

## EFFECTS OF FLUNIXIN MEGGLUMINE ADMINISTRATION ON ACUTE-PHASE AND PERFORMANCE RESPONSES OF TRANSPORTED FEEDER CATTLE

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**ABSTRACT:** The objective of this experiment was to evaluate the effects of flunixin meglumine administration on physiological and performance responses of transported cattle during feedlot receiving. Forty-five Angus × Hereford steers were ranked by BW on d 0, and assigned to 1 of 3 treatments: 1) transport for 1,280 km in a commercial livestock trailer and administration of flunixin meglumine (1.1 mg/kg of BW; i.v.) at loading (d 0) and unloading (d 1; **FM**); 2) transport for 1,280 km in a commercial livestock trailer and administration of 0.9% saline (0.022 mL/kg of BW; i.v.) at loading (d 0) and unloading (d 1; **TRANS**), or 3) no transport and administration of 0.9% saline (0.022 mL/kg of BW; i.v.) concurrently with loading (d 0) and unloading (d 1) of FM and TRANS cohorts (**CON**). On d 1, steers were ranked by BW within each treatment and assigned to 15 feedlot pens. Full BW was recorded prior to (d -1 and 0) treatment application and at the end of experiment (d 28 and 29) for ADG calculation. Total DMI was evaluated daily from d 1 to 28. Blood samples were collected on d 0, 1, 4, 7, 10, 14, 21, and 28. Body weight shrink from d 0 to d 1 was reduced ( $P < 0.01$ ) and mean ADG was greater ( $P < 0.04$ ) in CON vs. FM and TRANS, but similar ( $P = 0.94$  and  $P = 0.69$ , respectively) between TRANS and FM. No treatment effects were detected on DMI, but CON had greater G:F vs. TRANS and FM ( $P < 0.08$ ). Mean plasma cortisol tended to be greater ( $P < 0.09$ ) in TRANS vs. FM and CON, but was similar ( $P = 0.87$ ) between CON and FM. Plasma NEFA were greater ( $P < 0.02$ ) for TRANS and FM vs. CON on d 1, and greater ( $P < 0.04$ ) for FM vs. TRANS and CON on d 4. Plasma ceruloplasmin concentrations were greater ( $P < 0.03$ ) for TRANS vs. CON on d 1, 4, and 7, greater ( $P < 0.05$ ) for TRANS vs. FM on d 4 and 7, and greater ( $P < 0.04$ ) for FM vs. CON on d 1 and 4. Plasma haptoglobin concentrations were greater ( $P < 0.01$ ) for TRANS vs. CON and FM on d 1 and 4, and greater ( $P < 0.05$ ) for FM vs. CON on d 1 and 4. In conclusion, flunixin meglumine reduced the cortisol and acute-phase protein responses elicited by road transport, but did not improve receiving performance of feeder cattle.

**Keywords:** Acute-phase proteins, cattle, flunixin meglumine, transport

### Introduction

Road transport is one of the most stressful events encountered by feeder cattle during their productive lives (Arthington et al., 2005). Upon long transportation periods and feedlot arrival, cattle experience inflammatory and acute-phase responses (Cooke et al., 2011) that often lead to

impaired health and productivity during feedlot receiving (Araujo et al., 2010). Accordingly, management strategies that lessen the magnitude of the acute-phase protein response during feedlot receiving have been shown to improve productivity of transported cattle (Arthington et al., 2008).

One alternative to reduce the acute-phase protein response elicited by road transport is to provide anti-inflammatory agents to cattle. As an example, feeder steers supplemented with linolenic acid had reduced acute-phase protein response and improved performance during feedlot receiving compared with non-supplemented cohorts (Cooke et al., 2011; Cappelozza et al., 2012). Another alternative includes administration of flunixin meglumine, a non-steroidal anti-inflammatory drug, when feeder cattle are processed for transport and feedlot arrival. Accordingly, Merrill et al. (2007) reported that flunixin meglumine administration prior to road transport alleviated transport-elicited inflammatory reactions in gestating beef cows. Based on this rationale, we hypothesized that administration of flunixin meglumine prior to transport and at feedlot entry alleviates the acute-phase protein response and improves performance of feeder cattle during feedlot receiving. Hence, the objective of this experiment was to evaluate the effects of flunixin meglumine administration on circulating concentrations of cortisol, NEFA, acute-phase proteins, and feedlot receiving performance of transported cattle.

