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Effects of vaccination against respiratory pathogens on feed intake, metabolic, and inflammatory responses in beef heifers[1]

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ABSTRACT: The objective of this study was to evaluate intake, metabolic, inflammatory, and acute-phase responses in beef heifers vaccinated against pathogens that cause bovine respiratory disease (BRD). Eighteen weaned Angus heifers (initial BW 257 ± 3 kg; initial age 245 ± 2 d) were ranked by BW and allocated to 2 groups, which were assigned to 2 experiments of 7 d and the following treatments on d 1 of each experiment: 1) revaccinated against infectious bovine rhinotracheitis virus, parainfluenza-3 virus, bovine respiratory syncytial virus, bovine viral diarrhea Types 1 and 2 viruses, and *Mannheimia haemolytica* (VAC; 2 mL [s.c.]) and 2) receiving a 2-mL s.c. injection of 0.9% sterile saline (CON). The group receiving VAC in Exp. 1 was assigned to CON in Exp. 2 and vice versa. Heifers were weaned 21 d before Exp. 1, when they all received the first dose of the aforementioned vaccine. Heifers were maintained in individual pens and offered free-choice mixed alfalfa–grass hay and 3.5 kg/d (DM basis) of a corn-based supplement throughout the study. During Exp. 1, hay and concentrate intake were evaluated daily. During Exp. 2, blood samples were collected before (–2 and 0 h) and at 2, 4, 6, 8, 12, 16, 24, 36, 48, 60, 72, 96, 120, 144, and 168 h after treatment administration. In Exp. 1, treatment × day interactions were detected ($P < 0.01$) for forage intake and total DMI; these parameters were reduced ($P \leq 0.05$) in VAC heifers compared with CON heifers on d 1 and 2 by an average of 1.7 and 0.8 kg (DM basis), respectively. In Exp. 2, mean serum tumor necrosis factor α (TNFα) concentration was greater ($P = 0.05$) in VAC heifers compared with CON heifers and treatment × hour interactions were detected for all plasma variables ($P \leq 0.02$), whereas a similar tendency was detected ($P = 0.09$) for blood *TNF*α mRNA expression. Haptoglobin concentrations were greater ($P \leq 0.05$) in VAC heifers compared with CON heifers from 16 to 120 h. Blood *TNF*α mRNA expression was greater ($P = 0.05$) in VAC heifers compared with CON heifers at 12 h. Cortisol concentrations were greater ($P \leq 0.05$) in VAC heifers compared with CON heifers from 2 to 16 h. Insulin concentration was greater ($P = 0.02$) in VAC heifers compared with CON heifers at 2 h. Leptin concentrations were greater ($P \leq 0.05$) in VAC heifers compared with CON heifers from 6 to 16 h. In conclusion, vaccinating beef heifers against BRD pathogens decreased forage intake and total DMI during the 2 d following vaccination in Exp. 1, which can be associated with transient metabolic, inflammatory, and acute-phase responses elicited by vaccination in Exp. 2.

Key words: beef cattle, inflammation, intake, vaccination

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INTRODUCTION

Vaccination against pathogens that cause bovine respiratory disease (**BRD**) is a common practice in beef production systems (Duff and Galyean, 2007); however, cattle performance can be reduced during the 2 wk subsequent to vaccination (Arthington et al., 2013). More specifically, adjuvants and antigens

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contained in commercially available BRD vaccines elicit innate immune responses associated with antigen presentation to T cell lymphocytes, including inflammatory and acute-phase reactions (Tizard, 2004). These reactions, characterized by proinflammatory cytokines and hepatic synthesis of acute-phase proteins, can impair cattle performance by increasing basal metabolism and tissue catabolism and by reducing DMI and G:F (Johnson, 1997). Nevertheless, research is still warranted to further elucidate the metabolic impacts of BRD vaccines and develop interventions that mitigate performance losses of vaccinated cattle.

Leptin is a hormone mainly produced by adipocytes, and its endocrine role in ruminants has been primarily associated with feed intake and energy metabolism (Keisler et al., 1999). However, leptin has been classified as proinflammatory cytokine due to its structure and pleiotropic role on the innate immune system (Lord, 2006), whereas immune cells such as leukocytes can also synthesize leptin during an inflammatory response (Schneiderman et al., 2012). However, the potential link between leptin and inflammatory responses in cattle still needs to be verified (Soliman et al., 2002; Waldron et al., 2003). Given that elevated leptin reduces feed intake and increases energy expenditure (Houseknecht et al., 1998), we hypothesized that the performance losses of BRD-vaccinated cattle are also modulated by increased circulating leptin concentrations, including leptin synthesized by leukocytes. Therefore, the objective of this study was to evaluate intake, metabolic, inflammatory, and acute-phase responses in beef heifers vaccinated against pathogens that cause BRD.

MATERIALS AND METHODS

This study was conducted at the Oregon State University – Eastern Oregon Agricultural Research Center (Burns Station; Burns, OR). The animals used were cared for in accordance with acceptable practices and experimental protocols reviewed and approved by the Oregon State University Institutional Animal Care and Use Committee (number 4592).

