Effects of timing of vaccination relative to weaning and post-weaning frequency of energy supplementation on growth and immunity of beef calves¹

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ABSTRACT: A 2 × 2 factorial design study evaluated the impact of pre- vs. post-weaning vaccination and different post-weaning frequency of energy supplementation (daily vs. 3X weekly) on growth and immunity of beef calves. At 14 d before weaning (d –14), 48 Angus calves (24 steers and 24 heifers; 244 ± 33 kg; 196 ± 20 d) were stratified by BW and age, and randomly assigned to receive vaccinations against bovine viral diarrhea virus 1a (BVDV-1a) and parainfluenza-3 (PI-3) on d –14 and 0 (**PRE**) or 7 and 21 (**POS**), relative to weaning. Calves were weaned on d 0 and offered daily concentrate DM supplementation (50:50 soybean hulls and corn gluten feed; 71% TDN, 15% CP of DM) at 0.5% of BW for 7 d. On d 7, calves were stratified by vaccination scheme and assigned into 1 of 16 drylot pens (3 calves of same sex/pen; 4 pens/treatment). Pens were randomly assigned to receive similar weekly concentrate DM supplementation (1% of BW multiplied by 7 d) that was divided and offered daily (**7X**) or three times weekly (**3X**; Mondays, Wednesdays, and Fridays) from d 7 to 43. From d 0 to 43, calves were provided ad libitum ground tall fescue hay (57% TDN, 13% CP of DM). Blood samples were collected from jugular vein on d 0, 1, 3, 7, and 14, relative to the respective

first vaccination, and on d 43 of the study. Effects of timing of vaccination × frequency of supplementation were not detected for any variable in this study ($P \geq 0.12$), except for overall ADG from d –14 to 43 ($P = 0.04$), which was less for PRE-3X vs. PRE-7X, POS-3X, and POS-7X calves (0.60, 0.70, 0.70, and 0.77 ± 0.04 kg/d, respectively; $P \leq 0.08$). Post-weaning total DMI and G:F did not differ among treatments ($P \geq 0.11$). Pre-weaning vaccination increased plasma concentrations of cortisol and haptoglobin from d 0 to 3, relative to first vaccination ($P \leq 0.03$), and decreased serum PI-3 titers on d 43 compared with post-weaning vaccination ($P < 0.0001$). Decreasing the supplementation frequency tended ($P = 0.10$) to increase overall plasma cortisol concentrations and reduce overall serum BVDV-1a titers. Hence, pre-weaning vaccination associated with reduced post-weaning frequency of energy supplementation caused the greatest reduction on calf growth performance. Post-weaning vaccination and daily energy supplementation alleviated inflammation and improved humoral immunity compared with pre-weaning vaccination and reduced post-weaning frequency of energy supplementation of recently weaned beef calves.

Key words: cattle, frequency of supplementation, immune system, vaccination

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INTRODUCTION

Pre-conditioning programs have been shown to reduce post-weaning incidence of bovine respiratory disease (BRD; Duff and Gaylean 2007; Lalman and Mourer, 2014), morbidity, and mortality in the feedlot (McNeill, 2001), and improve

growth of feedlot calves (Roeber et al., 2001; Lalman and Mourer, 2014). However, daily supplementation of concentrate can increase total pre-conditioning costs associated with labor, fuel, and equipment (Loy et al., 2007; Cooke et al., 2008). Decreasing the frequency of concentrate supplementation from daily to 3 times weekly reduced feeding costs, but may also impair post-weaning growth performance and vaccine-induced immune response of pre-conditioning beef steers (Artioli et al., 2015; Silva et al., 2017).

These potential negative impacts of reduced frequency of concentrate supplementation are associated with an exacerbation of the acute-phase response (APR) elicited by vaccination. Although APR is an essential early defense mechanism in response to cellular injury (Eckersall and Conner, 1988), nutrient demand is increased (Reeds and Jahoor, 2001), body tissues are mobilized (Jahoor et al., 1999), and nutrients partitioned away from growth to support the immune response (Reeds et al., 1994), leading to reduced calf growth performance and feed efficiency (Arthington et al., 2013; Moriel et al., 2015). We hypothesized that administering the vaccination against respiratory pathogens during pre- vs. post-weaning phase could be a strategy to prevent the negative impacts of reduced frequency of energy supplementation on growth and immunity of pre-conditioning beef calves. Therefore, the present study evaluated the effects of timing of vaccination against respiratory pathogens and post-weaning frequency of energy supplementation on measurements of growth, innate, and humoral immunity of beef calves during a 43-d pre-conditioning period.

MATERIALS AND METHODS

The Institutional Animal Care and Use Committee of NC State University (protocol #16-032-A) approved all procedures for the experiment conducted at the Mountain Research Station (Waynesville, NC; 35.48°N, 82.99°W; elevation = 659 m) from October to December 2016.

