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Meloxicam as an Alternative to Alleviate Inflammatory and Acute-Phase Reactions in Beef Cattle Upon Lipopolysaccharide Administration or Vaccination

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Abstract text: Twenty-one Angus steers (n = 11) and heifers (n = 10) were assigned to Exp. 1 (d -1 to 6) and Exp. 2 (d 6 to 20). On d -10, cattle were housed in individual pens and offered free-choice water, mineral-vitamin mix, and hay until d 20. In Exp. 1, calves were ranked on d -1 by gender and BW, and assigned to: 1) oral meloxicam administration (1 mg/kg of BW daily) from day -1 to 6 (**MEL7**), 2) oral meloxicam administration (1 mg/kg of BW) on d 0, and oral lactose monohydrate administration (1 mg/kg of BW) on d -1 and from d 1 to 6 (**MEL1**), and 3) oral lactose monohydrate administration (1 mg/kg of BW daily) from d -1 to 6 (**CON**). On d 0, all cattle received an intravenous lipopolysaccharide bolus (0.5 μg/kg of BW). From d -2 to d 6, cattle BW and hay DMI were recorded daily. Rectal temperature was assessed and blood samples collected every 2 h from -2 to 8 h, every 6 h from 12 to 72 h, and every 24 h from 96 to 144 h relative to lipopolysaccharide administration. Calves receiving MEL7 had greater (P = 0.03) hay DMI (kg/d) compared with MEL1 and CON. When DMI was evaluated as % of BW, MEL7 had greater (P = 0.05) hay DMI compared with CON. No treatment effects were detected (P = 0.90) for rectal temperature, plasma cortisol, insulin, leptin, haptoglobin and serum NEFA, although temperature and plasma variables increased after LPS administration (time effects; $P < 0.01$). For Exp. 2, calves were maintained and received the same treatments as in Exp. 1 from d 6 to 12. On d 7, cattle were vaccinated against respiratory pathogens. Cattle BW was recorded at the beginning (d 6 and 7) and end (d 20 and 21) of Exp. 2, whereas hay DMI was recorded daily. Rectal temperature was assessed and blood samples collected as in Exp. 1, in addition to 168, 240, and 336 h relative to vaccination. No treatment effects were detected ($P \geq 0.15$), although hay DMI decreased, plasma concentrations of cortisol, insulin, leptin, and haptoglobin increased, and serum antibodies against respiratory pathogens also increased (time effects, $P < 0.01$) after vaccination. Hence, meloxicam failed to modulate the physiological, inflammatory, and acute-phase protein reactions associated with lipopolysaccharide administration and vaccination in beef cattle.

Keywords: cattle, lipopolysaccharide, meloxicam, vaccination

INTRODUCTION

Oral meloxicam administration to feeder cattle alleviated the acute-phase protein reaction and prevented the decrease in receiving ADG, DMI, and G:F caused by transport and feedlot entry (Guarnieri Filho et al., 2014), indicating that meloxicam is an alternative to mitigate inflammatory reactions and performance losses elicited by stressful events. Nevertheless, research is still required to further understand the role of meloxicam during acute-phase and inflammatory reactions (Van Engen et al., 2014) to biologically support its benefits to highly-stressed beef cattle. One alternative to characterize the effects of meloxicam on the bovine innate immune system is through a bacterial lipopolysaccharide (**LPS**) challenge and subsequent evaluation of physiological and acute-phase variables (Carroll et al., 2009).

Vaccination is another stressful and mandatory procedure in cattle operations (Carroll and Forsberg, 2007). As an example, vaccination against *Mannheimia haemolytica*, the main bacterium isolated from bovine respiratory disease (**BRD**) in feedlot cattle (Rice et al., 2007), stimulated an acute-phase protein reaction and reduced ADG, DMI, and G:F during the 2 wk subsequent to vaccination (Arthington et al., 2013; Marques et al., 2014). Hence, research to develop management interventions that benefit vaccine-induced immune protection and cattle performance is warranted (Arthington et al., 2013). Based on the benefits of meloxicam administration to highly-stressed cattle (Guarnieri Filho et al., 2014; Van Engen et al., 2014) it can be hypothesized that oral meloxicam will alleviate the acute-phase response and prevent the decrease in performance caused by vaccination.

Based on this rationale, the objective of this experiment was to evaluate the effects of oral meloxicam administration on performance, inflammatory, and acute-phase parameters of beef cattle assigned to a bacterial LPS challenge (Exp. 1), or vaccinated against the BRD complex (Exp. 2).