### Materials and Methods

*Animals and diets.* Forty-five Angus × Hereford steers, weaned 35 d prior to the beginning of the experiment, were ranked by initial BW ( $228 \pm 3$  kg; initial age  $206 \pm 3$  d) and assigned to 1 of 3 treatments on d 0: 1) transport for 1,280 km (approximately 24 h) in a commercial livestock trailer and administration of flunixin meglumine (Banamine<sup>®</sup>; Merck Animal Health; Summit, NJ; 1.1 mg/kg of BW; i.v.) at loading (d 0) and unloading (d 1; **FM**); 2) transport for 1,280 km (approximately 24 h) in a commercial livestock trailer and administration of 0.9% saline (0.022 mL/kg of BW; i.v.) at loading (d 0) and unloading (d 1; **TRANS**), or 3) no transport and administration of 0.9% saline (0.022 mL/kg of BW; i.v.) concurrently with loading (d 0) and unloading (d 1) of FM and TRANS cohorts (**CON**). The flunixin meglumine dose used herein was based on the daily limit indicated by the manufacturer (2 injections of 1.1 mg/kg of BW within 24 h), whereas the CON treatment was included as a non-transport positive control for physiological and performance measurements.

From d -15 to 0, steers were maintained in a single drylot pen (50 × 100 m) and fed alfalfa-grass hay ad libitum and 2.3 kg/animal daily (DM basis) of a concentrate containing (as-fed basis) 84% cracked corn, 14% soybean meal, and 2% mineral mix. Steers assigned to FM and TRANS were transported at the same time and in the same double-deck commercial livestock trailer, while CON steers remained in the same drylot pen (50 × 100 m) with ad libitum access to alfalfa-grass hay and 2.3 kg/animal (DM basis) of the aforementioned concentrate. Immediately upon arrival of FM and TRANS cattle and treatment administration on d 1, steers were ranked by BW within each treatment and assigned to 15 feedlot pens (5 pens/treatment; 3 steers/pen; 7 × 15 m) for a 28-d feedlot receiving. During feedlot receiving, all pens were fed alfalfa-grass hay ad libitum and 2.3 kg/animal daily (DM basis) of the aforementioned corn-based concentrate, which was offered separately from hay at 0800 h. Water was offered for ad libitum consumption from d -15 to 28, except to FM and TRANS cattle during transport.

All cattle were vaccinated against clostridial diseases (Clostrishield 7; Novartis Animal Health; Bucyrus, KS) and bovine virus diarrhea complex (Virashield 6 + Somnus; Novartis Animal Health) at approximately 30 d of age. At weaning (d -35), cattle were vaccinated against clostridial diseases and *Mannheimia haemolytica* (One Shot Ultra 7; Pfizer Animal Health; New York, NY), infectious bovine rhinotracheitis, bovine viral diarrhea complex, and pneumonia (Bovi-Shield Gold 5 and TSV-2; Pfizer Animal Health), and administered an anthelmintic (Dectomax; Pfizer Animal Health). On d 0, 2 steers assigned to CON and 1 steer assigned to TRANS presented symptoms of pneumonia and required medication (0.1 mL/kg of BW of 300 PRO LA, Norbrook Inc.; Lenexa, KS); therefore, these steers were removed from the experiment. No other incidences of morbidity or mortality were observed from d 0 to d 28.

**Sampling.** Individual full BW was recorded and averaged over 2 consecutive days prior to treatment application (d -1 and 0) and at the end of the experiment (d 28 and 29) for ADG calculation. Individual BW was also collected on d 1, immediately after treatment application, to evaluate BW shrink as percentage change from the average BW recorded on d -1 and 0. Concentrate, hay, and total DMI were evaluated daily from d 1 to 28 from each pen by collecting and weighing orts 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. Hay, concentrate, and total daily DMI of each pen were divided by the number of animals within each pen, and expressed as kg per animal/d. Total BW gain and DMI of each pen from d 1 to 28 were used for feedlot receiving G:F calculation.

