

## GROWING BEEF STEERS DO NOT REQUIRE SUPPLEMENTAL DIETARY METHIONINE DURING AN ENDOTOXIN CHALLENGE

J.W. Waggoner\*, C. A. Löest, T.M. Thelen, M.K. Petersen, D.M. Hallford, M.D. Remmenga, and C.P. Mathis  
New Mexico State University, Las Cruces, NM

**ABSTRACT:** Acute phase response protein synthesis by the immune system during gram(-) bacterial infection increases metabolic amino acid demand in non-ruminants and limits efficiency of N utilization for growth in newly received feedlot calves. Therefore, we hypothesized that steers would have increased requirements for amino acids, such as Met, during an endotoxin challenge. This study evaluated the effects of supplemental dietary Met on N balance and blood metabolites in Angus-cross steers ( $n = 20$ ; BW =  $262 \pm 6.3$  kg) exposed to an endotoxin (LPS; gram(-) bacterial lipopolysaccharide, Sigma). Treatments ( $2 \times 2$  factorial) were LPS infusion and dietary Met addition (0 vs. 14 g/d rumen-protected; Smartamine M, Adisseo). Steers were adapted to a corn-based diet (DM intake = 1.4% of BW) and supplemental Met for 14 d, and were then infused (1 mL/min via i.v. catheter) with LPS on d 1 (LPS1; 2  $\mu\text{g/kg}$  BW) and 3 (LPS2; 1  $\mu\text{g/kg}$  BW) of a 5-d N balance collection period. Blood was collected prior to LPS infusions and every 2 h thereafter for 12 h. Serum cortisol peaked 4 h following LPS1, and remained elevated ( $P < 0.01$ ) for 12 h. Cortisol peaked 2 h after LPS2 and remained elevated ( $P < 0.01$ ) for 6 h (LPS  $\times$  hour interaction,  $P < 0.01$ ). Plasma Met was greater ( $P < 0.01$ ) for Met-supplemented steers prior to LPS administration, but declined ( $P < 0.01$ ) for steers infused with LPS such that plasma Met concentrations at 4 to 10 h post-LPS were not different ( $P > 0.09$ ) from both non-stressed and LPS-challenged steers that received no supplemental Met (LPS  $\times$  Met  $\times$  hour interaction,  $P < 0.01$ ). Infusion of LPS increased ( $P < 0.05$ ) urinary N excretion and decreased ( $P < 0.01$ ) N retention resulting in a negative N balance for LPS-challenged steers. Supplementation of Met did not affect ( $P = 0.49$ ) N retention, and the absence of an LPS  $\times$  Met interaction ( $P = 0.25$ ) for N retention indicates that supplemental dietary Met does not improve the efficiency of N utilization for growing beef steers during an endotoxin challenge.

**Key Words:** Methionine, Endotoxin challenge, Steers.

### Introduction

Morbidity and disease in feedlot cattle negatively impact performance and profitability (Waggoner et al., 2006). Gram(-) bacterial infection has been implicated in the pathology of many diseases, including shipping fever and pneumonia (Cullor, 1992). The inflammatory response to infection occurs due to recognition of lipopolysaccharide (LPS) within the bacterial cell wall. Administration of purified LPS stimulates the immune system and mimics symptoms of bacterial infection (Steiger et al., 1999).

During immunological stress, AA are directed away from tissue deposition to support the immune response. This occurs due to increased demands for AA to synthesize acute phase response proteins and glucogenic precursors (Le Floch et al., 2003). Grimble and Grimble (1998) suggested that insufficient supplies of sulfur AA (Met and Cys) limit acute phase response protein synthesis and contribute to increased N excretion during sepsis in humans. Furthermore, Met is a limiting AA in growing cattle (Greenwood and Titgemeyer, 2000). Therefore, it was hypothesized that Met requirements would increase for endotoxin-challenged steers.

The objective of this study was to evaluate effects of supplemental dietary Met on N balance and blood metabolites of growing steers during an endotoxin challenge.