MATERIALS AND METHODS

Twenty-one Angus halter-trained steers (n = 11) and heifers (n = 10) were assigned to the Exp. 1 (d -1 to 6) and subsequently to Exp. 2 (d 6 to 20). Cattle were weaned at 6 mo of age on d -33, and exposed daily to halter-training techniques until d -1. At weaning, cattle were vaccinated

against clostridial diseases, infectious bovine rhinotracheitis, bovine viral diarrhea complex, pneumonia, and *M. haemolytica* (One Shot Ultra 7, Bovi-Shield Gold One Shot, and TSV-2; Zoetis, Florham Park, NJ), and administered an anthelmintic (Dectomax; Zoetis). On d -10, cattle were housed in individual pens contained within an enclosed barn, and offered free choice water, mineral-vitamin mix, and mixed alfalfa-grass hay until the end of Exp. 2 (d 20).

Experiment 1

Animals and treatments. On d -1, calves were ranked by gender and BW (avg. BW = 232 ± 4 kg), and assigned to 1 of 3 treatments: 1) oral meloxicam administration (1 mg/kg of BW daily; Carlsbad Technologies, Inc., Carlsbad, CA) from day -1 to 6 (**MEL7**), 2) oral meloxicam administration (1 mg/kg of BW; Carlsbad Technologies, Inc.) on d 0, and oral lactose monohydrate administration (1 mg/kg of BW, excipient used in the manufacture of meloxicam tablets; Avantor Performance Materials, Center Valley, PA) on d -1 and from d 1 to 6 (**MEL1**), and 3) oral lactose monohydrate administration (1 mg/kg of BW daily; Avantor Performance Materials) from d -1 to 6 (**CON**).

Meloxicam was originally presented in 15 mg tablets, which were ground daily using a commercial food processor (Soho Food Processor; West Bend Housewares, West Bend, WI) to ensure that cattle received their exact dose. Lactose monohydrate was administered to account for potential placebo effects. Meloxicam or lactose monohydrate were manually mixed with 50 mL of 0.9% saline and administered individually to cattle via oral drench. The MEL7 and CON treatments are based on Guarnieri Filho et al. (2014), which also suggested that different lengths meloxicam administration should be investigated. Accordingly, the MEL1 treatment was included to determine if a single meloxicam administration can mitigate the inflammatory and acute-phase responses elicited by the LPS challenge, which occur within 24 h challenge (Carroll et al., 2009).

On d 0, all cattle received an intravenous bolus dose of bacterial LPS (0.5 µg/kg of BW, *Escherichia coli* 0111:B4, Sigma-Aldrich, St. Louis, MO) concurrently with treatment administration (Carroll et al., 2009). Bacterial LPS was dissolved into 10 mL of 0.9% saline immediately before administration.

Sampling. From d -2 to d 6, cattle were weighed daily. Hay DMI was evaluated daily from d -2 to 6 from each pen by collecting and weighing refusals daily. Samples of the offered and non-consumed feed were collected daily from each pen and dried for 96 h at 50°C in forced-air ovens for DM calculation.

Steer rectal temperature was assessed with a GLA M750 digital thermometer (GLA Agricultural Electronics, San Luis Obispo, CA) every 2 h from -2 to 8 h, every 6 h from 12 to 72 h, and every 24 h from 96 to 144 h relative to LPS administration. Blood samples were collected concurrently with rectal temperature assessment via jugular venipuncture into commercial blood collection tubes (Vacutainer, 10 mL; Becton Dickinson, Franklin Lakes, NJ) with or without freeze-dried sodium heparin for plasma and serum collection, respectively. Blood samples were placed immediately on ice, centrifuged (2,500 × g for 30 min; 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 concentrations (Guarnieri Filho et al., 2014). Samples collected from -2 to 48 h relative to LPS administration were analyzed for concentrations of serum NEFA, plasma cortisol, insulin, and leptin (Delavaud et al., 2000; Guarnieri Filho et al., 2014).

Experiment 2

Animals and treatments. Immediately after the last sampling of Exp. 1 on d 6, cattle (avg. BW = 228 ± 4 kg) were assigned to the same treatment scheme received in Exp. 1. Treatments (MEL7, MEL1, and CON) were administered from d 6 to d 12.