**Blood analysis.** Blood samples were collected on d 0 and 1 immediately before treatment application, and on d 4, 7, 10, 14, 21, and 28, via jugular venipuncture into commercial blood collection tubes (Vacutainer, 10 mL; Becton Dickinson, Franklin Lakes, NJ) with or without 158 USP units of freeze-dried sodium heparin for plasma and serum collection, respectively. Blood samples were collected prior to concentrate feeding, except for d 0 when

FM and TRANS cattle were transported after blood collection. All 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. Plasma concentrations of cortisol were determined in samples collected from d 0 to d 10 using a bovine-specific commercial ELISA kit (Endocrine Technologies Inc., Newark, CA). Plasma concentrations of ceruloplasmin and haptoglobin were determined in all samples according to colorimetric procedures previously described (Demetriou et al., 1974; Cooke and Arthington, 2012). Serum concentrations of NEFA were determined in samples collected from d 0 to d 10 using a colorimetric commercial kit (HR Series NEFA – 2; Wako Pure Chemical Industries Ltd. USA, Richmond, VA). The intra- and inter-assay CV were, respectively, 9.1 and 9.8% for cortisol, 6.7 and 7.3% for NEFA, 8.9 and 10.5% for ceruloplasmin, and 7.1 and 11.6% for haptoglobin.

**Statistical analysis.** Data were analyzed using animal as the experimental unit, given that treatments were individually administered to steers, with the PROC MIXED procedure of SAS (SAS Inst., Inc., Cary, NC) and Satterthwaite approximation to determine the denominator df for the tests of fixed effects. The model statement used for BW shrink from d 0 to d 1 and ADG contained the effects of treatment. Data were analyzed using animal(treatment × pen) as random variable. The model statement used for DMI and G:F contained the effects of treatment, as well as day and the treatment × day interaction for DMI only. Data were analyzed using pen(treatment) as the random variable. The model statement used for blood variables contained the effects of treatment, day, the treatment × day interaction, and values obtained on d 0 as covariate. Data were analyzed using animal(treatment × pen) as the random variable. The specified term for the repeated statements was day, pen(treatment) or animal(treatment × pen) as subject for DMI or blood variables, respectively, and the covariance structure utilized was based on the Akaike information criterion. Results are reported as least square means, as well as covariately adjusted least square means for blood variables, and were separated using PDIFF. Significance was set at  $P \leq 0.05$  and tendencies were determined if  $P > 0.05$  and  $\leq 0.10$ . Results are reported according to main effects if no interactions were significant, or according to the highest-order interaction detected.

## Results and Discussion

A treatment effect was detected ( $P < 0.01$ ) for BW shrink from d 0 to 1. As expected, BW shrink was greater ( $P < 0.01$ ) for both TRANS and FM compared with CON steers, but similar between TRANS and FM steers (Table 1). Supporting these findings, previous research from our group reported equivalent BW shrink rates in feeder cattle exposed to the same transportation schedule adopted herein (Marques et al., 2012). A treatment effect was also detected ( $P < 0.01$ ) for ADG (Table 1). Steers assigned to CON had greater ADG compared with TRANS ( $P = 0.04$ ) and FM ( $P = 0.01$ ) cohorts, whereas ADG was similar between ( $P = 0.69$ ) TRANS and FM steers. However, treatment effects

detected on ADG were not sufficient to impact ( $P = 0.37$ ) cattle BW at the end of the experimental period (Table 1). No treatment effects were detected ( $P \geq 0.94$ ) on hay, concentrate, and total DMI (Table 1). Nevertheless, a treatment effect was detected ( $P = 0.02$ ) for G:F because CON had greater G:F compared with FM ( $P = 0.02$ ) and tended to have greater G:F compared with TRANS steers ( $P = 0.08$ ), whereas G:F was similar ( $P = 0.68$ ) between TRANS and FM steers (Table 1). Hence, FM steers experienced a similar decrease in feedlot receiving performance compared with TRANS cohorts, indicating that flunixin meglumine administration failed to reduce the performance losses caused by road transport.

Table 1. Feedlot receiving performance (28 d) of steers transported for 1,280 km and administered flunixin meglumine (FM) or 0.9% saline (TRANS) at loading (d 0) and upon arrival (d 1), or non-transported steers administered 0.9% saline (CON)<sup>1</sup>.

Item	CON	FM	TRANS	SEM	$P =$
BW, kg					
Initial	229	229	227	5	0.93
Final	268	257	255	6	0.27
Shrink, %	0.46 <sup>a</sup>	8.85 <sup>b</sup>	8.89 <sup>b</sup>	0.43	< 0.01
ADG, kg/d	1.18 <sup>a</sup>	0.99 <sup>b</sup>	1.02 <sup>b</sup>	0.05	0.04
DMI, kg/d					
Hay	4.88	4.86	4.95	0.18	0.94
Concentrate	2.21	2.22	2.21	0.05	0.98
Total	7.09	7.08	7.16	0.21	0.96
G:F, g/kg	171 <sup>a</sup>	146 <sup>b</sup>	149 <sup>ab</sup>	6	0.02

<sup>1</sup>Within rows, values with different superscripts differ ( $P < 0.05$ ).