Animals, Diets, and Sample Collection

At 14 d before weaning (d -14), 48 Angus calves (24 steers and 24 heifers; 244 ± 33 kg; 196 ± 20 d) were stratified by BW and age, and randomly assigned, in a 2×2 factorial design, to receive pre- (PRE; d -14 and 0) or post-weaning (POS; d 7 and 21) vaccinations against BRD pathogens. Then, calves were randomly assigned to receive post-weaning energy supplementation 3 (3X) or 7 (7X) times weekly from d 7 to 43. From d -14 to

0, calves and their dams were managed as a single group in a 22-ha tall fescue pasture (*Lolium arundinaceum*; 16% CP and 59% TDN; DM basis), and provided free-choice access to water and no concentrate supplementation until weaning (d 0). On d 0, calves were also stratified by sex and BW, within their respective treatment, and then randomly allocated into 1 of 16 pens (3 steers or 3 heifers/pen; 4 pens/frequency of supplementation/timing of vaccination; 18×4 m; 24 m^2 and 1.33 m of bunk space per calf) in a half-covered, concrete floor drylot feeding facility. The second calf stratification was performed to ensure calf uniformity within each pen at the time of feedlot entry. From d 0 to 6, all calves were provided daily free-choice access to ground tall fescue hay and daily energy supplementation of 50:50 mixture of soybean hulls and corn gluten feed at 0.5% of BW (DM basis). From d 7 to 43, calves were provided free-choice access of ground tall fescue hay and similar weekly concentrate DM supplementation (1% of average calf BW of each pen multiplied by 7 d; DM basis; Table 1).

First round of vaccinations (d -14 for PRE calves and d 7 for POS calves) consisted of vaccination against infectious bovine rhinotracheitis (IBR), bovine viral diarrhea virus (BVDV) types 1a and 2, parainfluenza-3 virus (PI-3), *Mannheimia haemolytica* (2 mL s.c.; Bovi Shield Gold One Shot; Zoetis Inc., New York, NY), and *Clostridium* (2 mL s.c.; Ultrabac 7, Zoetis Inc., New York, NY). Second round of vaccinations (d 0 for PRE calves and d 21 for POS calves) consisted of boosters of Bovi Shield Gold 5 (2 mL s.c.; Zoetis

Table 1. Average weekly chemical composition of ground tall fescue hay and concentrate (50% soy hulls and 50% corn gluten feed; as-fed basis) provided to steers from d 0 to 43.^a

Item	Tall fescue hay	Concentrate ^b
DM, %	90.2	93.4
	DM basis	
CP, %	13.5	15.3
ADF, %	36.8	31.1
NDF, %	62.7	53.3
TDN ^c , %	56.5	71.0
NE _m ^d , Mcal/kg	1.09	1.61
NE _e ^d , Mcal/kg	0.54	1.01

^aHay and concentrate samples were collected daily, pooled within each wk, and sent in duplicate to a commercial laboratory for wet chemistry analysis (Dairy One Forage Laboratory, Ithaca, NY).

^bSame concentrate was used from weaning to end of study (d -14 to 43) and consisted of 50% soybean hulls pellets and 50% corn gluten feed pellets (DM basis).

^cCalculated as described by Weiss et al. (1992).

^dCalculated using the equations proposed by the NRC (2000).

Inc.) and Ultrabac 7 (2 mL s.c.; Zoetis Inc.). The post-weaning vaccination protocol described above was chosen to replicate the protocol utilized by the local pre-conditioning alliance (Mountain Cattle Alliance, Canton, NC; [Artioli et al., 2015](#); [Moriel et al., 2015, 2016a](#); [Silva et al., 2017](#)). On d 0, all calves were treated with doramectin for internal and external parasites (5 mL s.c.; Dectomax injectable, Zoetis Inc., Kalamazoo, MI).

Concentrate and hay were offered in separate sections of the same concrete feed bunk at 0800 h. Calves provided concentrate supplementation daily and 3 times weekly consumed 100% of their concentrate offer within 1 and 6 h after supplementation, respectively. Individual calf BW was measured before feeding on d -14 and 43, following 12 h of feed and water withdrawal. Weekly concentrate DM offered to each pen was adjusted to the average full BW of each pen collected, before morning supplementation, on 2 consecutive days (d 0 and 1, 7 and 8, and 21 and 22). Shrunk BW was not obtained on d 0 to 22 to not disturb feeding behavior and avoid an unnecessary exacerbation of physiological stress response caused by weaning and feedlot entry, which 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 provided free choice to all cow-calf pairs from d -14 to 0, and top-dressed daily over the supplement at a rate of 0.114 g/calf from d 0 to 43.

Hay was processed through a forage chopper (Balebuster 2100; Haybuster, Jamestown, ND) to an approximately 5-cm particle size immediately before feeding. Hay DM offered and refused were obtained daily for each pen by drying samples of hay offered and refused in a forced-air oven at 60°C for 48 h. Daily hay DMI was determined by subtracting the daily hay DM refused from the daily hay DM offered. Samples of hay and concentrate offered were collected weekly, pooled by 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. Samples were analyzed for concentrations of CP (method 984.13; [AOAC, 2006](#)), ADF (method 973.18 modified for use in an Ankom 200 fiber analyzer; Ankom Technology Corp., Fairport, NY; [AOAC, 2006](#)), and NDF ([Van Soest et al., 1991](#); modified for use in an Ankom 200 fiber analyzer; Ankom Technology

Corp.). Concentrations of TDN were calculated as proposed by [Weiss et al. \(1992\)](#), whereas NEm and NEg were calculated using equations from [NRC \(2000\)](#).

Blood samples (10 mL) were collected via jugular venipuncture into sodium-heparin (158 USP) containing tubes (Vacutainer, Becton Dickinson, Franklin Lakes, NJ) at 1300 h for plasma harvest on d -14, -13, -11, -7, 0, 7, 8, 10, 14, and 21 to assess the plasma concentrations of haptoglobin and cortisol. Additional blood samples (10 mL) from jugular vein were collected into tube containing no additives (Vacutainer, Becton Dickinson, Franklin Lakes, NJ) for serum harvest on d 0 and 14, relative to first vaccination, and at the end of the study (d 43) to evaluate serum antibody titers against BVDV-1a and PI-3 viruses. Blood samples were immediately placed on ice following collection, and then centrifuged at $3,000 \times g$ for 30 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 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 (Immulin 1000; Siemens Medical Solutions Diagnostics, Los Angeles, CA). Inter- and intra-assay CV for haptoglobin and cortisol analyses were 9.8 and 9.1, and 4.7 and 4.7%, respectively.