On d 7, cattle were re-vaccinated against infectious bovine rhinotracheitis, bovine viral diarrhea complex, pneumonia, and *M. haemolytica* (Bovi-Shield Gold One Shot; Zoetis) concurrently with treatment administration. As in Exp. 1, the MEL7 and CON treatments are based on Guarnieri Filho et al. (2014). Given that leukocytes responsible for inflammatory and acute-phase responses are directly involved with antigen presentation to T cells (Durum and Muegge. 1996), excessive meloxicam administration may impair the innate immune responses required for proper vaccine efficacy. Therefore, MEL1 was included to determine if a single meloxicam administration concurrently with handling for vaccination can mitigate the resultant inflammatory and acute-phase responses (Marques et al., 2014) without impairing vaccine efficacy.

Sampling. Cattle full BW was recorded at the beginning (d 6 and 7) and end (d 20 and 21) of the experiment. Hay DMI was recorded daily from d 5 to 20 as in Exp. 1, whereas G:F was calculated based on total BW gain and hay DMI during the experimental period. Rectal temperature was assessed and blood samples collected as in Exp. 1, with additional collected at 168, 240, and 336 h relative to vaccination for analysis of plasma haptoglobin (Guarnieri Filho et al., 2014). Plasma samples collected immediately prior (0 h) and at 168, 240, and 336 h following vaccination were also analyzed for concentrations of antibodies against *M. haemolytica* (Confer et al., 2009), as well as titers against *bovine respiratory syncytial virus* (**BRSV**), *bovine herpesvirus-1* (**BHV-1**), *bovine viral diarrhea virus-1* (**BVD-1**), and *parainfluenza 3 virus* (**PI3**) at the Oklahoma Animal Disease Diagnostic Laboratory (Stillwater, OK).

Statistical analysis

Data from both experiments were analyzed using the MIXED procedure of SAS (SAS Inst., Inc., Cary, NC) and Satterthwaite approximation to determine the denominator df for the tests of fixed effects. Animal was considered the experimental unit. The model statements contained the effects of treatment, time, gender, and all resultant interaction. Hay DMI was analyzed using values obtained on d -2 and -1 (Exp. 1) or d 5 and 6 (Exp. 2) as covariates. Animal(treatment × gender) was used as random variable. The specified term for the repeated statement was time, animal(treatment × gender) was included as subject, and the covariance structure utilized was autoregressive, which provided the lowest Akaike information criterion and

therefore the best fit. Results are reported as least square means, covariately adjusted means for hay DMI, and separated using PDIFF. Significance was set at $P \leq 0.05$, and tendencies were determined if $P > 0.05$ and ≤ 0.10.

Table 1. Performance and rectal temperature (**RT**) of steers and heifers challenged with lipopolysaccharide (**LPS**; Exp. 1) or vaccination (Exp. 2), and assigned to receive oral meloxicam (1 mg/kg of BW daily) for 7 d (**MEL7**), lactose monohydrate for 7 d (**CON**), or meloxicam for 1 d and lactose monohydrate for 6 d (**MEL1**) during the challenging period.[1]

Item	CON	MEL7	MEL1	SEM	*P* =
Exp. 1					
BW, kg	229	231	221	7	0.61
DMI					
Kg/d	4.73[a]	5.52[b]	4.75[a]	0.23	0.05
% of BW	1.97[a]	2.33[b]	2.10[ab]	0.12	0.09
RT	38.99	38.95	38.96	0.07	0.90
Exp. 2					
BW, kg					
Initial	230	234	222	7	0.48
Final	234	238	228	7	0.55
BW change	4.98	3.98	5.24	1.40	0.80
DMI					
Kg/d	5.50	5.72	5.30	0.19	0.38
% of BW	2.38	2.47	2.35	0.08	0.55
G:F, g/kg	71.4	53.5	80.4	21.0	0.66
RT	39.19	39.06	39.13	0.06	0.29

[1] In Exp. 1, all cattle received an intravenous bolus dose of bacterial LPS (0.5 µg/kg of BW, *Escherichia coli* 0111:B4, Sigma-Aldrich, St. Louis, MO). In Exp. 2, cattle were vaccinated against infectious bovine rhinotracheitis, bovine viral diarrhea complex, pneumonia, and *Mannheimia. haemolytica* (Bovi-Shield Gold One Shot; Zoetis, Florham Park, NJ).