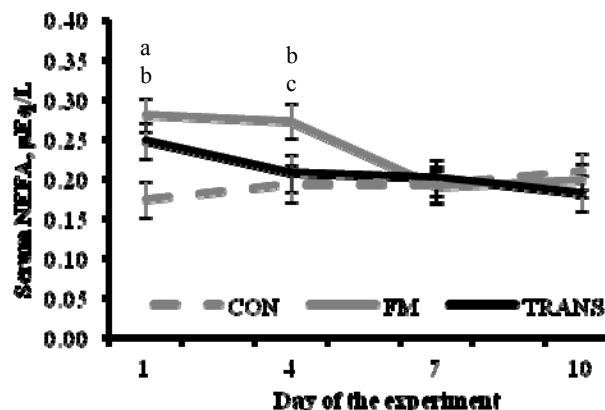


Figure 1. Serum NEFA concentration in steers transported for 1,280 km and administered flunixin meglumine (FM) or 0.9% saline (TRANS) at loading (d 0) and upon arrival (d 1), or non-transported steers administered 0.9% saline (CON). A treatment  $\times$  day interaction was detected ( $P = 0.03$ ). Within days, letters indicate the following treatment differences; a = TRANS vs. CON ( $P = 0.02$ ), b = FM vs. CON ( $P \leq 0.01$ ), c = TRANS vs. FM ( $P = 0.04$ ).

During feedlot receiving, mean plasma cortisol concentrations tended to be greater in TRANS vs. FM ( $P = 0.09$ ) and CON steers ( $P = 0.08$ ; 41.3, 35.9, and 35.4 ng/mL, respectively; SEM = 2.2), but was similar ( $P = 0.87$ ) between CON and FM steers (treatment effect;  $P = 0.09$ ). These results indicate that flunixin meglumine prevented the increase in circulating cortisol concentrations

elicited by long road transport (Cooke et al., 2011; Marques et al., 2012). Serum NEFA concentrations were greater ( $P < 0.02$ ) for TRANS and FM vs. CON on d 1, and greater ( $P < 0.04$ ) for FM vs. TRANS and CON on d 4 (Figure 1; treatment  $\times$  day interaction,  $P = 0.03$ ). These results corroborate that road transport stimulate fat tissue mobilization and increase circulating NEFA concentrations in cattle (Marques et al., 2012). In addition, flunixin meglumine administration further increased this response, given that serum NEFA concentrations in FM steers were still elevated on d 4 relative to CON and TRANS cohorts. The reason for the increased serum NEFA concentrations in FM steers compared to CON and TRANS cohorts is still unknown, given that the effects of flunixin meglumine on lipid metabolism in beef cattle still need investigation.

A treatment  $\times$  day interaction was detected for plasma haptoglobin ( $P < 0.01$ ; Figure 2), whereas a tendency ( $P = 0.10$ ; Figure 3) for the same interaction was detected for plasma ceruloplasmin. Plasma haptoglobin concentrations were greater ( $P < 0.01$ ) for TRANS vs. CON and FM on d 1 and 4, and greater ( $P < 0.05$ ) for FM vs. CON on d 1 and 4. Plasma ceruloplasmin concentrations were greater ( $P < 0.03$ ) for TRANS vs. CON on d 1, 4, and 7, greater ( $P < 0.05$ ) for TRANS vs. FM on d 4 and 7, and greater ( $P < 0.04$ ) for FM vs. CON on d 1 and 4. Previous research from our group also documented an acute-phase protein reaction in beef cattle upon a similar 24-h road transport (Araujo et al., 2010) that impaired feedlot receiving performance (Marques et al., 2012). Accordingly, circulating concentrations of acute-phase proteins in transported feeder cattle have been negatively associated with feedlot receiving performance (Araujo et al., 2010), and such outcome can be attributed to altered basal metabolism, increased tissue catabolism, and reduced feed efficiency during an acute-phase response (Johnson, 1997).