### Materials and Methods

*Animals, Facilities, and Diet.* Procedures were approved by the New Mexico State University Institutional Animal Care and Use Committee. Twenty Angus crossbred steers ( $262 \pm 6.3$  kg initial BW) were individually housed in tie stalls of a metabolism building with evaporative cooling and continuous lighting. Steers had free access to fresh water and were limit-fed a corn-based diet (Table 1) at 1.4% of BW (DM basis). The diet was divided into two equal portions and fed twice daily at 0700 and 1900.

*Design and Treatments.* The experiment was a randomized block design and lasted 20 d, which allowed 14 d for adaptation to diets and dietary treatments, and 5 d for collections. On d 14, indwelling jugular catheters (J-457A; Jorgensen Laboratories, Loveland, CO) were inserted. Treatments, in a  $2 \times 2$  factorial arrangement, were two levels of LPS infusion and two levels of dietary Met supplementation. Dietary Met levels included 0 (-MET) vs. 14 g/d (+MET) rumen-protected Met (Smartamine M, Adisseo, Alpharetta, GA). Rumen-protected Met was divided into two portions and mixed with each portion of the daily diet before feeding. Levels of LPS included no LPS (-LPS) vs. a prolonged low dose of LPS (+LPS; Steiger et al., 1999). At 3 h after feeding on d 1 of the collection period, LPS (*E. coli* O55:B55; Sigma Chem. Co., St. Louis, MO) dissolved in 100 mL of sterile saline was infused (1 mL/min via i.v. catheter) at 2  $\mu\text{g}$  LPS/kg BW. A second dose of LPS was administered at the same time on d 3 of the collection period. However, the dose was reduced to 1  $\mu\text{g}$  LPS/kg BW (dissolved in 50 mL of saline) due to the death of a steer on d 1. An equal volume of sterile saline was administered at a similar rate to -LPS steers.

Table 1. Diet composition

Item	% of DM
<i>Ingredient</i>	
Cracked corn	35.0
Alfalfa hay	25.0
Corn silage	20.0
Sorghum, sudan hay	12.4
Molasses	4.0
Supplement <sup>a</sup>	3.6
<i>Nutrient</i>	
CP	12.8
Ca	0.70
P	0.29

<sup>a</sup> Composition (% of supplement DM): Soybean meal (33), Sodium caseinate (28), Urea (17), Salt (8), Limestone (8), Dicalcium P (3), Vitamin E (0.56), Rumensin (0.53), Sodium selenite (0.42), Zinc sulfate (0.25), Vitamin A (0.24), Copper sulfate (0.10).

**Collections.** Dietary samples, total feed refusals (if any), and fecal and urinary excreta from each steer were collected daily during the 5-d collection period. Urine was collected via vacuum pouches into vessels containing 600 mL of 3 N HCl (to prevent NH<sub>3</sub> loss). Total fecal and urinary output was weighed, and representative samples of feces (10%) and urine (1%) were frozen for later analysis. Steers were fed Cr-EDTA containing (400 g) and Yb-labeled (120 g) diet on d 1 of the collection period. Fecal grab samples were obtained every 24 h for 5 d. Rectal temperatures were measured (Cooper TM99A digital thermometer, Cooper Atkins Corp., Middlefield, CT), and blood samples were collected via catheters prior to LPS infusion and every 2 h for 12 h post-LPS infusion on d 1 and 3 of the collection period. Blood samples were collected into vacuum tubes (Corvac serum separator and Monoject 15% EDTA, Kendall, Ontario, CA). Blood samples for serum were allowed to coagulate at room temperature for 30 min, whereas samples for plasma were immediately placed on ice. All samples were centrifuged at 1,500 × g for 20 min at 10°C and then frozen.

