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Effects of rumen-protected polyunsaturated fatty acid supplementation on performance and physiological responses of growing cattle after transportation and feedlot entry¹

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ABSTRACT: Two experiments were conducted to evaluate the effects of rumen-protected PUFA supplementation on performance and inflammation measures in beef calves after truck transportation and feedyard entry. In Exp. 1, 30 weaned Braford steers (BW = 218 \pm 4.3 kg) were transported for 1,600 km over a 24-h period in a commercial trailer and delivered to a feedlot (d 0). Upon arrival (d 1), steers were stratified by BW and allocated to receive 1 of 3 treatments (10 steers/ treatment), which consisted of grain-based concentrates without supplemental fat (NF), or with the inclusion of a rumen-protected SFA (SF; 2.1% as-fed basis) or PUFA source (PF; 2.5% as-fed basis). Shrunk BW was determined on d 1 and 30 for ADG calculation. Individual DMI was recorded from d 2 to 28. Blood samples were collected on d 0, 1, 4, 8, 15, 22, and 29 for determination of acute-phase protein concentrations. Steers fed PF had decreased (P = 0.04) mean DMI and tended to have reduced ADG (P = 0.07) compared with NF-fed steers (2.32 vs. 2.72% of BW, and 0.78 vs. 1.07 kg/d,respectively). No other treatment effects were detected. In Exp. 2, 48 weaned Brahman-crossbred heifers (BW $= 276 \pm 4.6$ kg) were stratified by initial BW and randomly allocated to 6 pastures (8 heifers/pasture) before

transportation (d -30 to 0). Pastures were randomly assigned (3 pastures/treatment) to receive (DM basis) 3.0 kg/heifer daily of NF, or 2.5 kg/heifer daily of a concentrate containing 5.7% (as-fed basis) of a rumenprotected PUFA source (PF). On d 0, heifers were transported as in Exp. 1. Upon arrival (d 1), 24 heifers were randomly selected (12 heifers/treatment), placed into individual feeding pens, and assigned the same pretransport treatment. Shrunk BW was recorded on d - 30, 1, and 30 to determine ADG. Individual DMI was recorded daily from d 2 to 28. Blood samples were collected on d 0, 1, 4, 8, 15, 22, and 29 for determination of acute-phase protein concentrations. A treatment \times day interaction was detected for haptoglobin (P < 0.01) because PF-fed heifers had decreased haptoglobin concentrations compared with NF-fed heifers on d 1, 4, and 8. No other treatment effects were detected. Data from this study indicate that PUFA reduces haptoglobin concentrations in beef calves after transport and feedlot entry when supplemented before and after transportation. Further, PUFA supplementation during the feedyard only appears to negatively affect cattle performance by decreasing ADG and DMI.

Key words: beef cattle, inflammation, performance, polyunsaturated fatty acid, transportation

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INTRODUCTION

Three of the most stressful events encountered by a feeder calf are weaning, transportation, and feedlot

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entry. These events, which may occur together or in a short period of time, lead to physiological, nutritional, and immunological changes that affect subsequent calf health and feedlot performance (Loerch and Fluharty, 1999). An example is the acute-phase protein response (Arthington et al., 2003, 2005), which is an important component of the innate immune system (Carroll and Forsberg, 2007), but that has been negatively associated with growth rates in cattle (Qiu et al., 2007; Cooke et al., 2009). Accordingly, management strategies that lessen the magnitude of the acute-phase protein re-

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sponse have been shown to benefit cattle productivity and overall efficiency of beef operations (Duff and Galyean, 2007; Arthington et al., 2008).

The inclusion of PUFA into diets has been shown to modulate the immune response in animals (Calder et al., 2002); however, the mechanisms involved in this process are not yet understood. Cullens et al. (2004) reported that diets containing a rumen-protected PUFA source decreased circulating concentrations of acutephase proteins in periparturient dairy cows. Therefore, we hypothesized that rumen-protected PUFA supplementation to transported beef calves would alleviate the acute-phase protein response during the feedlot receiving period and consequently benefit feedlot performance.

Two experiments were conducted to evaluate the effects of rumen-protected PUFA supplementation to growing cattle. Experiment 1 evaluated performance measures and concentrations of acute-phase proteins in transported beef steers supplemented or not with PUFA during the feedlot receiving period. Experiment 2 evaluated performance and concentrations of cortisol and acute-phase proteins in beef heifers supplemented or not with PUFA before and after transportation to the feedyard.

MATERIALS AND METHODS

All animals utilized in these experiments were cared for in accordance with acceptable practices and experimental protocols reviewed and approved by the University of Florida, Institutional Animal Care and Use Committee.