Serum antibody titers against BVDV-1a and PI-3 viruses were determined by the Oklahoma Animal Disease and Diagnostic Laboratory using a virus neutralization test ([Rosenbaum et al., 1970](#)). Serum titers were reported as the $\log_2$ of the greatest dilution of serum that provided complete protection of the cells (lowest and greatest tested dilution = 1:4 and 1:256, respectively). For the seroconversion analysis, samples with serum neutralization value of <4 were considered negative and assigned a value of 0, whereas samples with serum neutralization value ≥ 4 were considered positive and assigned a value of 1. Then, the assigned values (0 or 1) were used to calculate the positive seroconversion (% of steers with positive serum neutralization) to BVDV-1a and PI-3 viruses ([Richeson et al., 2008](#); [Artioli et al., 2015](#); [Moriel et al., 2015, 2016a](#)). Statistical analyses. Except for seroconversion, all data were analyzed as a 2×2 factorial design using the MIXED procedure of SAS (version 9.4, SAS

Institute Inc., Cary, NC, USA) with Satterthwaite approximation to determine the denominator degrees of freedom for the test of fixed effects. Pen was the experimental unit, whereas calf sex, calf(pen), and pen(timing of vaccination $\times$ frequency of energy supplementation) were included as random effects in all analyses, except for analyses of G:F, daily hay DMI, and total DMI from d 0 to 43 that included only calf sex and pen(timing of vaccination $\times$ frequency of energy supplementation) as random effects. Calf sex was included as random effects because: 1) the physical capacity of the drylot limited the number of pens of same calf sex for each treatment (2 pens of steers or heifers/supplementation frequency/timing of vaccination), which diminishes the statistical power to detect interactions among calf sex and treatments and 2) major goal of the study was to evaluate effects of frequency of supplementation and timing of vaccination, regardless of calf sex. Daily hay DMI data were pooled by days that all calves were provided concentrate (Monday, Wednesday, and Friday), and days that concentrate supplementation was offered only to 7X calves (Tuesday, Thursday, Saturday, and Sunday). Thereafter, daily hay DMI was analyzed as repeated measures and tested for fixed effects of supplementation frequency, timing of vaccination, week of the study, day of the week, and all resulting interactions, using pen(timing of vaccination $\times$ frequency of energy supplementation) as the subject. Total DMI, G:F, and ADG from d 0 to 43 were tested for fixed effects of frequency of energy supplementation, timing of vaccination, and the resulting interaction. Body weight, plasma, and serum measurements were analyzed as repeated measures and tested for fixed effects of frequency of supplementation, timing of vaccination, day of the study (or days relative to first vaccination), and all resulting interactions. Compound symmetry covariance structure was used for all the repeated-measures analyses as this covariance structures generated the lowest Akaike information criterion. Positive seroconversion to BVDV-1a and PI-3 viruses were tested for fixed effects of frequency of energy supplementation, timing of vaccination, day relative to first vaccination, and all resulting interactions using the GLIMMIX procedure of SAS and calf sex, calf(pen), and pen(timing of vaccination $\times$ frequency of supplementation) as random effects. All results are reported as least-squares means. Data were separated using PDIFP if a significant preliminary *F*-test was detected. Significance was set at $P \leq 0.05$, and tendencies if $P > 0.05$ and ≤ 0.10 .

RESULTS

Effects of day of the study, but not frequency of energy supplementation, timing of vaccination, frequency of energy supplementation $\times$ timing of vaccination, frequency of energy supplementation $\times$ timing of vaccination $\times$ day of the study ($P \geq 0.11$), were detected for calf BW ($P < 0.0001$; Table 2). Effects of timing of vaccination $\times$ frequency of energy supplementation were not detected for any measurements of calf ADG, total DMI, and G:F ($P \geq 0.12$), except for overall ADG from d -14 to 43 ($P = 0.04$). Overall ADG did not differ among PRE-7X, POS-3X, and POS-7X calves ($P \geq 0.19$). However, overall ADG tended to be less for PRE-3X vs. POS-7X and PRE-7X calves ($P = 0.09$), and was less ($P = 0.006$) for PRE-3X vs. POS-3X (0.60, 0.70, 0.70, and 0.78 ± 0.038 kg/d for PRE-3X, PRE-7X, POS-7X, and POS-3X calves, respectively). Pre-weaning vaccination decreased calf ADG from d -14 to 0 ($P = 0.03$), whereas post-weaning vaccination decreased calf ADG from d 7 to 21 ($P = 0.10$). However, calves provided post-weaning vaccination had greater ($P = 0.03$) ADG from d 21 to 43 compared with calves provided pre-weaning vaccination. Frequency of energy supplementation did not affect any measurement of calf ADG ($P \geq 0.21$), except for ADG from d 0 to 43, which tended to be less for 3X vs. 7X calves ($P = 0.10$; Table 3).