Animals and Diets

Eighteen Angus heifers (initial BW 257 ± 3 kg; initial age 245 ± 2 d) were used in the study (Exp. 1 and 2). Heifers were weaned 21 d before the beginning of the Exp. 1 and exposed daily to halter training to prevent, or at least alleviate, the impacts of human handling and weaning on the variables evaluated herein (Arthington et al., 2005; Cooke and Bohnert, 2011; Cooke et al., 2012). At weaning, heifers were vaccinated against *Clostridium* (2 mL [**s.c.**] injection of One Shot Ultra 7; Zoetis, Florham Park, NJ) and infectious bovine rhinotracheitis virus, parainfluenza-3 virus, bovine respiratory syncytial virus, bovine viral diarrhea Types 1 and 2 viruses, and *Mannheimia haemolytica* (2 mL s.c. injection of Bovi-Shield Gold One Shot; Zoetis) and administered an anthelmintic (s.c. injection at 1 mL/50 kg of BW of Dectomax; Zoetis). Beginning 15 d before and throughout the study, heifers were housed in individual pens (7 by 15 m) and received water and mixed alfalfa–grass hay for ad libitum consumption and 3.5 kg/heifer daily (DM basis) of a concentrate containing (as-fed basis) 84% cracked corn, 14% soybean meal, and 2% mineral mix, which was offered separately from hay in a different section of the same feed bunk at 0800 h.

At the beginning of the study, heifers were ranked by initial BW in a decreasing order and alternatingly assigned to 1 of 2 groups. Groups were assigned to 2 experiments of 7 d each and the following treatments at the start of each experiment (d 1): 1) revaccinated against infectious bovine rhinotracheitis virus, parainfluenza-3 virus, bovine respiratory syncytial virus, bovine viral diarrhea Types 1 and 2 viruses, and *M. haemolytica* (**VAC**; 2 mL s.c. of Bovi-Shield Gold One Shot; Zoetis) and 2) receiving a 2-mL s.c. injection of 0.9% sterile saline (**CON**). The group receiving VAC in Exp. 1 was assigned to CON in Exp. 2 and vice versa. This experimental length was selected because all the variables evaluated herein return to baseline levels within 7 d after vaccination (Arthington et al., 2013; Marques et al., 2014) or other challenges to the innate immune system, such as corticotropin-releasing hormone of liposaccharide administration (Carroll et al., 2009b; Cooke et al., 2012) and to maintain the interval between vaccination and revaccination against viral respiratory pathogens recommended by the manufacturer (Zoetis). Although revaccination against *M. haemolytica* is not required, this is a common practice in commercial feedlots due to the frequent lack of health history in high-risk receiving cattle (Richeson et al., 2008; Edwards, 2010).

Sampling

Individual full BW was recorded and averaged over 2 consecutive days before the beginning of Exp. 1 to establish initial BW and at the last day of Exp. 1 and first day of Exp. 2 to establish final BW of Exp. 1. Body weights were obtained immediately before concentrate and hay feeding. Experiment 1 focused on only DMI evaluation, whereas Exp. 2 focused on metabolic, inflammatory, and acute-phase responses, given that the sampling schedule adopted in Exp. 2 would most likely hinder proper assessment of heifer DMI.

Experiment 1. Concentrate and hay intake and total DMI were evaluated daily, beginning 5 d before and

during Exp. 1, by weighing and collecting samples of the offered and nonconsumed feed. All samples were dried for 96 h at 50°C in forced-air ovens for DM calculation. Samples of hay and concentrate ingredients were collected, pooled across all days, and analyzed for nutrient content by a commercial laboratory (Dairy One Forage Laboratory, Ithaca, NY). These samples were analyzed by wet chemistry procedures 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 an Ankom 200 fiber analyzer). Calculations for TDN used the equation proposed by Weiss et al. (1992), whereas NEm and NEg were calculated with the equations proposed by the NRC (2000). Hay nutritional profile was (DM basis) 63% TDN, 34% NDF, 24% ADF, 1.41 Mcal/kg of NEm, 0.83 Mcal/kg of NEg, and 20.0% CP. Based on the nutritional analysis of ingredients, the concentrate nutritional profile was (DM basis) 85% TDN, 9.0% NDF, 4.6% ADF, 2.12 Mcal/kg of NEm, 1.46 Mcal/kg of NEg, and 14.5% CP. The mineral mix (Cattleman's Choice; Performix Nutrition Systems, Nampa, ID), contained 14% Ca, 10% P, 16% NaCl, 1.5% Mg, 3,200 mg/kg of Cu, 65 mg/kg of I, 900 mg/kg of Mn, 140 mg/kg of Se, 6,000 mg/kg of Zn, 136,000 IU/kg of vitamin A, 13,000 IU/kg of vitamin D_3, and 50 IU/kg of vitamin E.