Experiment 1 was conducted from July to September 2006. Steers were originated from the University of Florida–IFAS, Range Cattle Research and Education Center, Ona, and transported to the University of Florida–IFAS, North Florida Research and Education Center, Marianna. Experiment 2 was conducted from September 2007 to February 2008 at the University of Florida–IFAS, Range Cattle and Education Center, Ona, and was divided into a pretransport phase (d -30 to 0) and a posttransport phase (d 1 to 29).

Animals

Exp.~1. Thirty weaned Braford steers (initial BW = 218 \pm 4.3 kg; initial age = 226 \pm 4.7 d) were utilized. On d 0 of the study, all steers were loaded into a commercial livestock trailer and transported 1,600 km. Steers remained in the trailer for 24 h before being received into the feedlot. Upon arrival (d 1), steers were ranked by BW, received electronic ear identification tags (Allflex USA Inc., Dallas-Ft. Worth, TX) for the measurement of individual feed intake with the GrowSafe System (model 4000 E, GrowSafe Systems Ltd., Airdrie, Alberta, Canada), and were allocated to

3 feedlot pens (10 steers/pen). Each pen (104.6 m² total area) was covered with a roof, had concrete flooring, and contained 2 similar feed bunks and 1 water source. Pens were assigned randomly to receive a grain-based concentrate without the inclusion of supplemental fat (NF), or with the addition of a rumen-protected SFA (SF; Energy Booster 100, MSC Co. Inc., Carpentersville, IL) or a rumen-protected PUFA source (**PF**; Megalac-R, Church & Dwight Co. Inc., Princeton, NJ). Because feed intake was determined individually, steer was considered the experiment unit (10 steers/treatment). Immediately after weaning until transportation to the feedlot facility (d -30 and 0, respectively), steers were maintained in a single bahiagrass (Paspalum notatum) pasture and were group-offered 4.1 kg/steer daily of the NF treatment (DM basis).

Exp. 2. Forty-eight weaned Brahman-crossbred heifers (initial BW = 276 ± 4.6 kg; initial age = 330 ± 2.4 d) were initially utilized. Heifers were weaned on d -37of the study. For the pretransport phase (d - 30 to d 0), heifers were ranked by BW and allocated to 6 bahiagrass (Paspalum notatum) pastures (1.07 ha/pasture; 8 heifers/pasture). Pastures were randomly allocated to receive NF or PF. On d 0, all heifers were loaded onto a commercial livestock trailer and transported 1,600 km. Heifers remained in the trailer for 24 h before unloading. After transport, 24 heifers were randomly selected (12 heifers/treatment) and placed into individual feeding pens (12 pens/treatment) from d 1 to 29. Heifer received the same pretransport treatment. Each individual pen (48 m² total area) was covered with a roof, had concrete flooring, and contained 2 feed bunks (1 for hay and 1 for concentrate) and 1 water trough. The remaining 24 heifers were removed from the experiment.

Diets

Exp. 1. Composition and nutritional profile of the treatments are described in Tables 1 and 2. From d 1 to d 4, steers were offered (as fed-basis) a 70:30 mixture of treatment:cottonseed hulls for ad libitum intake, in addition to 5 kg/d (as-fed basis) of Tifton 85 bermudagrass (Cynodon dactylon) in a separate feed bunk. Steers consumed all the hay provided. After this initial period, steers were offered (as-fed basis), for ad libitum intake, a 60:25:15 mixture of treatment:cottonseed hulls:bermudagrass hay from d 5 to 12, and followed by a 65:28:7 mixture of the same ingredients, for ad libitum intake, from d 13 to the end of the study. Random samples of all feedstuffs were collected at the beginning and during the experiment and were analyzed for nutrient content by a commercial laboratory (Dairy One Forage Laboratory, Ithaca, NY). All samples were analyzed by wet chemistry procedures for concentrations of CP, ADF, and NDF, whereas NE_m and NE_g were calculated using the equations proposed by NRC (1996). Nutrient profile of cottonseed hulls and bermudagrass hay is reported in Table 3.

Table 1. Ingredient composition and nutrient profile of treatments fed to steers during Exp. $1^{1,2}$

Item	NF	SF	PF
Ingredient, % as-fed			
Wheat middlings	30.63	29.97	29.85
Ground corn	20.00	19.58	19.50
Cracked corn	20.00	19.58	19.50
Cottonseed meal	11.85	11.6	11.55
Cottonseed hulls	10.00	9.80	9.75
Cane molasses	6.25	6.12	6.10
Calcium carbonate	1.25	1.22	1.22
Mineral and vitamin mix ³	0.03	0.03	0.03
Megalac-R	_	_	2.50
Energy booster		2.10	
Nutrient profile, ⁴ DM basis			
NE _g , ⁵ Mcal/kg	1.17	1.24	1.24
NE_{m}^{-5} Mcal/kg	1.99	2.11	2.11
CP, %	17.31	16.94	16.87
NDF, %	26.26	25.72	25.60
Ether extract, %	3.93	5.91	6.02
Ca, %	0.68	0.67	0.90
P, %	0.64	0.62	0.62

 $^1\mathrm{Treatments}$ were included (as-fed basis) at 70% of a total-mixed ration (TMR) from d 1 to 4, 60% of TMR from d 5 to 12, and 65% of TMR from d 13 to the end of the study.