Effects of timing of vaccination and frequency of energy supplementation were not detected for total DMI (hay plus concentrate) and G:F ($P \geq 0.11$), except for G:F from d 21 to 43 ($P = 0.05$) that was greater for POS vs. PRE calves (Table 3). Effects of timing of vaccination $\times$ week of the study and frequency of energy supplementation $\times$ week of the study $\times$ day of the week were detected for daily hay DMI ($P \leq 0.002$). Daily hay DMI did not differ between PRE and POS calves on wk 1, 3, 4, and 5 ($P \geq 0.23$), but decreased on wk 2 for POS vs. PRE calves ($P = 0.04$; Figure 1a). All calves received daily energy supplementation during wk 1, and hence, daily hay DMI during wk 1 did not differ ($P \geq 0.29$) among treatments. Daily hay DMI of 7X calves from wk 2 to 6 did not differ ($P \geq 0.11$) between days that 3X and 7X calves received energy supplementation and days that only 7X calves received energy supplementation (Figure 1b). However, daily hay DMI of 3X calves from wk 2 to 6 were always greater ($P \leq 0.05$) on days that only 7X calves received energy supplementation compared with days that 3X and 7X calves received energy supplementation. During wk 2, daily hay DMI of 3X calves on days that 3X calves did not

Table 2. Body weight of calves assigned, in a 2×2 factorial design, to receive pre- (d -14 and 0; PRE) or post-weaning (d 7 and 21; POS) vaccination against pathogens associated with respiratory disease, and then, post-weaning energy supplementation provided 3 (3X; Monday, Wednesday, and Friday) or 7 (7X; daily) times weekly during a 43-d pre-conditioning period (24 steers and 24 heifers; 4 pens/timing of vaccination/frequency of energy supplementation).^a

	Day of the study					SEM	P-value
	-14	0	7	21	43		
BW, kg							Vac. $\times$ day
PRE	245	251	272	282	282	3.9	0.20
POS	243	254	273	280	284		
P-value ^b	0.65	0.88	0.89	0.56	0.90		
							Freq. $\times$ day
3X	244	254	272	280	282	3.9	0.19
7X	244	252	273	282	284		
P-value ^b	0.99	0.64	0.94	0.69	0.76		

^aIndividual BW was measured on d -14 and 43, following 12 h of feed and water withdrawal. Full BW on d 0, 7, and 21 represent the average calf BW collected immediately before morning supplementation on d 0 and 1, 7 and 8, and 21 and 22.

^bP-value for the comparison of treatment within day of the study.

Table 3. Growth performance of calves assigned, in a 2×2 factorial design, to receive pre- (d -14 and 0; PRE) or post-weaning (d 7 and 21; POS) vaccination against pathogens associated with respiratory disease, and then, post-weaning energy supplementation provided 3 (3X; Monday, Wednesday, and Friday) or 7 (7X; daily) times weekly during a 43-d pre-conditioning period (24 steers and 24 heifers; 4 pens/timing of vaccination/frequency of energy supplementation).^a

	Timing of vaccination			<i>P</i> -value	Supplementation frequency			<i>P</i> -value
Item	POS	PRE	SEM	Timing of vaccination	3X	7X	SEM	Supplementation frequency
ADG, kg/d								
d −14 to 0	0.81	0.48	0.096	0.03	0.74	0.55	0.098	0.21
d 0 to 7	2.83	2.94	0.176	0.70	2.72	3.04	0.180	0.24
d 7 to 21	0.50	0.69	0.080	0.10	0.55	0.64	0.081	0.43
d 21 to 43	0.18	0.00	0.049	0.03	0.09	0.09	0.050	0.97
d 0 to 43	0.73	0.72	0.040	0.90	0.68	0.77	0.039	0.10
d −14 to 43	0.74	0.65	0.030	0.04	0.69	0.70	0.028	0.71
Total DMI, kg/d								
d −14 to 0	—	—	—	—	—	—	—	—
d 0 to 7	35	32	1.4	0.26	34	33	1.4	0.36
d 7 to 21	84	87	4.3	0.67	86	86	4.3	0.99
d 21 to 43	151	146	7.2	0.64	147	149	7.2	0.85
d 0 to 43	261	265	12.7	0.82	259	267	12.7	0.63
d −14 to 43	—	—	—	—	—	—	—	—
G:F ^b								
d −14 to 0	—	—	—	—	—	—	—	—
d 0 to 7	0.57	0.63	0.027	0.14	0.55	0.66	0.027	0.02
d 7 to 21	0.09	0.11	0.015	0.27	0.09	0.11	0.015	0.56
d 21 to 43	0.03	0.00	0.009	0.05	0.01	0.01	0.008	0.76
d 0 to 43	0.12	0.11	0.005	0.74	0.11	0.12	0.005	0.11
d −14 to 43	—	—	—	—	—	—	—	—

^aFrom d -14 to 0, calves and their dams were managed as a single group in a 22-ha tall fescue pasture until weaning (d 0). From d 0 to 43, all calves were provided daily free-choice access to ground tall fescue hay, and concentrate supplementation of 50:50 mixture of soybean hulls and corn gluten feed at 0.5 and 1.0% of BW (DM basis) from d 0 to 6 and 7 to 43, respectively.

^bCalculated by dividing total BW gain by total DMI from d 0 to 43.

Figure 1. Daily hay DMI of beef calves assigned, in a 2×2 factorial design, to receive pre- (d -14 and 0; PRE) or post-weaning (d 7 and 21; POS) vaccination against pathogens associated with respiratory disease, and then, post-weaning energy supplementation offered 3 (3X; Monday, Wednesday, and Friday) or 7 (7X; daily) times weekly during a 43-d pre-conditioning period (24 steers and 24 heifers; 4 pens/timing of vaccination/frequency of energy supplementation). Effect of timing of vaccination $\times$ week of the study (Figure 1a) and frequency of energy supplementation $\times$ day of the week $\times$ week of the study (Figure 1b) were detected for hay DMI ($P \leq 0.0002$). * $P = 0.04$. Within week, means without a common superscript (a–c) differ ($P \leq 0.05$).

receive energy supplementation did not differ ($P \geq 0.35$) compared with 7X calves (Figure 1b).

