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## IMMUNE RESPONSE IN FEEDER CATTLE FED DIFFERENT SOURCES OF DIETARY LIPID<sup>1</sup>

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### Summary

Two studies were conducted utilizing crossbred beef steers to evaluate immune response following endotoxin challenge. In Trial 1 steers (n = 20; 688 lb BW) were fed diets containing rolled full-fat soybeans (SOY) or tallow (TALLOW). In Trial 2, steers (n=18; 780 lb BW) were fed diets containing TALLOW, flaxseed (FLAX), or a micro-algae (ALGAE) top-dressed to the TALLOW diet. Both FLAX and ALGAE were sources of omega-3 polyunsaturated fatty acids. In both trials, diets were fed for a 14-day acclimation period prior to intravenous injection of a bacterial lipopolysaccharide(LPS) endotoxin. Injection of LPS in Trial 1 resulted in higher rectal temperatures for animals fed TALLOW compared to those fed SOY. In contrast, plasma concentrations of tumor necrosis factor- $\alpha$  (TNF) were higher for animals fed SOY. Haptoglobin and fibrinogen increased and total white blood cell count decreased in response to LPS, but these measures were not different ( $P>0.1$ ) between SOY and TALLOW. In Trial 2, rectal temperature was higher for TALLOW ( $P<0.05$ ) than for FLAX at 3, 4, 5, and 6 hours after the initial injection of LPS. In addition, rectal temperature for TALLOW was higher ( $P=0.05$ ) at hour 4 when compared to ALGAE and tended ( $P=0.1$ ) to be higher at hour 5. Serum haptoglobin concentration at 24 hours was higher ( $P<0.05$ ) for animals fed ALGAE

than those fed FLAX or TALLOW. Haptoglobin and fibrinogen concentrations increased at 24 hours after injection with LPS, but were not different at other times among treatments in either trial. Results show that source and type of dietary fatty acid may impact immune response in cattle.

(Key Words: Endotoxin Challenge, Immune Modulation, Lipids.)

### Introduction

Undifferentiated Bovine Respiratory Disease (BRD) is common among post-weaning cattle. Losses attributable to this disease complex exceed \$800 million annually. Gram-negative bacteria *Pasturella multocida* and *Pasturella haemolytica* are the most problematic pathogens involved in BRD. The animal's reaction to parts of the bacterial cell wall can lead to irreversible lung damage, resulting in reduced future productivity and disease resistance, expensive treatment costs, and even death. A disproportional, exaggerated inflammatory and immune response is the major cause of lung damage. Consequently, moderating the inflammatory response is of much interest. Studies with omega-3 polyunsaturated fatty acids have suggested that they have anti-inflammatory immunomodulatory effects in several species. Our objective was to evaluate responses to an endotoxin challenge in cattle fed diets with

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and without supplemental sources of omega-3 polyunsaturated fatty acids.

### Experimental Procedures

**Trial 1.** Twenty crossbred beef steers (688 lb BW) were stratified by weight and randomly assigned, within strata, to one of two treatments. Dietary treatments (Table 1) were TALLOW, a standard corn and soybean meal based diet with tallow as the added lipid source, and SOY, where soybean meal and tallow were replaced with rolled full-fat soybeans. Diets were fed once daily. Steers were fed and housed in individual pens with drinking water available at all times. They received equal levels of protein, vitamins, and minerals, and were fed their respective treatments for a 14-day acclimation period. On day 14, steers ( $n = 16$ ) were fitted with jugular catheters and injected intravenously with bacterial endotoxin ( $0.09 \mu\text{g}/\text{lb BW}$  *E. coli* 055:B55 lipopolysaccharide; Sigma-Chemical Company, St. Louis, MO). Two steers from each diet were injected with saline to establish baseline blood parameters and temperature readings. Blood samples and rectal temperatures were obtained immediately before (at 0 hours), and at 2, 3, 4, 5, and 24 hours after LPS challenge. All blood samples were analyzed for concentrations of tumor necrosis factor alpha (TNF), fibrinogen, haptoglobin, and total white blood cell count.

**Trial 2.** Eighteen crossbred beef steers (780 lb BW) were stratified by weight and randomly assigned, within strata, to one of three treatments. Dietary treatments (Table 2) were: TALLOW, FLAX, where a portion of the soybean meal and corn were replaced with ground flaxseed, and ALGAE, in which the TALLOW diet was top-dressed daily with an algae fermentation product containing a high proportion of docosahexanoic acid. The ALGAE was top-dressed to provide docosahexanoic acid at 10 g/animal daily.

Both FLAX and ALGAE are sources of omega-3 fatty acids. Steers were housed under the same conditions as in Trial 1 and were fed their respective dietary treatments once daily for a 14-day acclimation period. On day 14, steers were fitted with jugular catheters and injected intravenously with bacterial endotoxin ( $0.09 \mu\text{g}/\text{lb BW}$  *E. coli* 055:B55 lipopolysaccharide). Blood samples and rectal temperatures were obtained immediately before (0 hour), and at 1, 2, 3, 4, 6, and 24 hours following LPS challenge. Blood samples were analyzed as in Trial 1.