²NF = grain-based concentrate without the addition of a supplemental fat source; SF = grain-based concentrate with the addition (2.1%; as-fed basis) of a rumen-protected SFA source (Energy Booster 100, MSC Co. Inc., Carpentersville, IL); PF = grain-based concentrate with the addition (2.5%; as-fed basis) of a rumen-protected PUFA source (Megalac-R, Church & Dwight Co. Inc., Princeton, NJ).

 3 Cattle Select (Lakeland Animal Nutrition, Lakeland, FL) contained 14% Ca, 9% P, 64% NaCl, 0.2% K, 0.3% Mg, 0.3% S, 50 mg/kg of Co, 1,500 mg/kg of Cu, 210 mg/kg of I, 500 mg/kg of Mn, 40 mg/kg of Se, 3,000 mg/kg of Zn, 800 mg/kg of F, 800 mg/kg of Fe, 360,000 mg/kg of vitamin A.

⁴Values obtained from a commercial laboratory wet chemistry analysis (Dairy One Forage Laboratory, Ithaca, NY).

 5 Calculated with the following equations (NRC, 1996): NE $_{\rm m}=1.37$ ME-0.138 ME $^2+0.0105$ ME $^3-1.12;$ NE $_{\rm g}=1.42$ ME-0.174 ME $^2+0.0122$ ME $^3-0.165.$ Given that ME $=0.82\times$ DE, and 1 kg of TDN =4.4 Mcal of DE.

Exp. 2. Composition and nutritional profile of the treatments are described in Tables 2 and 4. Treatments were offered daily, at 0800 h, at a rate of 3.0 and 2.5 kg of DM per heifer for NF and PF, respectively. Treatment intakes were formulated to be isocaloric and isonitrogenous and balanced for Ca, given the increased concentration of Ca in the PUFA source (Table 4). Forage and concentrate samples were analyzed for nutrient content similarly as in Exp. 1. Nutritional quality of pastures utilized during the pretransport phase is reported in Table 3 and was estimated from samples collected at the beginning of the experiment according to the procedures described by Vendramini et al. (2006). The pastures utilized in this experiment were not fertilized before or during the experimental period. Stargrass (Cynodon nlemfuensis) hay was offered for ad libitum consumption during the posttransport phase. Hay nutritional quality is reported in Table 3 and was calculated from samples collected weekly. A complete commercial mineral/vitamin mix (14% Ca, 9% P, 24%) NaCl, 0.20% K, 0.30% Mg, 0.20% S, 0.005% Co, 0.15% Cu, 0.02% I, 0.05% Mn, 0.004% Se, 0.3% Zn, 0.08% F, and 82 IU/g of vitamin A; as-fed basis) and water were offered for ad libitum consumption throughout the experiment.

Sampling

Exp. 1. Steer shrunk BW was recorded on d 1 (after 24 h of truck transportation with feed and water restriction) and at the end of the experiment (d 30, after 16 h of feed and water restriction) for ADG calculation. Individual feed intake was recorded continuously and summarized daily, from d 2 to 28, using the GrowSafe feed intake system, which allows for continuous measure of individual feed consumption. Full BW was recorded on d 4, 8, 15, 22, and 29, whereas average full BW during the receiving period was utilized for calculation of DMI as a percentage of BW. Total BW gain achieved during the study (using shrunk BW values) was divided by total DM consumed for individual G:F calculation.

Blood samples were collected on d 0 (immediately before loading into truck), 1 (immediately after unloading), 4, 8, 15, 22, and 29 for determination of plasma fibrinogen and ceruloplasmin concentrations. Plasma samples collected on d 0 and 29 were also analyzed for fatty acid (**FA**) composition. Rectal temperatures were recorded on d 1, 15, and 29 using a GLA M750 digital thermometer (GLA Agricultural Electronics, San Luis Obispo, CA).

Exp. 2. Heifers were weighed on 2 consecutive days to determine full and shrunk (after 16 h of feed and water restriction) BW at the beginning (48 heifers; d -31 and -30) and at the end of the experiment (24 heifers; d 29 and 30). Shrunk BW was also recorded on d 1 immediately after unloading (48 heifers; after 24 h of truck transportation with feed and water restriction). Shrunk BW was utilized to calculate heifer ADG during the pre- and posttransport phases.