Experiment 2. Blood samples were collected before (–2 and 0 h) and at 2, 4, 6, 8, 12, 16, 24, 36, 48, 60, 72, 96, 120, 144, and 168 h after treatment administration via jugular venipuncture into commercial blood collection tubes (Vacutainer, 10 mL; Becton Dickinson, Franklin Lakes, NJ) with or without 158 U. S. Pharmacopeia units of freeze-dried sodium heparin for plasma and serum collection, respectively. Samples were immediately placed on ice, centrifuged (2,500 × *g* for 30 min at 4°C) for plasma or serum harvest, and stored at –80°C on the same day of collection. All plasma samples were analyzed for plasma haptoglobin concentration according to colorimetric procedures described by Cooke and Arthington (2013). Samples collected from 0 to 48 h were also analyzed for concentrations of plasma cortisol, insulin, and leptin as well as serum tumor necrosis factor α (**TNF**α). Plasma concentrations of cortisol and insulin were determined using a chemiluminescent enzyme immunoassay (Immulite 1000; Siemens Medical Solutions Diagnostics, Los Angeles, CA). Plasma concentrations of leptin were determined by radioimmunoassay according to procedures described by Delavaud et al. (2000). Serum concentrations of TNFα were determined using a bovine-specific commercial ELISA kit (RayBiotech, Inc., Norcross, GA), but the TNFα procedure was validated for bovine samples using pools of serum collected from yearling beef steers immediately before (0 h) and 2 and 4 h following an intravenous liposaccharide administration (0.5 μg/kg of BW, *Escherichia coli* 0111:B4; Sigma-Aldrich, St. Louis, MO), which is known to promptly increase serum TNFα concentrations in cattle (Carroll et al., 2009b). Mean TNFα concentrations were 0.26 ± 0.31, 2.86 ± 0.31, and 0.54 ± 0.31 ng/mL for pools collected at 0, 2, and 4 h relative to liposaccharide administration, respectively. The intra- and interassay CV were 1.9 and 6.8% for haptoglobin and 4.3 and 7.5% for TNFα, respectively. Plasma cortisol, insulin, and leptin concentrations were analyzed within a single assay. The intra-assay CV were 2.5% for cortisol, 4.0% for insulin, and 4.9% for leptin,

Additional blood samples were collected at –2, 2, 4, 12, and 24 h relative to treatment administration into PAXgene tubes (Qiagen, Valencia, CA) for subsequent RNA isolation and analysis of *leptin*, *TNFα*, and β-*actin* mRNA expression in blood cells via real-time quantitative reverse transcription (**RT-**) PCR. Upon collection, PAXgene tubes were stored at room temperature overnight and then at –80°C until RNA isolation. Total RNA was extracted from blood samples using the PAXgene Blood RNA Kit (Qiagen). Quantity and quality of isolated RNA were assessed via UV absorbance (UV Mini 1240; Shimadzu Scientific Instruments, Inc., Columbia, MD) at 260 nm and 260:280 nm ratio, respectively (Fleige and Pfaffl, 2006). Extracted blood RNA (1.0 μg) was reverse transcribed using the High Capacity cDNA Reverse Transcription Kit with random hexamers (Applied Biosystems, Foster City, CA). Real-time RT-PCR was completed using the Fast SYBR Green Master Mix (Applied Biosystems) and gene-specific primers (20 p*M* each; Table 1) with the StepOne Real-time PCR system (Applied Biosystems), according to procedures described by Cooke et al. (2008). At the end of each RT-PCR, amplified products were subjected to a dissociation gradient (95°C for 15 s, 60°C for 30 s, and 95°C for 15 s) to verify the amplification of a single product by denaturation at the anticipated temperature. Responses were quantified based on the threshold cycle (**CT**), the number of PCR cycles required for target amplification to reach a predetermined threshold. The CT responses from *leptin* and *TNFα* were normalized to β-*actin* (Ohtsuka et al., 2014), whereas the CV for β-*actin* CT values across all samples was 4.4%. Results are expressed as relative fold change ($2^{-\Delta\Delta CT}$), as described by Ocón-Grove et al. (2008).

Statistical Analysis

All analyses were performed within each experiment with the MIXED procedure of SAS (version 9.3; SAS Inst., Inc., Cary, NC) and Satterthwaite approximation to determine the denominator degrees of freedom for the tests of fixed effects, using heifer as the experimental unit and heifer(treatment) as random

Table 1. Primer sequences and accession number for all gene transcripts analyzed by quantitative real-time reverse transcription PCR

Target gene	Primer sequence[1]	Accession no.
β-actin		
Forward	5′-CTTCTACAACGAGCTCCGTG-3′	NM_173979.3
Reverse	5′-CACGCTCCGTGAGGATCTTC-3′	
Leptin		
Forward	5′-TCGTGACCTTCTTTGGGATTT-3′	NM_173928.2
Reverse	5′-CACACTGGAATACTTCCCTCTC-3′	
Tumor necrosis factor alpha		
Forward	5′-AACAGCCCTCTGGTTCAAAC-3′	NM_173966.3
Reverse	5′-TCTTGATGGCAGACAGGATG-3′	

[1]Primer sequences obtained from **β**-*actin* (Ohtsuka et al., 2014), *leptin* (Perkins et al., 2014), and *tumor necrosis factor alpha* (Riollet et al., 2000).