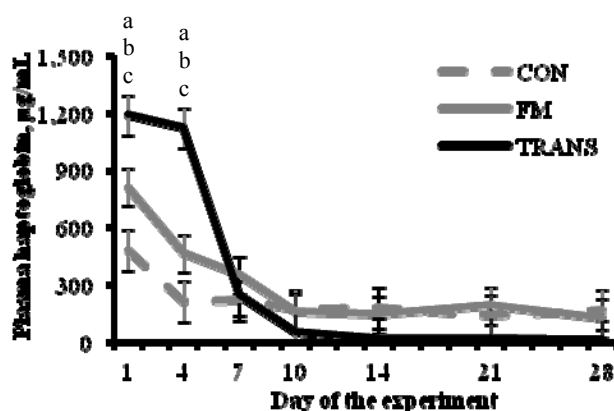


Figure 2. Plasma haptoglobin concentrations in steers transported for 1,280 km and administered flunixin meglumine (FM) or 0.9% saline (TRANS) at loading (d 0) and upon arrival (d 1), or non-transported steers administered 0.9% saline (CON). A treatment  $\times$  day interaction was detected ( $P < 0.01$ ). Within days, letters indicate the following treatment differences; a = TRANS vs. CON ( $P < 0.01$ ), b = FM vs. CON ( $P \leq 0.05$ ), c = TRANS vs. FM ( $P < 0.01$ ).

Supporting our hypothesis, flunixin meglumine administration alleviated the acute-phase protein response elicited by transport. This outcome can be attributed to the

decreased plasma cortisol concentrations during feedlot receiving in FM steers, given that cortisol stimulates the bovine acute-phase protein reaction (Cooke and Bohnert, 2011). In addition, flunixin meglumine inhibits cyclooxygenase, an enzyme that regulates synthesis of inflammatory eicosanoids associated with the acute-phase response such as PGE<sub>2</sub> (Odensvik, 1995). However, in the present experiment, FM and TRANS steers had a similar decrease in feedlot receiving performance compared to that of CON cohorts, indicating that flunixin meglumine administration reduced the acute-phase protein response but did not alleviate the performance losses caused by road transport. Still, the acute-phase reaction during feedlot receiving may negatively impact performance (Cooke et al., 2009) and increases the incidence of respiratory diseases in overtly healthy cattle. Therefore, the development of management strategies that prevent or alleviate the acute-phase response during feedlot receiving, including flunixin meglumine administration, is warranted for optimal performance, health, and efficiency parameters in feedlot systems. Perhaps a greater dosage of flunixin meglumine, such as 2.2 mg/kg of BW at loading and upon feedlot arrival, is necessary to further reduce the transport-elicited acute-phase protein response and enhance performance parameters.

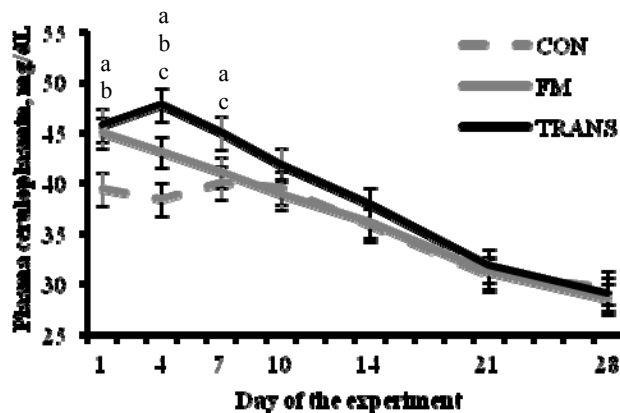


Figure 3. Plasma ceruloplasmin concentrations in steers transported for 1,280 km and administered flunixin meglumine (FM) or 0.9% saline (TRANS) at loading (d 0) and upon arrival (d 1), or non-transported steers administered 0.9% saline (CON). A tendency for treatment  $\times$  day interaction was detected ( $P = 0.10$ ). Within days, letters indicate the following treatment differences; a = TRANS vs. CON ( $P < 0.03$ ), b = FM vs. CON ( $P \leq 0.04$ ), c = TRANS vs. FM ( $P \leq 0.05$ ).

### Implications

Flunixin meglumine administration to feeder steers prior to road transport and at feedlot arrival prevented the increase in circulating cortisol and alleviated the acute-phase protein response elicited by transport, but did not improve feedlot receiving performance. Hence, flunixin meglumine appears to be a viable alternative to reduce neuroendocrine and acute-phase protein responses during feedlot receiving. Therefore, additional research is warranted to further assess the benefits of flunixin meglumine administration, including greater dosages, on health and productive responses of transported cattle.

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