During the posttransport phase, individual voluntary hay intake was recorded daily from d 2 to 28. Star-

Table 2. Fatty acid profile of rumen-protected fatty acid sources fed to steers or heifers in Exp. 1 and 2, respectively

Fatty acid	PUFA source ²	${\rm SFA~source}^3$	
Lauric acid (12:0), %	0.1	0.1	
Mystiric acid (14:0), %	0.9	2.7	
Palmitic acid (16:0), %	36.3	41.5	
Palmitoleic acid (16:1), %	0.2	1.4	
Stearic acid (18:0), %	3.9	38.7	
Oleic acid (18:1), %	26.8	12.7	
Linoleic acid (18:2), %	28.5	1.7	
Linolenic acid (18:3), %	3.0	0.1	
Other	0.3	1.1	

¹As percentage of total fatty acids. Values provided by manufacturers.

 $^{^2\}mathrm{Megalac\text{-}R}$ (Church & Dwight Co. Inc., Princeton, NJ).

³Energy Booster 100 (MSC Co. Inc., Carpentersville, IL).

Table 3. Nutrient profile (DM basis) of feedstuffs fed to steers and heifers in Exp. 1 and 2, respectively

Item	Exp. 1	Exp. 1		
	Cottonseed hulls	Hay^2	$Pasture^3$	Hay^4
NE _g , Mcal/kg	0.00	0.55	0.44	0.37
NE _m , Mcal/kg	0.44	1.11	0.99	0.92
CP, %	6.5	12.5	9.6	12.8
NDF, %	84.0	77.3	73.2	77.8
Ether extract, %	2.2	2.5	1.9	2.1
Ca, %	0.17	0.35	0.18	0.45
P, %	0.13	0.21	0.21	0.28

¹Values obtained from a commercial laboratory wet chemistry analysis (Dairy One Forage Laboratory, Ithaca, NY).

grass hay was offered in amounts to ensure ad libitum intake, and orts were collected and weighed daily. Samples of the offered and the nonconsumed hay were collected daily from each pen and dried for 96 h at

Table 4. Ingredient composition, nutrient profile, and intake of treatment concentrates fed to heifers during Exp. 2^1

Item	NF	PF
Ingredient, % (as-fed)		
Soybean hulls	74.3	72.9
Cracked corn	10.5	
Cottonseed meal	14.0	21.4
Megalac-R	_	5.7
Limestone	1.2	
Nutrient profile, ² DM basis		
NE _g , Mcal/kg	0.88	1.04
NE_{m}^{3} , $Mcal/kg$	1.46	1.70
CP, %	16.2	18.5
NDF, %	56.6	56.2
Ether extract, %	2.7	7.5
Ca, %	1.0	1.1
P, %	0.3	0.3
Daily intake ⁴		
DM, kg	3.00	2.50
NE _g , ³ Mcal	2.64	2.60
${ m NE}_{ m m}^{{ m 5}'}, { m ^3 Mcal}$	4.38	4.25
CP, kg	0.48	0.46
NDF, kg	1.69	1.40
Ether extract, kg	0.08	0.18
Ca, kg	0.03	0.03
P, kg	0.01	0.01

¹NF = grain-based concentrate without the addition of a supplemental fat source; PF = grain-based concentrate with the addition (150 g per heifer daily; as-fed basis) of a rumen-protected PUFA source (Megalac-R, Church & Dwight Co. Inc., Princeton, NJ).

50°C in forced-air ovens for DM calculation. Full BW was recorded on d 4, 8, 15, 22, and 29, whereas average full BW during the posttransport phase was utilized for calculation of DMI as a percentage of BW. Total BW gain achieved during the study (using shrunk BW values) was divided by total DM consumed for G:F calculation. Blood samples were collected on d 0, 1, 4, 8, 15, 22, and 29 for analysis of plasma concentrations of cortisol, ceruloplasmin, and haptoglobin.

Blood Analysis

Blood samples were collected via jugular venipuncture into commercial blood collection tubes (Vacutainer, 10 mL; Becton Dickinson, Franklin Lakes, NJ) containing sodium heparin, placed on ice immediately, and centrifuged at $2,400\times g$ for 30 min at 4°C for plasma collection. Plasma was frozen at -20°C on the same day of collection.