Table 2. Intake, metabolic, inflammatory, and acute-phase variables obtained from heifers before treatment application[1,2]

Item	CON	VAC	SEM	*P*-value
Experiment 1				
Hay DMI, kg/d	3.87	3.98	0.20	0.68
Concentrate DMI, kg/d	3.39	3.46	0.05	0.23
Total DMI, kg/d	7.24	7.44	0.20	0.49
Experiment 2				
Serum TNFα,[3] ng/mL	0.63	0.64	0.50	0.99
Blood TNFα mRNA expression,[3] fold change	1.64	1.65	0.26	0.99
Plasma haptoglobin, mg/mL	0.14	0.23	0.10	0.52
Plasma cortisol, ng/mL	40.4	34.8	3.1	0.23
Plasma insulin, μIU/mL	3.74	3.24	0.82	0.68
Plasma leptin, ng/mL	8.84	8.25	0.59	0.49

[1]Treatments were 1) VAC = revaccinated against infectious bovine rhinotracheitis virus, parainfluenza-3 virus, bovine respiratory syncytial virus, bovine viral diarrhea Types 1 and 2 viruses, and *Mannheimia haemolytica* (2 mL [s.c.] of Bovi-Shield Gold One Shot; Zoetis, Florham Park, NJ), or 2) CON = receiving a 2-mL s.c. injection of 0.9% sterile saline.

[2]In Exp. 1, hay and concentrate intake and total DMI during the 5 d preceding treatment application were averaged. In Exp. 2, blood samples were obtained before treatment application (average of –2 and 0 h for plasma and serum variables and –2 h for mRNA expression variables).

[3]TNF**α** = tumor necrosis factor alpha

variable. All model statements contained the effects of treatment, time (day for intake or hour for blood variables), and the resultant interaction. Intake data were analyzed using the average hay or concentrate intake or total DMI during the 5 d preceding the beginning of Exp. 1 as independent covariate. Blood variables in Exp. 2 were analyzed using values obtained before treatment application (average of –2 and 0 h for plasma and serum variables and –2 h for mRNA expression variables) as independent covariate. The specified term for the repeated statements was day for intake or hour for blood variables and heifer(treatment) as subject, and the covariance structure used was autoregressive based on the Akaike information criterion. Results are reported as covariately adjusted least squares means and separated using LSD. Significance was set at $P \leq 0.05$, and tendencies were determined if $P > 0.05$ and ≤ 0.10. Results are reported according to treatment effects if no interactions were significant or according to the highest-order interaction detected.

RESULTS AND DISCUSSION

The diet provided to heifers during the study was formulated (NRC, 2000) to yield a forage:concentrate ratio of 60:40 and simulate a feedlot receiving diet (Fluharty and Loerch, 1996), when feeder cattle are often revaccinated against BRD pathogens (Richeson et al., 2008). In addition, ADG and G:F were not assessed herein because this study adopted a sample size, experimental length, and sampling schedule that were adequate to evaluate vaccination effects on intake and physiological variables but not for the assessment of growth and feed efficiency parameters (Faul et al., 2007).

During the 5 d preceding Exp. 1, average hay and concentrate intake and total DMI were similar ($P \geq 0.23$) between CON and VAC heifers (Table 2), whereas average hay intake and total DMI before Exp. 1 were significant covariates ($P < 0.01$) within their respective analyses. During Exp. 2, average concentrations of serum TNFα, blood *TNF*α mRNA expression, plasma haptoglobin, cortisol, insulin, and leptin before treatment application were significant covariates ($P \leq 0.04$) within their respective analyses but did not differ ($P \geq 0.23$) between CON and VAC heifers (Table 2). Therefore, all heifers had similar DMI and were in similar physiological status before the beginning of Exp. 1 and 2, respectively, indicating that there were no treatment carryover effects from Exp. 1 to Exp. 2, as expected based on our experimental design and previous research within this subject (Carroll et al., 2009b; Arthington et al., 2013; Marques et al., 2014)

Experiment 1 – Hay and Concentrate Intake and Total DMI Evaluation

A treatment × day interaction was detected ($P < 0.01$) for hay intake and total DMI, whereas no treatment differences were detected for concentrate DMI (Table 3). On d 1 and 2, VAC heifers had reduced ($P \leq 0.02$) hay intake and total DMI compared with CON heifers, which were similar ($P \geq 0.13$) between treatments from d 3 to 7 (Table 3). Given that initial and final BW during Exp. 1 did not differ ($P \geq 0.52$) among treatments (255 vs. 259 kg of initial BW [SEM 5] and 271 vs. 273 kg of final BW [SEM 5] for CON and VAC heifers, respectively), similar outcomes were detected (data not shown) for intake

Table 3. Feed intake parameters of heifers revaccinated against infectious bovine rhinotracheitis virus, parainfluenza-3 virus, bovine respiratory syncytial virus, bovine viral diarrhea Types 1 and 2 viruses, and *Mannheimia haemolytica* (VAC; 2 mL [s.c.] of Bovi-Shield Gold One Shot; Zoetis, Florham Park, NJ) or receiving a 2-mL s.c. injection of 0.9% sterile saline (CON)[1,2]