RESULTS AND DISCUSSION

Experiment 1

No treatment effects were detected ($P = 0.61$) for calf BW during the experimental period (Table 1). Calves assigned to MEL7 had greater ($P = 0.03$) actual hay DMI compared with MEL1 and CON calves (Table 1). When DMI was evaluated as % of BW, MEL7 had greater ($P = 0.05$) hay DMI compared with CON calves only (Table 1), whereas hay DMI of MEL1 calves was similar ($P \geq 0.45$) compared with MEL7 and CON cohorts (Table 1). Therefore, these results indicate that oral meloxicam administration for 7 d alleviated the decrease in hay DMI caused by LPS administration and the stress of handling for sample collection (day effect, $P < 0.01$; Figure 1).

No treatment effects were detected ($P = 0.90$) for rectal temperature, which increased for all treatments (time effect, $P < 0.01$; data not shown) from 2 to 6 h and returned to baseline levels 8 h relative to LPS administration. No treatment effects were detected ($P \geq 0.74$) for plasma cortisol, insulin, leptin, haptoglobin and serum NEFA (Table 2). Time effects were detected ($P < 0.01$; data not shown) for all plasma variables. Plasma cortisol concentrations increased ($P < 0.05$) from 2 to 6 h and returned to baseline levels 8 h relative to LPS administration. Plasma insulin concentrations increased ($P < 0.05$) from 2 to 12 h and returned to baseline levels 16 h relative to LPS administration. Plasma leptin concentrations increased ($P < 0.05$) from 8 to 16 h and returned to baseline levels 24 h relative to LPS administration. Plasma haptoglobin concentrations increased ($P < 0.05$) beginning at 16 h and returned to baseline levels only at 144 h relative to LPS administration. Carroll et al. (2009) reported similar time effects for rectal temperature and plasma cortisol in weaned steers receiving LPS at 1.0 µg/kg of BW. The time effect detected for haptoglobin demonstrates that LPS administration effectively induced an acute-phase protein reaction (Carroll et al., 2009). Time effects detected for plasma insulin and leptin indicate changes in energy metabolism to cope with the stress of inflammation (Waggoner et al., 2009). Nevertheless, all these variables were similar among CON, MEL7, and MEL1, suggesting that oral meloxicam administration failed to modulate the physiological and inflammatory responses triggered by LPS administration.

Table 2. Blood variables from steers and heifers challenged with lipopolysaccharide (**LPS**; Exp. 1) or vaccination (Exp. 2), and assigned to receive oral meloxicam (1 mg/kg of BW daily) for 7 d (**MEL7**), lactose monohydrate for 7 d (**CON**), or meloxicam for 1 d and lactose monohydrate for 6 d (**MEL1**) during the challenging period.[1]

Item	CON	MEL7	MEL1	SEM	*P* =
Exp. 1					
Cortisol, ng/mL	30.4	30.6	30.1	2.7	0.98
Insulin, µIU/mL	2.72	3.04	2.75	0.66	0.93
NEFA, µEq/L	0.43	0.42	0.43	0.05	0.98
Leptin, ng/mL	4.89	4.93	5.23	0.34	0.74
Haptoglobin, ng/mL	0.26	0.29	0.28	0.05	0.95
Exp. 2					
Cortisol, ng/mL	26.6	26.1	24.4	1.6	0.61
Insulin, µIU/mL	1.84	1.92	1.91	0.38	0.98
NEFA, µEq/L	0.37	0.29	0.35	0.06	0.68
Leptin, ng/mL	5.00	5.29	5.44	0.25	0.46
Haptoglobin, ng/mL	0.57	0.52	0.56	0.09	0.92

[1] In Exp. 1, all cattle received an intravenous bolus dose of bacterial LPS (0.5 µg/kg of BW, *Escherichia coli* 0111:B4, Sigma-Aldrich, St. Louis, MO). In Exp. 2, cattle were vaccinated against infectious bovine rhinotracheitis, bovine viral diarrhea complex, pneumonia, and *Mannheimia haemolytica* (Bovi-Shield Gold One Shot; Zoetis, Florham Park, NJ).

Experiment 2

No treatment effects detected ($P = 0.80$) BW change during the experimental period (Table 1). No treatment effects were detected ($P \geq 0.38$; Table 1) for hay DMI parameters, although DMI decreased (day effect, $P < 0.01$; Figure 2) upon vaccination as previously reported by Marques et al. (2014).