Concentrations of cortisol were determined using Coat-A-Count solid phase ¹²⁵I RIA kit (DPC Diagnostic Products Inc., Los Angeles, CA). Concentrations of ceruloplasmin were determined according to procedures described by Demetriou et al. (1974). Concentrations of haptoglobin were determined by measuring haptoglobin/hemoglobin complex by the estimation of differences in peroxidase activity (Makimura and Suzuki, 1982), and results are expressed as arbitrary units from the absorption reading at 450 nm \times 100. Plasma fibrinogen concentrations were determined using a fibrinogen determination kit (Sigma procedure No. 880, Sigma Diagnostics, St. Louis, MO). Plasma FA composition was determined using gas-liquid chromatography (CR-3800 Gas Chromatograph, Varian Inc., Palo Alto, CA) according to the procedures described by Kramer et al. (1997). All samples were analyzed in duplicate. The intra- and interassay CV for Exp. 1 were, respectively, 4.8 and 6.4% for fibringen and 3.2 and 6.7% for ceruloplasmin. The intra- and interassay CV for Exp. 2 were,

²Tifton 85 bermudagrass (Cynodon dactylon) hay.

³Bahiagrass (*Paspalum notatum*) pastures.

⁴Stargrass (Cynodon nlemfuensis) hay.

⁵Calculated with the following equations (NRC, 1996): $NE_m = 1.37 \text{ ME} - 0.138 \text{ ME}^2 + 0.0105 \text{ ME}^3 - 1.12$; $NE_g = 1.42 \text{ ME} - 0.174 \text{ ME}^2 + 0.0122 \text{ ME}^3 - 0.165$. Given that $ME = 0.82 \times DE$, and 1 kg of TDN = 4.4 Meal of DE

²Values obtained from a commercial laboratory wet chemistry analysis (Dairy One Forage Laboratory, Ithaca, NY).

 $^{^3\}text{Calculated}$ with the following equations (NRC, 1996): NE $_m=1.37$ ME-0.138 ME $^2+0.0105$ ME $^3-1.12;$ NE $_g=1.42$ ME-0.174 ME $^2+0.0122$ ME $^3-0.165.$ Given that ME $=0.82\times$ DE, and 1 kg of TDN =4.4 Mcal of DE.

⁴Estimated from the concentrate consumption of individual heifers.

respectively, 12.6 and 7.1% for haptoglobin, 4.6 and 8.8% for ceruloplasmin, and 5.5 and 6.4% for cortisol.

Statistical Analysis

Exp. 1. Performance and physiological data were analyzed using the PROC MIXED procedure (SAS Inst. Inc., Cary, NC) and Satterthwaite approximation to determine the denominator degrees of freedom for the tests of fixed effects. The model statement used for plasma measurements, DMI, and rectal temperature contained the effects of treatment, day, and the interaction. Further, plasma measurements were analyzed using values from d 0 as covariate. Data were analyzed using steer(treatment) as the random variable. The specified term for the repeated statement was day, and the covariance structure utilized was autoregressive, which provided the best fit for these analyses according to the Akaike information criterion. The model statement used for ADG and G:F analysis contained only the effects of treatment. Data were analyzed using steer(treatment) as the random variable. Results are reported as least squares means and were separated using preplanned pairwise contrasts (PDIFF). Pearson correlations (CORR procedure of SAS) were calculated among ADG, mean concentrations of plasma measurements, mean DMI, and mean rectal temperature of steers during the study. The GLM procedure was utilized to determine effects of treatment on correlation coefficients. No treatment effects or interactions were detected; therefore, correlation coefficients reported herein were determined across treatments. Significance was set at P < 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.

Exp. 2. Performance and physiological data were analyzed using the PROC MIXED procedure of SAS and Satterthwaite approximation to determine the denominator degrees of freedom for the tests of fixed effects. The model statement used for posttransport plasma measurements, and DMI contained the effects of treatment, day, and the interaction. Data were analyzed using pretransport arrangements of pen(treatment) and heifer(pen) as random variables. The specified term for the repeated statement was day, and the covariance structure utilized was autoregressive, which provided the best fit for these analyses according to the Akaike information criterion. The model statement used for ADG and G:F analysis contained only the effects of treatment, whereas the pretransport arrangement of pen(treatment) was the random variable. Results are reported as least squares means and were separated using LSD. Pearson correlations (CORR procedure of SAS) were calculated among ADG, mean concentrations of plasma measurements, and mean DMI of heifers during the posttransport phase of the study. The GLM procedure was utilized to determine effects of treatment on correlation coefficients. No effects or interactions were detected; therefore, correlation coefficients reported herein were determined across treatments. 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