**Sample Analysis.** Diet, feed refusals, and fecal samples were dried at 55°C in a forced air oven, and ground to pass a 2-mm screen. Samples were analyzed for DM (105°C for 24 h) and N by total combustion (Leco FP-528, Leco Corp., St. Joseph, MI). Urine samples were also analyzed for N. Fecal grab samples were analyzed for Cr and Yb via inductively coupled plasma (Optima 4300; Perkin Elmer, Wellesley, MA). Liquid (Cr) and solid (Yb) passage rates (%/h) were determined from the slope of the natural log of Cr (24 to 96 h) and Yb (48 to 120 h) concentrations regressed against hour.

Serum samples were analyzed for cortisol by RIA using components of commercially available kit validated for cattle (Kiyama et al., 2004). Tumor necrosis factor alpha (TNF $\alpha$ ) concentrations were determined via double antibody RIA in serum samples obtained prior to LPS infusion and at 2, 4, and 6 h post-LPS on d 1 and 3 of the collection period. Antisera to bovine TNF $\alpha$  was obtained from Serotec Inc. (Raleigh, NC). Recombinant bovine TNF $\alpha$  (R & D Systems, Minneapolis, MN) was used as the standard and to prepare <sup>125</sup>I-b-TNF $\alpha$  (chloramine T method).

Assay sensitivity (90% displacement) was 0.4 ng/tube and addition of increasing amounts of bovine serum resulted in TNF $\alpha$  values that paralleled the standard curve. Within and between assay CV were less than 15% for cortisol and TNF $\alpha$ . Plasma AA concentrations were determined via gas chromatography (Varian CP-3800, Varian, Walnut Creek, CA) using a commercially available kit (EZ:FAAST; Phenomenex, Torrance, CA).

**Statistics.** All data were analyzed using the Mixed procedure of SAS (SAS Inst. Inc., Cary, NC). The model included effects of Met, LPS, and Met × LPS for all dietary measures. Rectal temperature, serum cortisol and TNF $\alpha$ , and plasma AA were analyzed as repeated measures (covariance structure = autoregressive order one). The model included all possible combinations of Met, LPS, hour, and day. Effects of day were evaluated for serum cortisol and TNF $\alpha$  only, to characterize LPS dose effects between d 1 and 3. Data are presented as least squares means with differences considered significant at  $P < 0.05$ .

## Results

An LPS × hour × day interaction ( $P < 0.05$ ) was observed for serum concentrations of cortisol (Figure 1) and TNF $\alpha$  (Figure 2). Serum cortisol peaked 4 h following LPS infusion on d 1, and remained elevated ( $P < 0.01$ ) for 12 h; cortisol peaked 2 h after LPS infusion on d 2 and remained elevated ( $P < 0.01$ ) for 6 h. Serum TNF $\alpha$  peaked 2 h following LPS infusion, but peak concentrations were greater ( $P < 0.01$ ) on d 1 (57.2 ng/mL) than d 3 (6.5 ng/mL). Supplemental Met reduced serum cortisol in +LPS steers, but not -LPS steers (LPS × Met interaction,  $P < 0.05$ ). Rectal temperature (Table 2) was elevated ( $P < 0.05$ ) in response to LPS, but not affected by Met addition.

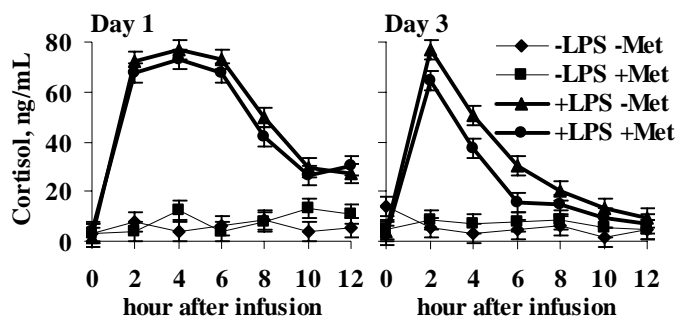


Figure 1. Serum cortisol concentrations in response to Met supplementation and endotoxin challenge in steers. Effects of LPS × hour × day ( $P < 0.01$ ), and LPS × hour ( $P < 0.01$ ).

