioning phase (d 226 to 266)

Item	Supplementation frequency <sup>1</sup>		SEM	P-value	
	S3	S7		Treatment	Treatment × day
Preconditioning BW, <sup>2</sup> kg					
d 226	190	199	15.7	0.51	0.37
d 266	212	228			
d 226 to 266 <sup>3</sup>					
ADG, kg/d	0.72	0.78	0.054	0.46	–
Hay DMI, kg/d	1.96	2.03	0.154	0.75	0.98
Concentrate DMI, kg/d	1.96	2.06	0.134	0.62	0.42
Total DMI, kg/d	3.87	4.05	0.278	0.66	0.95
G:F	0.19	0.20	0.011	0.80	–
Blood measurements <sup>4</sup>					
Plasma cortisol, ng/mL	15.4	16.0	2.23	0.84	0.64
Plasma haptoglobin, <sup>5</sup> mg/mL	0.89	0.80	0.07	0.37	0.85
Serum IBR titers, <sup>5</sup> log <sub>2</sub>	0.62	0.76	0.295	0.73	0.37
Serum BVDV-1a titers, <sup>5</sup> log <sub>2</sub>	3.13	2.46	0.399	0.33	0.50
Serum BVDV-2 titers, <sup>5</sup> log <sub>2</sub>	4.62	4.63	0.398	0.99	0.94

<sup>1</sup>Calves were born to mature cows that received similar weekly wet brewers grains supplement amount (0.5% of BW on d 0 multiplied by 7 d) offered either daily (wet brewers grains supplementation daily [S7]) or 3 times weekly (S3; Mondays, Wednesdays, and Fridays) from d 0 until calving.

<sup>2</sup>Calf BW was obtained on d 226 and 266 following 12 h of feed and water withdrawal.

<sup>3</sup>Calves were allocated to drylot pens on d 226 ( $n = 1$  to 2 calves/pen and 7 pens/treatment; same pen distribution assigned to cows on d 0) and were limit-fed ground fescue hay at 1.2% of BW (DM basis) and concentrate DM at 1.0% of BW (50:50% soy hulls pellets:corn gluten pellets) from d 226 to 266.

<sup>4</sup>Overall concentrations of plasma and serum measurements obtained from d 226 to 266.

<sup>5</sup>Covariate adjusted to calf gender ( $P \leq 0.08$ ).

( $P \geq 0.33$ ) for postweaning calf BW, DMI (hay, concentrate, and total), vaccine-induced plasma concentrations of cortisol and haptoglobin, and serum titers against IBR and BVDV-1a and -2 (Table 6). Effects of supplementation frequency were also not detected for calf ADG and G:F from d 226 to 266 (Table 6). Only serum titers against BVDV-2 ( $P = 0.02$ ) and IBR ( $P = 0.08$ ) were covariate adjusted to calf gender. 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, 1995; Richeson et al., 2008). The ability of an animal to respond to vaccination differs from animal to animal and depends on environmental and genetic factors, maternal antibody concentrations (Downey et al., 2013), calf age (Kirkpatrick et al., 2008), timing of vaccination after feedlot entry (Richeson et al., 2008), MP supply (Moriel et al., 2015), and also frequency of concentrate supplementation (Artioli et al., 2015, 2016). In humans, prenatal undernutrition impaired cell-mediated immunity and antibody responses to vaccination (Chandra, 1975, 1981), suggesting an association between prenatal nutrition and adult immune function (Moore et al., 1999b). Also, prenatal glucocorticoid exposure permanently increased basal plasma corticosterone concentrations in adult rats (Welberg et al., 2001). Contrary to our hypothesis, calf growth performance and antibody response

after vaccination did not differ between calves born to S3 cows and calves born to S7 cows, which might be partially associated with the lack of differences on cow plasma concentrations of cortisol during late gestation. Also, age of offspring could also affect the cortisol response to corticotropin-releasing hormone (CRH) challenge (Long et al., 2010b). In sheep, the HPA axis is most sensitive to CRH at 2 mo of age and then becomes less sensitive with increased postnatal age (Chadio et al., 2007). Hence, the greater plasma cortisol concentrations of S3 calves vs. S7 calves at birth but not during the postweaning phase might be associated with a less responsive stress reaction as calf age increased. However, further studies are needed to validate this rationale.

In summary, decreasing the frequency of wet brewers grains supplementation (from daily to 3 times weekly) caused oscillations in the plasma concentrations of glucose and insulin but did not affect plasma cortisol concentrations of beef cows during late gestation. Also, decreasing the frequency of wet brewers grains supplementation to beef cows during late gestation did not induce carryover effects on postcalving growth and reproductive performance of cows and postnatal calf growth and immunity. Hence, decreasing the frequency of supplementation to beef cows with wet brewers grains could be used to reduce cow-feeding costs without causing subsequent effects on postnatal calf performance.

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