Item	CON	VAC	SEM	*P*-value
Hay DMI, kg/d				
d 1	4.01	2.38	0.20	<0.01
d 2	4.05	3.20	0.20	<0.01
d 3	4.52	4.08	0.20	0.13
d 4	4.77	4.47	0.20	0.29
d 5	5.13	5.10	0.20	0.92
d 6	5.46	5.16	0.20	0.31
d 7	5.16	4.75	0.20	0.16
Concentrate DMI, kg/d	3.34	3.34	0.08	0.93
Total DMI, kg/d				
d 1	7.24	5.44	0.25	<0.01
d 2	7.47	6.65	0.25	0.02
d 3	7.62	7.39	0.25	0.53
d 4	8.22	7.82	0.25	0.26
d 5	8.61	8.38	0.25	0.52
d 6	8.92	8.60	0.25	0.38
d 7	8.66	8.19	0.25	0.21

[1]Treatments were administered on d 1. All heifers were vaccinated against *Clostridium* (2 mL s.c. injection of One Shot Ultra 7; Zoetis) and infectious bovine rhinotracheitis virus, parainfluenza-3 virus, bovine respiratory syncytial virus, bovine viral diarrhea Types 1 and 2 viruses, and *Mannheimia haemolytica* (2 mL s.c. injection of Bovi-Shield Gold One Shot; Zoetis) and administered an anthelmintic (s.c. injection at 1 mL/50 kg of BW of Dectomax; Zoetis) 20 d before the beginning of the study.

[2]Average hay and concentrate intake and total DMI during the 5 d preceding the experiment were similar ($P > 0.23$) between CON and VAC heifers (3.87 vs. 3.98 kg/d of hay DMI [SEM 0.20], 3.39 vs. 3.46 kg/d of concentrate DMI [SEM 0.05], and 7.24 vs. 7.44 kg/d of total DMI [SEM 0.20], respectively), whereas average hay intake and total DMI before Exp. 1 were significant covariates ($P < 0.01$) within their respective analysis. Therefore, hay intake and total DMI results reported are covariately adjusted least squares means.

variables as percent of heifer BW (hay intake and total DMI = treatment × day interaction, $P < 0.01$; concentrate intake = treatment effect, $P = 0.77$).

These results indicate that vaccination against BRD pathogens impacted only voluntary forage DMI, perhaps by impacting satiety, feeding behavior, and/or by one or more physical regulators of roughage DMI, such as ruminal digestibility, motility, and passage rate (Allison, 1985; Allen, 2000). Supporting our findings, Stokka et al. (1994) also reported that DMI decreased by nearly 20% in feedlot cattle on booster vaccination against *Clostridium*. Conversely, Arthington et al. (2013) reported similar DMI between feeder cattle vaccinated or not against *M. haemolytica*. Nevertheless, Arthington et al. (2013) handled cattle frequently after vaccination for sample collection, offered a total mixed ration with a 23:77 forage:concentrate ratio, and vaccinated cattle against only *M. haemolytica*. These factors may explain, at least partially, the difference in vaccination effects on DMI reported herein and by Arthington et al. (2013).

Based on the dietary nutrient profile and DMI results, VAC heifers consumed less ($P \leq 0.02$) TDN, NEm, NEg, and CP than CON heifers during the 2 d subsequent to treatment administration (4.57 vs. 5.31 kg of TDN/d [SEM 0.20], 10.9 vs. 12.6 Mcal of NEm/d [SEM 0.4], 7.1 vs. 8.1 Mcal of NEg/d [SEM 0.3], and 1.04 vs. 1.27 kg of CP/d [SEM 0.05], respectively) but not ($P \geq 0.21$; data not shown) from d 3 to 7 (treatment × day interaction, $P \leq 0.03$). Given that concentrate intake was not impacted by treatments, increasing concentrate offered during the 2 d following vaccination against BRD pathogens, such as 1 kg/heifer daily of the concentrate used herein, may be an alternative to mitigate the decrease in overall nutrient intake and alleviate the performance losses in vaccinated feeder cattle (Arthington et al., 2013).

Experiment 2 – Metabolic, Inflammatory, and Acute-Phase Responses

The vaccine administered in this study contained a freeze-dried preparation of modified live virus strains, a product from whole cultures of inactivated *M. haemolytica*, and a proprietary adjuvant formulation (Zoetis) to elicit a greater immune protection to target antigens (McKee et al., 2007; Coffman et al., 2010). In general, adjuvants assist in recruiting antigen-presenting leukocytes to the site of vaccine delivery, which engulf, process, and present the target antigen to T cell lymphocytes to begin the cascade conferring protective immunity to the host (Tizard, 2004). Upon recruitment and activation, leukocytes also start synthesizing proinflammatory cytokines such as TNFα, which, in turn, stimulate hepatic synthesis of acute-phase proteins including haptoglobin (Carroll and Forsberg, 2007). In addition, the viral fraction of the vaccine used in the current study also stimulates antigen-presenting leukocytes and elicits inflammatory and acute-phase protein responses in cattle (Arthington et al., 1996; Heegaard et al., 2000). In agreement with this rationale, a treatment effect was detected ($P = 0.05$) for plasma concentrations of the proinflammatory cytokine TNFα, whereas a treatment × hour interaction was detected ($P < 0.01$) for plasma haptoglobin (Fig. 1).