No LPS × Met interactions ( $P = 0.25$  to  $0.99$ ) were observed for dietary DM and N intake, feces, and urine, or passage rates. Also, DM intake and fecal DM were not affected ( $P = 0.08$  to  $0.95$ ) by LPS and Met (Table 2). However, DM digested decreased ( $P < 0.05$ ) in response to LPS, likely due to a tendency ( $P = 0.13$ ) for lower DM intake in +LPS steers. Infusion of LPS did not affect ( $P = 0.23$  to  $0.82$ ) N intake, fecal N excretion, and N digested. However, LPS increased ( $P < 0.05$ ) urinary N excretion and decreased ( $P < 0.01$ ) N retention resulting in a negative N balance for +LPS steers. Supplementation of Met increased

( $P < 0.05$ ) fecal N excretion of steers, but did not affect ( $P = 0.42$  to  $0.99$ ) N intake and urinary N excretion. Supplemental Met did not alter ( $P = 0.42$ ) N digested and N retained regardless of increased fecal N excretion. Liquid (Cr) and solid (Yb) passage were decreased ( $P < 0.01$ ) by LPS, but increased ( $P < 0.05$ ) in response to Met supplementation.

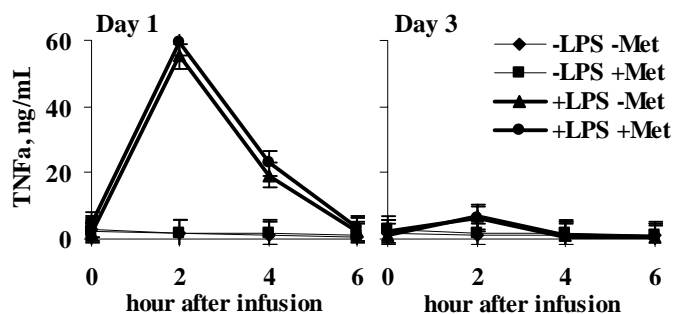


Figure 2. Serum tumor necrosis factor alpha (TNF $\alpha$ ) concentrations in response to Met supplementation and endotoxin challenge in steers. Effects of LPS  $\times$  hour  $\times$  day ( $P < 0.01$ ), and LPS  $\times$  hour ( $P < 0.01$ ).

Plasma Met (Figure 3) was greater ( $P < 0.01$ ) for +Met steers prior to LPS infusion, but declined ( $P < 0.01$ ) for +LPS steers such that their plasma Met at 4 to 10 h post-LPS were not different ( $P > 0.09$ ) from non-supplemented steers (LPS  $\times$  Met  $\times$  hour interaction,  $P < 0.01$ ). An LPS  $\times$  hour interaction ( $P < 0.01$ ) occurred for plasma concentrations of Lys, Leu, Ile, Phe, Trp, and total nonessential AA (NEAA). Plasma concentrations of Lys decreased ( $P < 0.05$ ) at h 4, 8, and 12 after LPS. Plasma concentrations decreased from 4 to 8 h for Leu ( $P < 0.05$ ), from 2 to 12 h for Ile ( $P < 0.05$ ), from 4 to 10 h for Phe ( $P < 0.05$ ), and from 2 to 12 h for Trp ( $P < 0.01$ ). Total NEAA decreased ( $P < 0.01$ ) from 4 to 12 h post-LPS. The pattern of decline for total NEAA is due to LPS  $\times$  hour interactions ( $P < 0.01$ ) for plasma concentrations of Gly, Ser, Pro, and Asn (data not shown). Infusion of LPS decreased ( $P < 0.01$ ) plasma concentrations of Val (154.4 vs. 137.2  $\mu$ M) and Thr (59.0 vs. 40.8  $\mu$ M). Plasma His concentrations were not affected by LPS and Met.