No treatment effects were detected ($P = 0.29$) for rectal temperature, which increased for all treatments (time effect, $P < 0.01$; data not shown) from 2 to 16 h and returned to baseline levels 24 h relative to vaccination. No treatment effects were detected ($P \geq 0.61$) for plasma cortisol, insulin, leptin, haptoglobin and serum NEFA

(Table 2). Time effects were detected ($P < 0.01$; data not shown) for all plasma variables. Plasma cortisol concentrations increased ($P < 0.05$) from 4 to 12 h and returned to baseline levels 16 h relative to vaccination. Plasma insulin concentrations increased ($P < 0.05$) from 8 to 16 h and returned to baseline levels 24 h relative to vaccination. Plasma leptin concentrations increased ($P < 0.05$) from 8 to 16 h and returned to baseline levels 24 h relative to vaccination. Plasma haptoglobin concentrations increased ($P < 0.05$) beginning at 16 h and returned to baseline levels only at 168 h relative to vaccination. Similar outcomes were reported by Marques et al. (2014), illustrating the physiological changes, including energy metabolism, inflammatory and stress reaction, caused by vaccination against BRD pathogens in beef cattle.

Table 3. Serum concentrations of antibodies against M. *haemolytica* (ng bound), as well as serum titers (log) against bovine respiratory syncytial virus (**BRSV**), bovine herpesvirus-1 (**BHV-1**), bovine viral diarrhea virus-1 (**BVD-1**), and parainfluenza 3 virus (**PI3**) in steers and heifers challenged with a vaccine against these pathogens and assigned to receive oral meloxicam (1 mg/kg of BW daily) for 7 d (**MEL7**), lactose monohydrate for 7 d (**CON**), or meloxicam for 1 d and lactose monohydrate for 6 d (**MEL1**) during the challenging period.[1]

Item	CON	MEL7	MEL1	SEM	*P* =
M. haemolytica	0.87	0.70	0.96	0.17	0.53
BRSV	1.70	1.57	1.32	0.16	0.26
BHV-1	0.79	0.66	0.75	0.22	0.90
BVD-1	1.35	1.45	1.17	0.21	0.62
PI3	1.65	1.01	1.25	0.23	0.16

[1] Cattle were vaccinated against infectious bovine rhinotracheitis, bovine viral diarrhea complex, pneumonia, and *Mannheimia haemolytica* (Bovi-Shield Gold One Shot; Zoetis, Florham Park, NJ).

No treatment effects were detected ($P \geq 0.16$) for serum concentrations of antibodies against *M. haemolytica*, or serum titers against BRSV, BHV-1, BVD-1, and BVD-1. Time effects were detected ($P < 0.01$) for all titers but for BVD ($P = 0.40$), given that values increased ($P \leq 0.04$) when comparing samples collected prior to and after vaccination (0.68 vs. 0.89, SEM = 0.05 for *M. haemolytica*, 1.03 vs. 1.70, SEM = 0.11 for BRSV, 0.22 vs. 0.90, SEM = 0.15 for BHV-1, and 0.81 vs. 1.50, SEM = 0.14 for PI3. These results indicate that vaccination induced the expected antibody response but for BVD-1, whereas meloxicam administration did not impact these outcomes.

IMPLICATIONS

Oral meloxicam administration to beef cattle for 7 consecutive d at 1 mg/kg of BW prevented the decrease in DMI caused by LPS administration, but did not alleviate the resultant inflammatory and acute-phase protein reactions. Similarly, oral meloxicam administration to beef cattle at 1 mg/kg of BW upon vaccination against the BRD complex did not prevent the vaccine-induced decrease in DMI, did not alleviate the resultant inflammatory and acute-phase protein responses, and did not impact serum concentrations of antibodies against these pathogens. Hence, meloxicam administration failed to modulate the physiological challenges associated with LPS administration and vaccination in beef cattle.

LITERATURE CITED

Arthington, J. D., R. F. Cooke, T. D. Maddock, D. B. Araujo, P. Moriel, N. DiLorenzo, and G. C. Lamb. 2013. Effects of vaccination on the acute-phase protein response and measures of performance in growing beef calves. J. Anim. Sci. 91:1831-1837.

Carroll, J. A., and N. E. Forsberg. 2007. Influence of stress and nutrition on cattle immunity. Vet. Clin. Food. Anim. 23:105-149.

Carroll, J. A., Reuter, R.R., Chase Jr., C.C., Coleman, S.W., Riley, D.G., Spiers, D.E., Arthington, J.D. and Galyean, M.L. 2009. Profile of the bovine acute-phase response following an intravenous lipopolysaccharide challenge. Innate Immun. 15:81–89.