Following treatment application, mean serum TNFα concentration was greater ($P = 0.05$) in VAC heifers compared with CON heifers (0.86 vs. 0.63 ng/mL, respectively [SEM 0.08]). A treatment × hour interaction was not detected ($P = 0.79$) for serum TNFα concentrations, although this interaction was expected based on the bovine

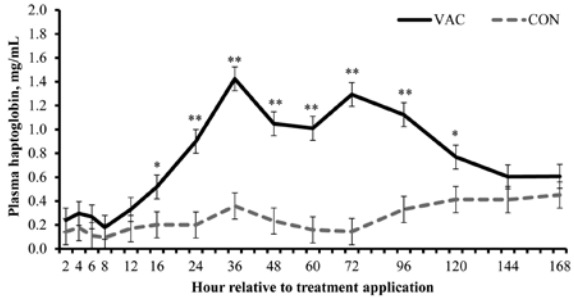

Figure 1. Plasma haptoglobin concentrations of heifers revaccinated against infectious bovine rhinotracheitis virus, parainfluenza-3 virus, bovine respiratory syncytial virus, bovine viral diarrhea Types 1 and 2 viruses, and *Mannheimia haemolytica* (VAC; 2 mL [s.c.] of Bovi-Shield Gold One Shot; Zoetis, Florham Park, NJ) or receiving a 2-mL s.c. injection of 0.9% sterile saline (CON). Average of values obtained from samples collected before treatment application (–2 and 0 h) served as covariate ($P < 0.01$) but did not differ ($P = 0.52$) between CON and VAC heifers (0.14 vs. 0.23 mg/mL, respectively [SEM 0.10]). Therefore, results reported are covariately adjusted least squares means. A treatment × hour interaction was detected ($P < 0.01$). Treatment comparison within hour; *$P \leq 0.05$; **$P \leq 0.01$.

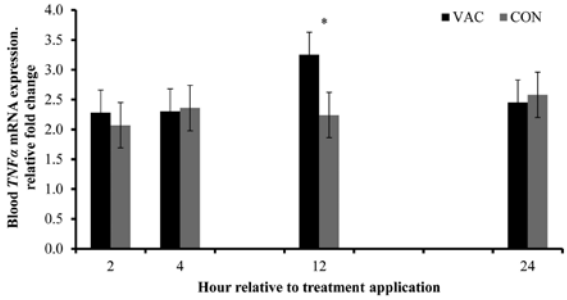

Figure 2. Blood mRNA expression of tumor necrosis factor α (TNFα) in heifers revaccinated against infectious bovine rhinotracheitis virus, parainfluenza-3 virus, bovine respiratory syncytial virus, bovine viral diarrhea Types 1 and 2 viruses, and *Mannheimia haemolytica* (VAC; 2 mL [s.c.] of Bovi-Shield Gold One Shot; Zoetis, Florham Park, NJ) or receiving a 2-mL s.c. injection of 0.9% sterile saline (CON). Values from samples collected before treatment application (–2 h) served as covariate ($P = 0.04$) but did not differ ($P = 0.98$) between CON and VAC heifers (1.64 vs. 1.65 relative fold change, respectively [SEM 0.26]). Therefore, results reported are covariately adjusted least squares means. A tendency for a treatment × hour interaction was detected ($P = 0.09$). Treatment comparison within hour; *$P = 0.05$.

proinflammatory cytokine response following a liposaccharide injection described by Carroll et al. (2009a,b). Perhaps the BRD vaccine used herein does not yield a peak response in serum TNFα concentrations similar to liposaccharide stimuli or the sampling schedule adopted in Exp. 2 was not frequent enough to properly assess this variable. Supporting this latter rationale, a tendency for a treatment × hour interaction was detected ($P = 0.09$) for blood *TNF*α mRNA expression (Fig. 2). Following treatment application, blood *TNF*α mRNA expression was greater ($P = 0.05$) in VAC heifers compared with CON heifers at 12 h (Fig. 2). Therefore, one can speculate that serum TNFα concentrations peaked in VAC heifers 12 h after vaccination, given that mRNA translation into the active protein requires time (Clancy and Brown, 2008), although research with a sampling schedule and frequency based on these findings is warranted to properly characterize the proinflammatory cytokine response in cattle vaccinated against BRD pathogens.

Following treatment application, plasma haptoglobin concentrations were greater ($P \leq 0.05$) in VAC heifers compared with CON heifers from 16 to 120 h (Fig. 1). Arthington et al. (2013) also reported that steers vaccinated against *M. haemolytica* had greater plasma haptoglobin concentrations compared with nonvaccinated cohorts on d 1 and 3 after vaccination. Collectively, these results support that the BRD vaccine used herein stimulated TNFα synthesis from blood cells including leukocytes, which resulted in an acute-phase reaction characterized by elevated plasma concentrations of TNFα and haptoglobin. These outcomes also corroborate the treatment effects detected for DMI in Exp. 1, given that TNFα is known to reduce feed intake by modulating the central nervous and endocrine systems and inhibiting digestive function (Klasing and Korver, 1997), whereas plasma haptoglobin concentrations were negatively associated with DMI in feeder cattle (Qiu et al., 2007; Araujo et al., 2010).