## Discussion

Observed increases in serum cortisol, and TNF $\alpha$  are indicative of the level of immunostimulation invoked by infusion of LPS. Cortisol increased as a result of stimulation of the hypothalamic-pituitary-adrenal axis. The rise in TNF $\alpha$ , a cytokine, is attributed to local stimulation of activated immune cells. Cytokines stimulate hepatic synthesis of acute phase response proteins, which increases metabolic AA demand (Le Floc' h et al., 2004).

The increase in N excretion following LPS likely arises due to mobilization of tissue protein to meet the AA demands of the activated immune system. During immunological stress, AA are partitioned away from tissue accretion to support the immune response. Reeds and Jahoor (2001) speculated that increased N excretion in humans during sepsis occurred due to imbalances between

the supply of AA from mobilized tissue protein and the AA composition of acute phase response proteins. These imbalances result in mobilization of excess tissue protein which is catabolized and excreted as N. The absence of an interaction between LPS and rumen protected Met for N retention indicates that LPS-challenged steers did not exhibit increased requirements for Met and that Met was likely not limiting for steers exposed to LPS. The decline in plasma Met of Met-supplemented steers following LPS, and lack of a similar decline in non-supplemented steers suggests, that the Met provided was either utilized by the immune system or catabolized by the liver.

Acute phase response proteins are primarily composed of Phe, Trp, Lys, Cys, and Ser in humans (Reeds and Jahoor, 2001). Therefore the observed decline in plasma concentrations of Phe, Trp, Lys, Leu, Ile, and NEAA may partially be due to increased acute phase response protein synthesis. However the decline in plasma concentrations may also be indicative of increased demand for glucogenic precursors.

## Implications

These findings imply that supplemental dietary methionine does not alleviate the negative effects of bacterial lipopolysaccharide on nitrogen utilization, and that metabolic demands for other amino acids may increase following exposure to bacterial lipopolysaccharide.

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Table 2. Effects of Met supplementation and endotoxin challenge on rectal temperature and dietary intakes, feces, urinary N, and passage rates of growing beef steers

Item	Treatments <sup>a</sup>				SEM <sup>b</sup>	<i>P</i> value		
	-LPS		+LPS			LPS	Met	LPS × Met
	-MET	+MET	-MET	+MET				
n	5	5	5	4				
Rectal Temp, °C	38.7	38.7	39.0	38.9	0.10	0.03	0.90	0.77
DM, g/d								
Intake	3755	3755	3474	3494	160	0.13	0.95	0.95
Fecal	993	1141	971	1087	68.2	0.60	0.08	0.82
Digested	2762	2614	2503	2408	102	0.04	0.27	0.81
N, g/d								
Intake	78.2	78.2	73.2	73.3	4.98	0.36	0.99	0.99
Fecal	26.1	30.9	27.8	30.0	1.36	0.82	0.03	0.38
Digested	52.0	47.2	45.5	43.3	4.00	0.23	0.42	0.75
Urinary	30.8	32.3	50.4	47.2	5.81	0.01	0.88	0.70
Retained	21.2	14.9	-5.0	-3.9	2.96	< 0.01	0.42	0.25
Passage, %/h								
Cr	4.81	5.29	2.88	3.85	0.30	< 0.01	0.03	0.44
Yb	2.49	2.88	1.38	2.08	0.21	< 0.01	0.02	0.47

<sup>a</sup> Infusion (i.v.) of sterile saline (-LPS) vs. lipopolysaccharide (+LPS), and supplementation of 0 g/d (-MET) vs. 14 g/d (+MET) of rumen protected Met.

<sup>b</sup> Standard error of the mean for 5 steers per treatment.