### Results and Discussion

**Trial 1.** Injection of the bacterial lipopolysaccharide (LPS) resulted in dramatic increases in rectal temperature, TNF, haptoglobin, and reductions in white blood cell count (Tables 3 and 4). Rapid changes in rectal temperatures, as well as changes in white blood cell count, TNF, and haptoglobin, relative to saline-injected animals, indicate that the model was effective in emulating a disease challenge.

After injection of LPS, rectal temperatures at hour 3 were greater ( $P < 0.03$ ) for TALLOW animals than for SOY and tended ( $P = 0.08$ ) to be higher at hour 4.

After injection of LPS, TNF was greater ( $P < 0.01$ ) for SOY than TALLOW at hour 2, but had returned to baseline by hour 5. White blood cell numbers were significantly reduced by 1 hour after injection of LPS, and by hour 24 the reduction was stabilized and white blood cell numbers returned to baseline. However, no differences between dietary treatments were observed. Changes in acute phase proteins (fibrinogen and haptoglobin) were not apparent other than in the sample obtained 24 hours after injection of LPS. That indicated a delayed response to the endotoxin challenge by these acute phase proteins. Fibrinogen and haptoglobin increased in response to LPS, but were not different for

SOY and TALLOW ( $P>0.1$ ). This study indicates that manipulating dietary lipid source may alter immune and inflammatory responses in challenged cattle.

**Trial 2.** As in Trial 1, injection of cattle with LPS resulted in elevated rectal temperature, TNF, acute phase proteins, and reduced white blood cell count (Tables 3 and 5).

Changes in rectal temperature following LPS administration were highest for TALLOW, intermediate for ALGAE, and lowest for FLAX. Rectal temperatures for animals fed TALLOW were higher ( $P<0.05$ ) at hours 3, 4, 5, and 6, compared to those fed FLAX. Furthermore, rectal temperature for TALLOW was higher ( $P=0.05$ ) at hour 4 and tended to be higher ( $P=0.10$ ) at hour 5 than for animals fed ALGAE. ALGAE led to higher rectal temperatures than FLAX at hours 3 and 6 ( $P<0.09$ ).

Rapid increases in TNF production were evident immediately after LPS injection, but returned to baseline by hour 4. Peripheral blood white blood cell count fell immediately after LPS injection on both days 14 and 17, and returned to pre-LPS injection levels by hour 24. This observation is in agreement with Trial 1. However, dietary treatments did not cause differences in white blood cell numbers after LPS injection.

Changes in acute phase proteins, fibrinogen and haptoglobin, were not evident other than in the sample taken 24 hours after LPS injection, indicating a delayed response to endotoxin challenge. Serum haptoglobin concentration for ALGAE was higher ( $P<0.05$ ) at hour 24 when compared to TALLOW and FLAX.

These studies indicated that the source of dietary lipids may have a significant impact on immune response in cattle.

**Table 1. Composition of Experimental Diets in Trial 1 (100% Dry Basis)**

Ingredient, %	TALLOW	SOY
Full-fat soybeans	-	20.0
Tallow	3.8	-
Dry-rolled corn	22.3	22.0
Soybean meal	15.9	-
Alfalfa hay	25.0	25.0
Prairie hay	25.0	25.0
Cane molasses	5.0	5.0
Vitamin-mineral premix <sup>a</sup>	3.0	3.0

<sup>a</sup>Provided 1000 IU/lb vitamin A, 0.1 ppm Co, 10 ppm Cu, 0.60 ppm I, 60 ppm Mn, 0.1 ppm Se, 60 ppm Zn, and 25 grams/ton Rumensin on a dry matter basis.

**Table 2. Composition of Experimental Diets in Trial 2 (100% Dry Basis)**

Ingredient, %	TALLOW	FLAX	ALGAE
Tallow	4.0	-	4.0
Microalgae <sup>a</sup>	-	-	daily top-dress
Ground flaxseed	-	12.9	-
Flaked corn	32.9	29.4	32.9
Alfalfa hay	39.4	39.4	39.4
Soybean meal	15.9	10.5	15.9
Vitamin mineral premix <sup>b</sup>	3.0	3.0	3.0
Cane molasses	4.8	4.8	4.8

<sup>a</sup>Top-dressed to provide docosahexaenoic acid at 10 g/animal daily.

<sup>b</sup>Provided 1000 IU/lb vitamin A, 0.1 ppm Co, 10 ppm Cu, 0.60 ppm I, 60 ppm Mn, 0.1 ppm Se, 60 ppm Zn, and 25 grams/ton Rumensin on a dry matter basis.