Treatment × hour interactions were also detected ($P \leq 0.01$) for plasma cortisol (Fig. 3) and insulin concentrations (Fig. 4). Plasma cortisol concentrations were greater ($P \leq 0.05$) in VAC heifers compared with CON heifers from 2 to 16 h following treatment application (Fig. 3). These results indicate that early physiological responses following vaccination against BRD pathogens also modulated cortisol, which appears to function as an effector molecule on subsequent proinflammatory and acute-phase reactions (Steiger et al., 1999; Carroll et al., 2009b). Accordingly, research from our group reported that administering corticotropin-releasing hormone, as a mean to stimulate adrenal cortisol synthesis, increased circulating concentrations of TNFα and haptoglobin in overtly healthy cattle (Cooke and Bohnert, 2011; Cooke et al., 2012). Plasma insulin concentrations were greater ($P = 0.02$) in VAC heifers compared with CON heifers at 2 h after treatment application (Fig. 4). Others have also reported immediate and transient increases in circulating insulin concentrations on a pathogen stimulus (Steiger et al., 1999; Waldron et al., 2003), given that insulin synthesis is enhanced during an inflammatory response (Eizirik et al., 1995; Andersson et al,, 2001) with the intent of increasing energy utilization by the body to restore homeostasis (Waggoner et al., 2009). Nevertheless, treatment differences in plasma cortisol and insulin concentrations were detected herein without a marked increase in serum TNFα concentrations and before the observed increase in blood *TNF*α mRNA expression, indicating that the

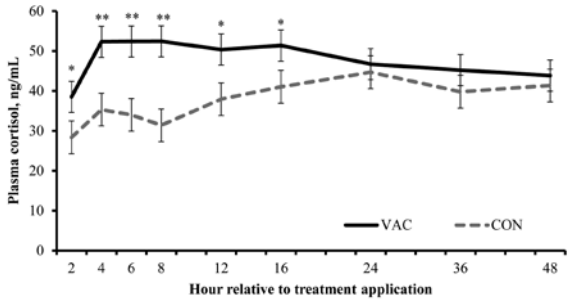

Figure 3. Plasma cortisol concentrations of heifers revaccinated against infectious bovine rhinotracheitis virus, parainfluenza-3 virus, bovine respiratory syncytial virus, bovine viral diarrhea Types 1 and 2 viruses, and *Mannheimia haemolytica* (VAC; 2 mL [s.c.] of Bovi-Shield Gold One Shot; Zoetis, Florham Park, NJ) or receiving a 2-mL s.c. injection of 0.9% sterile saline (CON). Average of values from samples collected before treatment application (–2 and 0 h) served as covariate ($P < 0.01$) but did not differ ($P = 0.23$) between CON and VAC heifers (40.4 vs. 34.8 ng/mL, respectively [SEM 3.1]). Therefore, results reported are covariately adjusted least squares means. A treatment × hour interaction was detected ($P < 0.01$). Treatment comparison within hour; *$P \leq 0.05$; **$P \leq 0.01$.

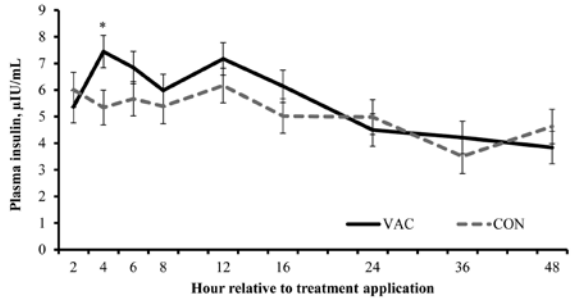

Figure 4. Plasma insulin concentrations of heifers revaccinated against infectious bovine rhinotracheitis virus, parainfluenza-3 virus, bovine respiratory syncytial virus, bovine viral diarrhea Types 1 and 2 viruses, and *Mannheimia haemolytica* (VAC; 2 mL [s.c.] of Bovi-Shield Gold One Shot; Zoetis, Florham Park, NJ) or a receiving 2-mL s.c. injection of 0.9% sterile saline (CON). Average of values from samples collected before treatment application (–2 and 0 h) served as covariate ($P < 0.01$) but did not differ ($P = 0.13$) between CON and VAC heifers (6.9 vs. 3.3 µIU/mL, respectively [SEM 1.6]). Therefore, results reported are covariately adjusted least squares means. A treatment × hour interaction was detected ($P < 0.01$). Treatment comparison within hour; *$P \leq 0.05$; **$P \leq 0.01$.

vaccination effects on plasma cortisol and insulin were not exclusively dependent on the resultant proinflammatory cytokine response (Steiger et al., 1999; Carroll and Forsberg, 2007). In addition, elevated circulating cortisol and insulin may also depress DMI in ruminants (Foster et al., 1991; Allen et al., 2009), although treatment differences for these hormones were detected within 16 h after vaccination whereas vaccination reduced DMI during Exp. 1 for at least 48 h.