Confer, A. W., S. Ayalew, M. Montelongo, D. L. Step, J. H. Wray, R. D. Hansen, and R. J. Panciera. 2009. Immunity of cattle following vaccination with a *Mannheimia haemolytica* chimeric PlpE–LKT (SAC89) protein. Vaccine 27:1771–1776.

Delavaud, C., F. Bocquier, Y. Chilliard, D. H. Keisler, A. Gertler, and G. Kann. 2000. Plasma leptin determination in ruminants: Effect of nutritional status and body fatness on plasma leptin concentration assessed by a specific RIA in sheep. J. Endocrinol. 165:519–526.

Guarnieri Filho, T. A., R. F. Cooke, B. I. Cappellozza, M. M. Reis, R. S. Marques, and D. W. Bohnert. 2014. Effects of meloxicam administration on physiological and performance responses of transported feeder cattle. J. Anim. Sci. 92:4137-4144.

Marques, R. S., R. F. Cooke, B. I. Cappellozza, T. Guarnieri Filho, M. M. Reis, D. H. Keisler, and D. W. Bohnert. 2014. A vaccine-induced acute-phase reaction increases plasma leptin concentrations in beef cattle. J. Anim. Sci. 92(E-Suppl. 2):258.

Rice, J. A., L. Carrasco-Medina, D. C. Hodgins, and P. E. Shewen. 2007. *Mannheimia haemolytica* and bovine respiratory disease. Anim. Health Res. Rev. 8:117–128.

Van Engen, N. K., M. L. Stock, T. Engelken, R. C. Vann, L. W. Wulf, L. A. Karriker, W. D. Busby, J. Lakritz, A. J. Carpenter, B. J. Bradford, W. H. Hsu, C. Wang, and J. F. Coetzee. 2014. Impact of oral meloxicam on circulating physiological biomarkers of stress and inflammation in beef steers after long-distance transportation. J. Anim. Sci. 92:498–510.

Waggoner, J. W., C. A. Löest, J. L. Turner, C. P. Mathis, and D. M. Hallford. 2009. Effects of dietary protein and bacterial lipopolysaccharide infusion on nitrogen metabolism and hormonal responses of growing beef steers. J. Anim. Sci. 2009. 87:3656–3668.

Figure 1. Hay DMI, expressed as kg/d (1A) or % of BW (1B), of steers and heifers administered intravenous bacterial liposaccharide (**LPS**; 0.5 µg/kg of BW, *Escherichia coli* 0111:B4, Sigma-Aldrich, St. Louis, MO) on d 0 of the experiment. A day effect was detected ($P < 0.01$) for both variables. Values with different letters differ at $P < 0.05$.

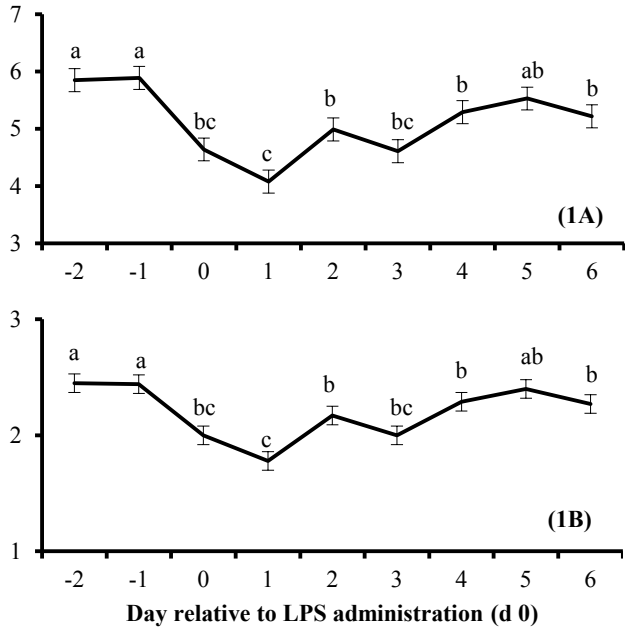

Figure 2. Hay DMI, expressed as kg/d (2A) or % of BW (2B), of steers and heifers vaccinated against infectious bovine rhinotracheitis, bovine viral diarrhea complex, pneumonia, and *Mannheimia haemolytica* (Bovi-Shield Gold One Shot; Zoetis, Florham Park, NJ) on d 7 of the experiment. A day effect was detected ($P < 0.01$) for both variables. Values with different letters differ at $P < 0.05$.