Exp. 1

Steers fed PF tended to have reduced (P = 0.07) ADG (Table 5) compared with NF-fed steers, but similar (P = 0.17) ADG compared with SF-fed steers. No differences were detected in ADG between SF- and NF-fed steers (P = 0.67). A treatment \times day interaction was detected (P < 0.01) for DMI (Figure 1). Steers fed PF often had reduced DMI compared with the other treatments, particularly compared with NF-steers during the initial 15 d of the study. As a result, PF-fed steers had reduced (P = 0.04) mean DMI (Table 5) during the study compared with NF-fed steers, but not compared with SF-fed steers (P = 0.23). Mean DMI was not different between SF- and NF-fed steers (P = 0.37; Table 5). No treatment effects $(P \ge 0.33)$ were detected for G:F and rectal temperatures (Table 5). These results indicate that PF supplementation decreased growth rates mainly by impairing DMI. Other authors have also reported decreased DMI and BW gain when cattle are supplemented with fat sources (Harrison et al., 1995; Gibb et al., 2004; Pavan et al., 2007), particularly when Ca-soaps of FA (CSFA), such as the PF source, are utilized (Simas et al., 1995; Bateman et al., 1996; Ngidi et al., 1990). Further, PUFA sources, as CSFA or not, are more detrimental to feed intake compared with SFA sources (Elliott et al., 1996; Allen, 2000).

In the present experiment (after d 5), treatment concentrates were combined with hay and cottonseed hulls as a total mixed ration and were formulated to prevent potential negative effects of supplemental fat on ruminal digestibility and subsequent DMI. According to Hess et al. (2008), ruminal digestibility is not impaired if high-concentrate diets contain less than 6% (DM basis) of fat. Similarly, inclusion (DM basis) of SFA-based CSFA up to 6% of the diet, or PUFA sources up to 9.4% of the diet, did not impair ruminal digestibility parameters in sheep and cattle fed high-concentrate diets (Ngidi et al., 1990; Kucuk et al., 2004; Atkinson et al., 2006). The maximum fat content observed in the diets offered in this study was (DM basis) 3.6, 4.9, and 4.8% for NF, PF, and SF, respectively. Therefore, treatment effects on DMI should not be attributed to impaired DM digestibility due to fat intake of PF-fed steers, particularly because DMI was similar between SF and NF steers. On the other hand, PUFA supplementation as CSFA can impair DMI by affecting other factors such as dietary palatability, gut motility, and

Table 5. Body weight, ADG, DMI, feed efficiency, rectal temperature, and plasma concentrations of fibrinogen and ceruloplasmin of steers offered diets without (NF; n = 10) or with the inclusion of a rumen-protected SFA (SF; n = 10) or PUFA (PF; n = 10) source during the 29-d feedlot receiving period in Exp. 1

Item		$\mathrm{Treatment}^1$		_	P-value		
	NF	SF	PF	SEM	NF vs. SF	NF vs. PF	SF vs. PF
Initial shrunk BW, kg	234	235	231	8	0.95	0.75	0.71
Final shrunk BW, kg	265	264	253	8	0.91	0.33	0.38
ADG, ² kg/d	1.07	1.00	0.78	0.11	0.67	0.07	0.17
G:F, kg/kg	0.15	0.15	0.13	0.01	0.97	0.33	0.32
DMI, ⁴ % of BW	2.72	2.55	2.32	0.13	0.37	0.04	0.23
Rectal temperature, °C	39.04	38.96	38.98	0.10	0.56	0.68	0.86
Fibrinogen, mg/dL	318	372	365	24	0.12	0.17	0.85
$Ceruloplasmin, {}^{5}mg/dL$	17.6	18.4	18.1	0.7	0.41	0.65	0.73

¹NF = grain-based concentrate without the addition of a supplemental fat source; SF = grain-based concentrate with the addition (2.1%; as-fed basis) of a rumen-protected SFA source (Energy Booster 100, MSC Co. Inc., Carpentersville, IL); PF = grain-based concentrate with the addition (2.5%; as-fed basis) of a rumen-protected PUFA source (Megalac-R, Church & Dwight Co. Inc., Princeton, NJ).

release of gut hormones that control satiety (Allen, 2000). Grummer et al. (1990) reported that CSFA is less acceptable to cattle compared with prilled sources of FA, such as the SF source, when these fat sources are

offered alone, top-dressed, or mixed with other dietary components. In addition, PUFA might decrease gut motility and enhance cholecystokinin release at a greater extent compared with SFA (Drackley et al., 1992;