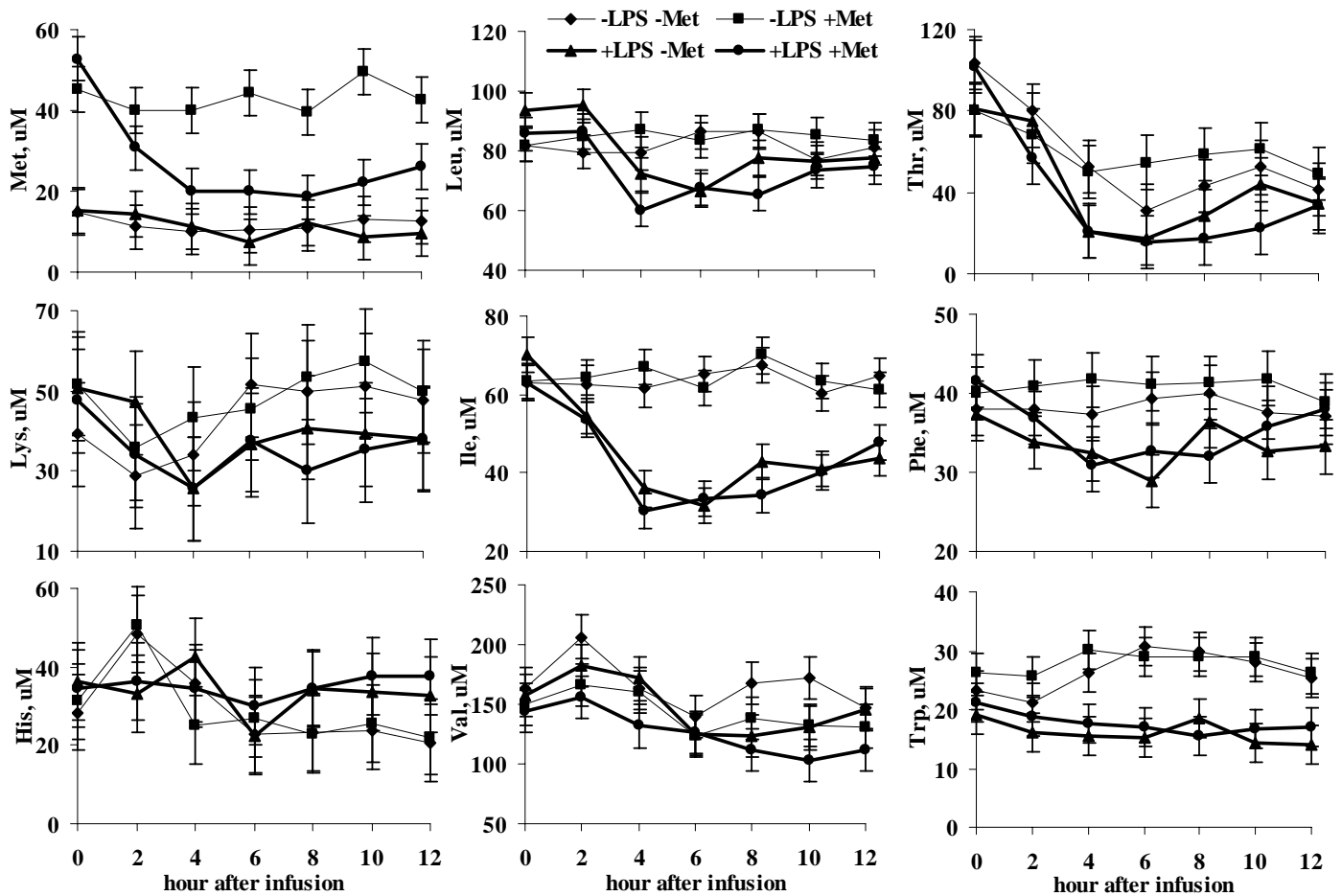


Figure 3. Plasma amino acid concentrations in response to Met supplementation and endotoxin challenge in steers. Effects of LPS × Met × hour ( $P < 0.01$ ) for plasma Met, LPS × hour ( $P < 0.01$ ) for plasma Lys, Leu, Ile, Phe, and Trp, and LPS ( $P < 0.01$ ) for Val and Thr.