Effects of timing of vaccination $\times$ day of the study were detected for plasma concentrations of cortisol and haptoglobin, relative to days of first vaccination ($P < 0.0001$; Figures 2a and 3a, respectively) or relative to weaning ($P \leq 0.03$; Figures 2b and 3b, respectively). Effects of frequency of energy supplementation $\times$ timing of vaccination, frequency of energy supplementation $\times$ day of the study, and frequency of energy supplementation $\times$ timing of vaccination $\times$ day of the study were not detected for plasma concentrations of cortisol and haptoglobin ($P \geq 0.17$). Plasma concentrations of cortisol were greater for PRE vs. POS calves from d 0 to 14, relative to first vaccination ($P \leq 0.04$), and d -13, 7, 8, 10, and 14, relative to weaning ($P \leq 0.05$). Plasma concentrations of haptoglobin were greater ($P \leq 0.05$) for PRE vs. POS calves from d 0 to 3, relative to first vaccination, but less for PRE vs. POS calves on d 7 relative to first vaccination ($P = 0.04$). Plasma concentrations of haptoglobin were greater ($P < 0.0001$) for PRE vs. POS calves on d -13 and -11, relative to weaning, but less for PRE vs. POS calves on d 8, 10, and 14, relative to weaning ($P \leq 0.03$). Pre-weaning vaccination increased overall plasma concentrations of cortisol ($P < 0.0001$) and tended

to increase overall plasma concentrations of haptoglobin ($P = 0.10$) compared with post-weaning vaccination (Table 4). Decreasing the frequency of energy supplementation did not affect overall plasma concentrations of haptoglobin ($P = 0.17$), but tended ($P = 0.10$) to increase plasma concentrations of cortisol compared with daily energy supplementation (Table 4).

Effects of frequency of energy supplementation $\times$ timing of vaccination $\times$ day of the study and frequency of energy supplementation $\times$ timing of vaccination were not detected for serum titers and positive seroconversion against PI-3 and BVDV-1a viruses ($P \geq 0.15$). Effects of frequency of energy supplementation $\times$ day of the study, timing of vaccination $\times$ day of the study, and timing of vaccination were not detected for serum titers and positive seroconversion to BVDV-1a ($P \geq 0.27$). However, overall serum BVDV-1a titers tended to be greater for 7X vs. 3X calves ($P = 0.10$; Table 5). Effects of frequency of energy supplementation $\times$ day of the study and timing of vaccination $\times$ day of the study tended to be detected for serum titers and positive seroconversion to PI-3 virus ($P \leq 0.09$; Table 5). Serum titers and positive seroconversion to PI-3 virus were greater for 7X vs. 3X calves on d 43 ($P \leq 0.04$), but not at the time of their first and second rounds of vaccination ($P \geq 0.31$). Serum titers and

Figure 2. Plasma concentrations of cortisol of calves assigned, in a 2×2 factorial design, to receive pre- (d -14 and 0; PRE) or post-weaning (d 7 and 21; POS) vaccination against pathogens associated with respiratory disease, and then, post-weaning energy supplementation offered 3 (3X; Monday, Wednesday, and Friday) or 7 (7X; daily) times weekly during a 43-d pre-conditioning period (24 steers and 24 heifers; 4 pens/timing of vaccination/frequency of energy supplementation). Effect of timing of vaccination $\times$ day ($P \leq 0.03$) were detected for plasma concentrations of cortisol, relative to day of first round of vaccination (Figure 2a) and relative to weaning (Figure 2b). * $P \leq 0.05$.

positive seroconversion to PI-3 virus were greater for POS vs. PRE calves on d 43 ($P \leq 0.003$), but not at the time of first and second rounds of vaccination ($P \geq 0.31$).

DISCUSSION

Weaning, feedlot entry, and vaccination of beef cattle elicit an APR that triggers the production of a large and varied group of hepatic proteins (Suffredini et al., 1999). Although APR is an essential early defense mechanism in response to cellular injury (Eckersall and Conner, 1988), nutrient demand is increased to support the synthesis of acute-phase proteins, immune cells, and gluconeogenic precursors (Reeds and Jahoor, 2001). Consequently, muscle protein and fat reserves are mobilized (Jahoor et al., 1999), and absorbed AA are shifted from growth towards hepatic uptake (Reeds et al., 1994), leading to reduced feed intake, ADG, and feed efficiency of beef calves (Arthington et al., 2013; Moriel et al., 2015, 2016a; Artioli et al.,

2015). Recent studies demonstrated that reducing the frequency of energy supplementation exacerbated the vaccination- and feedlot entry-induced APR (Artioli et al., 2015; Moriel et al., 2016a; Silva et al., 2017), causing further decrease on calf growth performance (Artioli et al., 2015; Moriel et al., 2016a; Silva et al., 2017). Therefore, we hypothesized that allocating the timing of vaccination against BRD-associated pathogens to the pre-weaning phase could be a strategy to overcome these negative impacts of reduced frequency of concentrate supplementation on post-weaning growth and immunity of recently weaned beef calves. However, contrary to our hypothesis, overall calf ADG (d -14 to 43) was least for calves administered pre-weaning vaccination and post-weaning concentrate supplementation offered 3 times weekly. Reasons for this response will be discussed below.