A treatment × hour interaction was detected ($P = 0.02$) for plasma leptin (Fig. 5). Plasma leptin concentrations were greater ($P \leq 0.05$) in VAC heifers compared with CON heifers from 6 to 16 h following treatment application, supporting our hypothesis that vaccination against BRD pathogens increases circulating leptin concentrations in cattle. Leptin is known to limit feed intake and increase energy expenditure in ruminants by directly acting on the central nervous system (Houseknecht et al., 1998). Hence, elevated leptin concentrations on vaccination against BRD pathogens may have contributed to the reduced DMI detected in Exp. 1 (Table 3) and the decrease in G:F reported by Arthington et al. (2013). However, vaccination reduced DMI during Exp. 1 for at least 48 h (Table 3) whereas treatment differences for plasma leptin were detected only within 16 h after vaccination in Exp. 2 (Fig. 5), which may indicate that additional mechanisms besides increased leptin concentrations also mediate the decreased DMI in cattle vaccinated against BRD pathogens. These include proinflammatory cytokines as mentioned herein as well as inflammatory compounds such as eicosanoids (Klasing and Korver, 1997).

Leptin has also been associated with the innate immune system based on its similar structure to proinflammatory cytokines and its modulatory role in inflammatory responses (Fantuzzi, 2006). More specifically, leptin stimulates activation and maturation of leukocytes and production of proinflammatory cytokines (Matarese et al., 2005; Fernández-Riejos et al., 2010), whereas circulating leptin concentrations were increased on liposaccharide administration to rodents (Grunfeld et al., 1996; Sarraf et al., 1997; Roelfsema et al., 2001). Although previous studies failed to demonstrate a link between circulating leptin and immune responses in cattle (Soliman et al., 2002; Waldron et al., 2003), results from this experiment provide novel evidence that leptin is involved with inflammatory and acute-phase responses elicited by vaccination against BRD pathogens. Leptin is mainly synthesized by adipocytes on endocrine stimuli such as insulin, cortisol, and proinflammatory cytokines (Houseknecht et al., 2000; Matarese, 2000; Faggioni et al., 2001), although leptin can also be locally synthesized by leukocytes during an inflammatory response (Schneiderman et al., 2012; Tao et al., 2013). In Exp. 2, the greater plasma leptin concentrations in VAC heifers compared with CON heifers can be associated with the vaccine-induced increases in plasma cortisol, insulin, and TNFα concentrations. However, *leptin* mRNA was not expressed in the blood of VAC or CON heifers (no data to show), given that *leptin* mRNA was not amplified in any of the samples analyzed via real-time RT-PCR (Freeman et al., 1999). Therefore, vaccination against BRD pathogens appears to stimulate leptin synthesis by adipocytes, which deserves investigation and was not evaluated herein because biopsy of fat tissues in cattle (Rule and Beitz, 1986) may result in injuries known to stimulate inflammatory and acute-phase reactions that would be confounded with the treatments evaluated by the present experiment (Carroll and Forsberg, 2007).

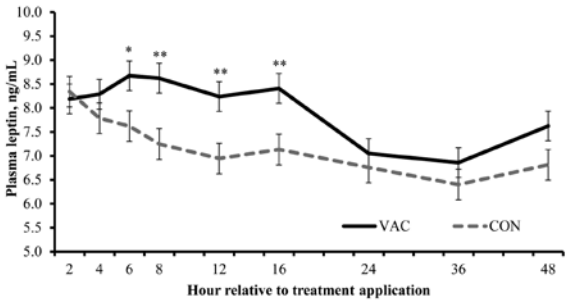

Figure 5. Plasma leptin concentrations of heifers revaccinated against infectious bovine rhinotracheitis virus, parainfluenza-3 virus, bovine respiratory syncytial virus, bovine viral diarrhea Types 1 and 2 viruses, and *Mannheimia haemolytica* (VAC; 2 mL [s.c.] of Bovi-Shield Gold One Shot; Zoetis, Florham Park, NJ) or receiving a 2-mL s.c. injection of 0.9% sterile saline (CON). Average of values from samples collected before treatment application (–2 and 0 h) served as covariate ($P < 0.01$) but did not differ ($P = 0.49$) between CON and VAC heifers (8.8 vs. 8.2 ng/mL, respectively [SEM 0.6]). Therefore, results reported are covariately adjusted least squares means. A treatment × hour interaction was detected ($P < 0.01$). Treatment comparison within hour; *$P \leq 0.05$; **$P \leq 0.01$.

Overall Conclusions

Results from Exp. 1 demonstrated that vaccinating beef cattle against BRD pathogens using a commercial vaccine based on modified live virus strains, inactivated *M. haemolytica*, and an adjuvant formulation decreased forage and total nutrient intake during the 2 d following vaccination. This outcome may be associated, at least in part, to metabolic, inflammatory, and acute-phase responses triggered by vaccination but required for proper acquirement of protective immunity (Tizard, 2004). More specifically, vaccination increased serum concentrations of TNFα for at least 48 h and transiently increased blood *TNF*α mRNA expression and plasma concentrations of cortisol, insulin, leptin, and haptoglobin during Exp. 2, which are all known to impact feed intake (Foster et al., 1991; Klasing and Korver, 1997; Houseknecht et al., 1998). These results can be used in formulating interventions that maximize immune protection and optimize performance of cattle vaccinated against BRD pathogens (Arthington et al., 2013), including strategic concentrate feeding during the first 2 d following vaccination.

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