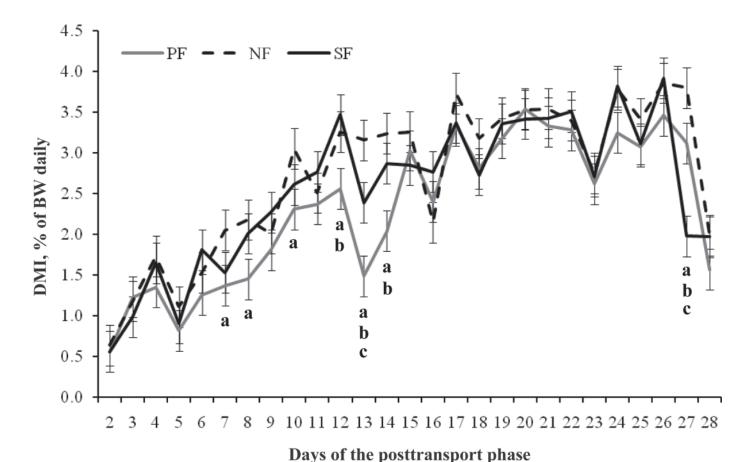


Figure 1. Daily DMI, as a percentage of full BW, of steers offered diets without (NF; n = 10) or with the inclusion of a rumen-protected SFA [SF (Energy Booster 100, MSC Co. Inc., Carpentersville, IL); 2.1% as-fed basis; n = 10] or PUFA [PF (Church & Dwight Co. Inc., Princeton, NJ); 2.5% as-fed basis; n = 10] source during the 29-d feedlot receiving period in Exp. 1. A treatment \times day interaction was detected (P < 0.01). Within day, letters indicate the following treatment differences (P < 0.05): a = NF vs. PF, b = SF vs. PF, and c = NF vs. SF.

²Calculated using initial (d 1) and final (d 30) shrunk BW.

³Calculated by dividing the total DM consumed from d 2 to 28 into the total BW gain achieved over this time period.

⁴Based on average full BW obtained during the receiving period (262, 258, and 249 kg of BW for NF, SF, and PF, respectively).

⁵Least squares means adjusted covariately to values obtained before transportation and feedlot entry (d 0).

Table 6. Pearson correlation coefficients among ADG, plasma measurements, DMI, and rectal temperature of steers from Exp. 1¹

Item	ADG	Rectal temperature	Ceruloplasmin	DMI	
Tueni		temperature	Certhopiasinin		
Rectal temperature	0.05				
-	0.76				
Ceruloplasmin	-0.29	-0.05			
	0.14	0.79			
DMI	0.79	0.11	-0.49		
	< 0.01	0.55	< 0.01		
Fibrinogen	-0.32	-0.11	0.56	-0.44	
_	0.10	0.59	< 0.01	0.02	

¹Within an item, upper row = correlation coefficient; lower row = P-value.

Allen, 2000), although further research is required to investigate this matter. Lastly, steers were not adapted to treatments before transportation and feedlot entry. Fat sources, particularly PUFA-based CSFA, should be introduced into cattle diets gradually to facilitate acceptance and adaptation to these ingredients (Zinn, 1988, 1989; Grummer et al., 1990). Therefore, the reduced acceptability and potential effects of PF on gut motility and satiety control, combined with the lack of a proper adaptation period, were likely the causes for the reduced DMI detected in PF-fed steers compared with the other treatments.

No treatment effects $(P \geq 0.12)$ were detected for concentrations of plasma fibringen and ceruloplasmin (Table 5), indicating that treatment differences detected for performance measures were not associated with the acute-phase protein response. Nevertheless, fibringen and ceruloplasmin concentrations peaked during the initial 8 d after transport and feedlot entry (day effect, P < 0.01; data not shown), indicating that steers experienced an acute-phase reaction during the initial part of the feedlot receiving period (Arthington et al., 2008). Furthermore, independent of treatments, ceruloplasmin and fibringen were negatively correlated with DMI (P < 0.01 and P = 0.02, respectively; Table 6). Positive correlations were detected between concentrations of ceruloplasmin and fibringen (P <0.01), and between heifer DMI and ADG (P < 0.01)across treatments. These data, in addition to previous efforts from our group (Qiu et al., 2007; Cooke et al., 2009), indicate that the acute-phase protein response is negatively correlated with feed intake and consequently growth rates in cattle. Supporting our results, Klasing and Korver (1997) reported that the proinflammatory cytokines synthesized during the acute-phase protein response, such as IL-1, IL-6, and tumor necrosis factor- α (TNF- α), can reduce feed intake of livestock by modulating the central nervous and endocrine systems, and also by inhibiting gastric motility, emptying, and gastric acid secretion.