Temporary reductions on calf growth performance following vaccination have been reported consistently in previous studies (Arthington et al., 2013; Moriel and Arthington, 2013;

Table 4. Plasma and serum measurements of calves assigned, in a 2×2 factorial design, to receive pre- (d -14 and 0; PRE) or post-weaning (d 7 and 21; POS) vaccination against pathogens associated with respiratory disease, and then, post-weaning energy supplementation provided 3 (3X; Monday, Wednesday, and Friday) or 7 (7X; daily) times weekly during a 43-d pre-conditioning period (24 steers and 24 heifers; 4 pens/timing of vaccination/frequency of energy supplementation).^a

Item	Timing of vaccination			P-value Timing of vaccination	Supplementation frequency			P-value Supplementation frequency
	PRE	POS	SEM		3X	7X	SEM	
Overall plasma concentrations								
Cortisol, ng/mL	21.0	10.3	0.87	<0.0001	16.7	14.6	0.87	0.10
Haptoglobin, mg/mL	0.49	0.42	0.031	0.10	0.49	0.43	0.031	0.17
Parainfluenza-3 virus								
Serum titers, log ₂	1.72	2.31	0.161	0.01	1.85	2.18	0.161	0.17
Positive seroconversion ^b , %	25.0	36.1	3.60	0.04	29.2	31.9	3.70	0.59
Bovine viral diarrhea virus 1a								
Serum titers, log ₂	2.56	2.49	0.109	0.68	2.41	2.65	0.109	0.10
Positive seroconversion ^b , %	33.3	44.4	6.94	0.27	33.3	44.4	6.94	0.27

^aFirst round of vaccinations (d -14 and 7 for PRE and POS calves, respectively) consisted of vaccination against infectious bovine *rhinotracheitis*, bovine viral diarrhea virus types 1a and 2, parainfluenza-3 virus, *Mannheimia haemolytica* (2 mL s.c.; Bovi Shield Gold One Shot; Zoetis Inc), and *clostridium* (2 mL s.c.; Ultrabac 7, Zoetis Inc.). Second round of vaccinations (d 0 and 21 for PRE and POS calves, respectively) consisted of boosters of Bovi Shield Gold 5 (2 mL s.c.; Zoetis Inc.) and Ultrabac 7 (2 mL s.c.; Zoetis Inc.).

^bSerum titers were reported as the log₂ of the greatest dilution of serum that provided complete protection of the cells (lowest and greatest tested dilution = 1:4 and 1:256, respectively). For the seroconversion analysis, samples with serum neutralization value of < 4 were considered negative and assigned a value of 0, whereas samples with serum neutralization value ≥ 4 were considered positive and assigned a value of 1.

Lippolis et al., 2016). In the current study, pre-weaning vaccination decreased pre-weaning calf ADG by 41% compared with calves assigned to post-weaning vaccination, whereas post-weaning vaccination decreased calf ADG from d 7 to 21 (first 14 d after first round of vaccination) by 28% compared with calves that received pre-weaning vaccinations. These results can be partially explained by the negative effects of vaccination in forage DMI (Rodrigues et al., 2015). Although pre-weaning forage DMI was not measured, daily hay DMI during wk 2 of the pre-conditioning period decreased by 16% after post-weaning vaccination. The magnitude of reduction was greater for ADG vs. hay DMI, indicating that factors beyond DMI (i.e., increased nutrient demand to support the immune system; Reeds and Jahoor, 2001) also modulated calf growth performance following vaccination. Nonetheless, our results demonstrated that vaccine-induced decrease in calf ADG were greater during pre- vs. post-weaning phases. Arthington et al. (2013) and Moriel and Arthington (2013) showed that vaccination against *Mannheimia haemolytica* decreased ADG of early-weaned beef calves by 25–26% during the first 14 d after vaccination compared with unvaccinated calves, despite the similar total DMI. In contrast, Lippolis et al. (2016) reported that pre-weaning calf vaccination,

administered 15 d before weaning, decreased calf pre-weaning ADG by 37% compared with calves assigned to vaccination immediately at or after weaning.

Upon activation of APR, plasma concentrations of acute-phase proteins (i.e., haptoglobin) and cortisol increase, reach peak concentrations between 3 to 7 d post-stimulus, and gradually decline during the subsequent 7 d (Moriel and Arthington, 2013). Haptoglobin is one of the major proteins associated with APR in cattle (Carroll and Forsberg, 2007) and is detectable only in injured or stressed animals (Petersen et al., 2004). Thus, haptoglobin can be used as an indicator of bovine acute and chronic inflammation when plasma concentrations are ≥ 0.11 mg/mL (Tourlomoussis et al., 2004). In addition, an increase in plasma concentrations of cortisol reduced DMI (Allen et al., 2009), elicited an APR, and enhanced plasma haptoglobin concentrations (Cooke and Bohnert, 2011). In the present study, plasma concentrations of cortisol and haptoglobin on days relative to first vaccination, and d 7 to 14 relative to weaning, were greater for calves administered pre- vs. post-weaning vaccinations, which was unexpected, but partially explains the greater post-vaccination reduction in calf ADG for calves administered pre- vs. post-weaning vaccinations. The exact reason for the greater plasma

Table 5. Serum titers and positive seroconversion against parainfluenza 3 virus of calves assigned, in a 2×2 factorial design, to receive pre- (d -14 and 0; PRE) or post-weaning (d 7 and 21; POS) vaccination against pathogens associated with respiratory disease, and then, post-weaning energy supplementation provided 3 (3X; Monday, Wednesday, and Friday) or 7 (7X; daily) times weekly during a 43-d pre-conditioning period (24 steers and 24 heifers; 4 pens/timing of vaccination/frequency of energy supplementation).^a