**Table 3. Rectal Temperature Profiles, °F**

Item	Hour after LPS injection							SEM
	0	1	2	3	4	5	6	
Trial 1 <sup>1</sup>								
TALLOW-LPS	102.2	103.6	104.2	104.9 <sup>a</sup>	104.7 <sup>c</sup>	-	103.2	.35
SOY-LPS	102.1	103.3	103.5	103.8 <sup>b</sup>	103.8 <sup>d</sup>	-	103.1	.35
TALLOW-no LPS	102.6	101.7	101.9	101.9	101.6	-	101.9	.69
SOY-no LPS	102.1	102.2	102.1	102.3	101.7	-	101.6	.69
Trial 2								
TALLOW	102.4	103.7	104.0	104.9 <sup>a</sup>	104.8 <sup>a</sup>	103.9 <sup>a,c</sup>	103.1 <sup>a</sup>	.29
FLAX	102.2	103.6	103.7	103.6 <sup>b,c</sup>	103.4 <sup>b</sup>	102.8 <sup>b</sup>	102.1 <sup>b</sup>	.29
ALGAE	102.6	103.6	103.8	104.3 <sup>d</sup>	104.0 <sup>b</sup>	103.2 <sup>d</sup>	102.9 <sup>a</sup>	.29

<sup>1</sup>Contrast LPS vs. NO-LPS (P<0.05).

<sup>a,b</sup>TALLOW different from SOY at hour 3 (P<0.03).

<sup>c,d</sup>TALLOW tended to be different from SOY at hour 4 (P=0.08).

<sup>a,b</sup>Means within a column with different superscripts are different (P<0.05).

<sup>c,d</sup>Means within a column with different superscripts tended to be different (P<0.1).

**Table 4. Blood Constituents From Trial 1**

Item	Hour After LPS Injection						SEM
	0	2	3	4	5	24	
TNF $\alpha$ , ng/ml <sup>1</sup>							
TALLOW-LPS	0.17	1.36 <sup>a</sup>	0.48	0.26	0.18	-	0.15
SOY-LPS	0.17	2.04 <sup>b</sup>	0.60	0.26	0.20	-	0.15
TALLOW-no LPS	0.15	0.16	0.16	0.11	0.12	-	0.31
SOY-no LPS	0.17	0.16	0.20	0.21	0.18	-	0.31
Fibrinogen, mg/dl							
TALLOW-LPS	438	463	438	450	338	538	53
SOY-LPS	350	300	325	288	263	425	53
TALLOW-no LPS	400	400	400	350	300	350	107
SOY-no LPS	350	400	400	300	200	300	107
Total white blood cell count, $\times 10^3$ /ml							
TALLOW-LPS	9.9	2.5	1.7	2.4	3.2	10.6	0.44
SOY-LPS	10.5	2.4	1.5	1.7	2.4	10.5	0.44
TALLOW-no LPS	9.1	8.7	8.6	8.4	9.0	9.0	0.88
SOY-no LPS	9.6	9.2	8.9	9.2	8.9	8.8	0.88
Haptoglobins, mg % <sup>1</sup>							
TALLOW-LPS	7.9	9.6	12.4	13.3	8.9	27.9	0.98
SOY-LPS	7.5	9.0	12.8	12.8	9.6	29.1	0.92
TALLOW-no LPS	7.5	8.5	12.0	13.0	9.0	10.0	1.84
SOY-no LPS	10.5	9.0	13.0	13.0	9.5	10.5	1.84

<sup>a,b</sup>Means within columns with different superscripts are different (P<0.01).

<sup>1</sup>Contrast LPS vs. no-LPS (P<0.05).

**Table 5. Blood Constituents From Trial 2**

Item	Hour after LPS Injection							SEM
	0	1	2	3	4	6	24	
TNF- $\alpha$ , ng/ml								
TALLOW	0.17	0.88	0.51	0.24	0.20			0.13
FLAX	0.19	0.79	0.64	0.34	0.19			0.15
ALGAE	0.20	1.00	0.70	0.40	0.20			0.13
Fibrinogen, mg/dl								
TALLOW	350	350	317	300	267	317	400	48
FLAX	350	350	317	250	300	333	400	48
ALGAE	283	250	300	250	300	283	367	48
Total white blood cell count, $\times 10^3$ /ml								
TALLOW	9.7	2.8	3.1	2.7	3.3	5.0	9.7	0.88
FLAX	10.5	3.1	3.7	3.6	3.8	6.3	10.3	0.88
ALGAE	11.5	3.3	3.2	3.0	3.2	5.3	10.3	0.88
Haptoglobin, mg/dl								
TALLOW	5.2	5.7	5.8	5.3	5.5	8.0	10.8 <sup>a</sup>	0.86
FLAX	5.3	5.5	5.5	6.3	6.5	6.8	9.0 <sup>a</sup>	1.05
ALGAE	6.5	5.7	5.7	5.8	7.0	6.5	15.0 <sup>b</sup>	0.86

<sup>a,b</sup>Means within a column with different superscripts are different (P<0.05).