	Day of sample collection				
Item	First vaccination	Second vaccination	End of study	SEM	<i>P</i> -value
Parainfluenza-3 virus					
Serum titers, log ₂					Freq. × day
3X	0.00	0.63	4.94	0.269	0.09
7X	0.01	0.61	5.91	0.269	
<i>P</i> -value ^b	0.98	0.97	0.01		
					Vac. timing × day
PRE	0.00	0.61	4.54	0.269	0.0007
POS	0.10	0.63	6.31	0.269	
<i>P</i> -value ^b	0.98	0.97	<0.0001		
Positive seroconversion % ^c					Freq. × day
3X	0.0	12.5	75.0	5.91	0.08
7X	0.0	4.2	91.7	5.91	
<i>P</i> -value ^b	1.00	0.31	0.04		
					Vac. timing × day
PRE	0.0	4.2	70.8	5.91	0.08
POS	0.0	12.5	95.8	5.91	
<i>P</i> -value ^b	1.00	0.31	0.003		

^aFirst round of vaccinations (d -14 and 7 for PRE and POS calves, respectively) consisted of vaccination against infectious bovine rhinotracheitis, bovine viral diarrhoea virus types 1a and 2, parainfluenza-3, Mannheimia haemolytica (2 mL s.c.; Bovi Shield Gold One Shot; Zoetis Inc), and clostridium (2 mL s.c.; Ultrabac 7, Zoetis, Inc.). Second round of vaccinations (d 0 and 21 for PRE and POS calves, respectively) consisted of boosters of Bovi Shield Gold 5 (2 mL s.c.; Zoetis, Inc.) and Ultrabac 7 (2 mL s.c.; Zoetis, Inc.).

^bP-value for the comparison of treatment within day of blood sample collection.

^cSerum titers were reported as the log₂ of the greatest dilution of serum that provided complete protection of the cells (lowest and greatest tested dilution = 1:4 and 1:256, respectively). For the seroconversion analysis, samples with serum neutralization value of < 4 were considered negative and assigned a value of 0, whereas samples with serum neutralization value ≥ 4 were considered positive and assigned a value of 1.

concentrations of cortisol and haptoglobin of PRE vs. POS calves is unknown. However, calves in the present study were not exposed to human handling and cattle chute restraint from birth until the start of the study. Therefore, it is plausible that the pre-weaning blood results reported herein were affected by the first experience in cattle handling facilities at the start of the study. In support of this rationale, plasma concentrations of cortisol and haptoglobin of POS calves were greater during pre- vs. post-weaning phase, indicating that these calves also experienced a relatively greater physiological stress during pre- vs. post-weaning phase.

Vaccine-induced neutralizing antibody titers provide an indication of immune protection (Bolin and Ridpath, 1990) and vaccine efficacy in calves (Callan, 2001; Richeson et al., 2008). The ability of an animal to respond to vaccination varies from animal to animal and depends on environmental and genetic factors, maternal antibody concentrations (Downey et al., 2013), stress and previous exposure to pathogens (Loerch and Fluharty, 1999),

frequency of energy supplementation (Artoli et al., 2015; Silva et al., 2017), metabolizable protein supply (Moriel et al., 2015), maternal energy-restriction during late gestation (Moriel et al., 2016b), and timing of vaccination (Richeson et al., 2008; Lippolis et al., 2016). However, it is still poorly understood how acquired antibody levels may be affected by the timing of vaccination associated with other stressful calf management practices.

In the present study, serum titers and positive seroconversion against BVDV-1a virus was not impacted by pre- or post-weaning vaccinations. However, serum titers and positive seroconversion to PI-3 virus at the end of the pre-conditioning period were less for calves administered pre- vs. post-weaning vaccinations, suggestive of diminished immune protection against this pathogen (Bolin and Ridpath, 1990; Callan, 2001). Cortisol can cause immune suppression and block the cytokine secretion by T helper cells with Cluster of differentiation 4 (CD4+T), which are involved in antibody production (Salak-Johnson and McGlone, 2007).

Figure 3. Plasma concentrations of haptoglobin of calves assigned, in a 2×2 factorial design, to receive pre- (d -14 and 0; PRE) or post-weaning (d 7 and 21; POS) vaccination against pathogens associated with respiratory disease, and then, post-weaning energy supplementation offered 3 (3X; Monday, Wednesday, and Friday) or 7 (7X; daily) times weekly during a 43-d pre-conditioning period (24 steers and 24 heifers; 4 pens/timing of vaccination/frequency of energy supplementation). Effects of timing of vaccination $\times$ day ($P < 0.0001$) were detected for plasma haptoglobin concentrations, relative to first vaccination (Figure 3a) and relative to weaning (Figure 3b). * $P \leq 0.05$.

Hence, the greater plasma cortisol concentrations of PRE vs. POS calves immediately at and after 14 d of first round of vaccination (and from d 7 to 14 of the post-weaning phase) may have decreased the communication between innate and humoral immunity (Artioli et al., 2015), leading to suppressed antibody production against PI-3 virus. Also, maternal antibodies can bind to the virus antigen and prevent recognition by the adaptive immune system (Zimmerman et al., 2006). Thus, it is possible that maternal antibodies present in calf bloodstream at the time of pre-weaning vaccination may have limited the activation of humoral immunity and antibody production. However, the level of maternal antibody titers declines as calf age increases (Downey et al., 2013), and serum titers against BVDV-1a and PI-3 viruses at the time of first vaccination (d -14) was nearly zero, which makes this rationale of maternal antibody interference on pre-weaning calf vaccine response less plausible.

It has been shown that reducing the frequency of energy supplementation from daily to 3 times weekly decreased ADG by 10% to 21% (Cooke

et al., 2008; Loy et al., 2008; Artioli et al., 2015; Silva et al., 2017) or did not impact ADG of pre-conditioning calves (Moriel et al., 2016a). Discrepancies among these results can be associated to differences in supplement composition (high-moisture vs. grain pellet-based supplements), animal breed, and gender, location of the study, forage species, and quality, and the potential interactions among those factors (Artioli et al. 2015). In the present study, ADG during pre-conditioning (d 0 to 43) was 12% less for calves that received concentrate supplementation 3 times weekly vs. daily, regardless if calves were administered pre- or post-weaning vaccinations. This reduction in growth performance due to reduced frequency of concentrate supplementation occurred despite the similar total DMI from d 0 to 43 between 3X and 7X calves, which supports previous evidences that factors beyond reduced DMI impact BW gain of calves supplemented infrequently (Artioli et al., 2015; Moriel et al. 2016a; Silva et al., 2017). However, the magnitude of decrease in calf ADG due to reduced frequency of supplementation was similar to the 10% reduction

reported by [Silva et al. \(2017\)](#), but less than the 21% reduction in calf ADG during pre-conditioning reported by [Artioli et al. \(2015\)](#).

From wk 2 to 6, daily hay DMI of 3X calves decreased in average 35% on days that all calves received supplementation (Monday, Wednesday, and Friday), and increased by 20% on days that only 7X steers received concentrate supplementation (Tuesday, Thursday, Saturday, and Sunday) compared with 7X calves. Likewise, [Silva et al. \(2017\)](#) observed that daily hay DMI of calves supplemented 3 times weekly decreased by 27% on the days that 3X and 7X were supplemented, and increased by 14% on days that only 7X calves were supplemented. Despite the low-starch supplements used in this study, this response on daily hay DMI was expected because supplements often decrease forage DMI when supplemental TDN is greater than 0.7% of BW ([Moore et al., 1999](#)). However, total hay DMI did not differ among treatments, which partially explains the smaller than expected differences in calf overall ADG. Compared to calves offered daily concentrate supplementation, [Artioli et al. \(2015\)](#) reported that daily hay DMI of steers supplemented 3 times weekly decreased by 53% on the days that 3X and 7X were supplemented, and increased by 10% on days that only 7X steers were offered concentrate, leading to an overall reduction on intake of hay DM, CP, and NEg. Concentrate supplementation-induced depression in forage intake is greater as forage quality increases ([Horn and McCollum, 1987](#)). Hence, the greater hay DMI reduction after concentrate supplementation in the study of [Artioli et al. \(2015\)](#) may be attributed to the greater forage nutritive value compared to the present study (17.4 vs. 11.8% CP; 58.0 vs. 54.0% TDN; 34.4 vs. 39.8% ADF; 57.7 vs. 64.2% NDF of DM, respectively).

As described above, decreasing the frequency of energy supplementation exacerbated the vaccine- and feedlot entry-induced APR of pre-conditioning calves ([Artioli et al., 2015](#); [Moriel et al., 2016a](#); [Silva et al., 2017](#)), leading to greater nutrient demand to support the immune system and nutrient partitioning away from growth ([Reeds and Jahoor, 2001](#)). In agreement, calves supplemented 3 times weekly tended to have greater overall plasma cortisol concentrations and numerically greater plasma haptoglobin concentrations compared to calves provided daily concentrate supplementation, which partially explains their diminished post-weaning BW gain. In addition, overall plasma haptoglobin concentrations of 3X and 7X calves ranged between 0.43 and 0.49 mg/mL, whereas overall plasma cortisol

concentrations ranged between 14.6 and 16.7 ng/mL. [Artioli et al. \(2015\)](#) observed that plasma haptoglobin concentrations ranged between 0.69 and 0.91 mg/mL and were 31.8% greater for steers supplemented 3 vs. 7 times weekly, whereas overall plasma cortisol concentrations ranged between 19.6 and 23.4 ng/mL and were 19% greater for steers supplemented 3 vs. 7 times weekly. Hence, the greater overall plasma concentrations of cortisol and haptoglobin (and magnitude of differences between 3X vs. 7X steers) indicates that calves experienced greater physiological stress and inflammatory responses in the study of [Artioli et al. \(2015\)](#), and partially explains the smaller than expected differences in overall pre-conditioning ADG between 3X and 7X steers reported herein.

Contrary to our hypothesis, overall calf ADG (d -14 to 43) was least for calves administered pre-weaning vaccination and post-weaning concentrate supplementation offered 3 times weekly. This response is likely the result of multiple factors that elicited multiple APR in PRE-3X calves, including: 1) pre-weaning vaccination triggering APR and reducing pre-weaning ADG ([Arthington et al., 2013](#); [Moriel and Arthington, 2013](#)); and 2) the exacerbation of weaning- and feedlot entry-induced APR compared with daily concentrate supplementation, which likely increased nutrient demand and nutrient partitioning towards the immune system ([Reeds and Jahoor, 2001](#)) and decreased post-weaning growth performance ([Arthington et al., 2013](#)).

In summary, the present study demonstrated that pre-weaning vaccination led to the least humoral immune response to parainfluenza-3 virus at the end of a 43-d pre-conditioning period, and provided further evidence that reducing the frequency of energy supplementation exacerbated the acute-phase response and reduced calf post-weaning growth performance and humoral immunity. In addition, the combination of pre-weaning vaccination and reduced post-weaning frequency of concentrate supplementation caused the greatest reduction on calf growth performance, indicating that allocating the vaccination against respiratory pathogens to the pre-weaning phases (14 d before weaning) does not prevent the negative impacts of reduced frequency of energy supplementation on calf growth performance and measurements of innate and humoral immunity during pre-conditioning.

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