The main hypothesis of the present study was that supplementing PUFA during the receiving period of the feedlot would alleviate the acute-phase protein response stimulated by transport and feedlot entry, and consequently benefit steer performance. Dietary PUFA has been reported to modulate the immune response in animals (Calder et al., 2002). Conversely, SFA affects immune competence to a lesser degree (Miles and Calder, 1998), whereas Farran et al. (2008) observed no difference in the acute-phase reaction of LPS-challenged steers supplemented with tallow (increased SFA) compared with nonsupplemented steers. Therefore, the SF treatment of the present study was included as an iso-lipidic, iso-caloric, and iso-nitrogenous control treatment because energy and protein intake also can modulate the immune system (Carroll and Forsberg, 2007). Cullens et al. (2004) reported that periparturient dairy cows offered diets containing the same rumen-protected PUFA source utilized in the present study had decreased fibringen concentrations during the first 10 wk postpartum compared with cows offered no supplemental fat. The mechanisms involved in this process are not yet understood, but PUFA are believed to modulate the immune system by altering the synthesis of eicosanoids and cytokines (Miles and Calder, 1998). More specifically, linolenic acid serves as precursor of eicosapentaenoic and docosahexaenoic acids, which promote synthesis of eicosanoids that do not elicit the acute-phase protein response, such as PGE₃. Conversely, linoleic acid serves as precursors of arachdonic acid, which promotes the synthesis of PGE₂, a potent stimulator of the acute-phase and other inflammatory responses (Yagoob and Calder, 2007; Schmitz and Ecker, 2008).

Farran et al. (2008) reported that beef steers supplemented with a full-fat soybeans (increased linoleic acid) had increased TNF- α concentrations after an LPS challenge compared with steers supplemented with a SFA source. Rezamand et al. (2009) reported that lactating dairy cows supplemented with camelina meal (increased linolenic acid) had reduced expression of IL-1 and TNF- α in peripheral blood mononuclear cells compared with nonsupplemented cohorts. The PUFA source offered to cattle in the present study contained greater amounts of linoleic acid compared with linolenic acid (Table 2). However, cattle requirements for these FA are still unknown, whereas linolenic acid might be required in reduced amounts to trigger an anti-inflam-

Table 7. Plasma fatty acids profile (on d 29) of steers offered diets without (NF; n = 10) or with the inclusion of a rumen-protected SFA (SF; n = 10) or PUFA (PF; n = 10) source during the 29-d feedlot receiving period in Exp. 1^{1}

Fatty acid, $^2 \mu g/mL$		Treatment ³				P-value	
	NF	SF	PF	SEM	NF vs. SF	NF vs. PF	SF vs. PF
Stearic (18:0)	207	214	225	23	0.83	0.60	0.74
Linoleic (18:2)	454	537	582	56	0.31	0.13	0.57
Linolenic (18:3)	6.3	7.6	8.6	0.95	0.36	0.11	0.45
Arachidonic (20:4n-6)	20.6	19.9	16.5	1.5	0.73	0.08	0.12
EPA (20:5n-3)	1.4	0.8	1.6	0.4	0.24	0.74	0.14
DHA (22:6n-3)	1.8	2.0	4.7	1.1	0.89	0.09	0.12
Total SFA	354	382	403	36	0.58	0.35	0.68
Total MUFA	89	96	97	8	0.53	0.48	0.89
Total PUFA	485	571	612	58	0.30	0.14	0.61
Total fatty acids	927	1,051	1,108	96	0.37	0.20	0.67

¹Treatments were offered from d 1 (feedlot entry) until d 29 of the feedlot receiving period.

matory response and overcome the proinflammatory effects of linoleic acid. Further, the PUFA source offered herein contained both linoleic and linolenic acids in greater amounts compared with the other treatments (Table 2), what could explain the lack of treatment effects on the acute-phase protein response of steers. Still, Cullens et al. (2004) reported differences in postpartum concentrations of acute-phase proteins in dairy cows supplemented or not with the same PUFA source utilized herein, as described previously. However, Farran et al. (2008), Cullens et al. (2004), and Rezamand et al. (2009) supplemented cattle with PUFA for at least 2 wk before the LPS challenge, calving, or sampling, respectively. Continuous feeding of FA sources is required to manipulate the FA content of body tissues in cattle (Mattos et al., 2000; Bilby et al., 2006). Therefore, a preliminary period of supplementation might be necessary to elicit the immunomodulatory effects of PUFA after stressful events such as endotoxin challenge, parturition, weaning, transport, and feedlot entry. In the current experiment, treatments were offered immediately after transportation and feedlot entry, whereas plasma concentrations of acute-phase proteins typically peak during the first 7 d after transport and feedlot arrival, as detected in the present study and by previous efforts from our group (Arthington et al., 2005, 2008). Consequently, the lack of treatment effects on plasma concentrations of acute-phase proteins can be attributed, at least in part, to the absence of a pretransport treatment feeding to ensure that PF-fed steers had the opportunity to incorporate dietary PUFA into their tissues and circulation, and therefore benefit immunologically during the feedlot receiving period.

Previous studies reported that PUFA supplementation increases plasma concentrations of linoleic and linolenic acids in cattle (Lessard et al., 2003, 2004; Farran et al., 2008). However, in the present study, no

treatment effects $(P \ge 0.08)$ were detected for plasma FA content (Table 7), even though samples for FA analysis were collected 29 d after feedlot entry, which should provide adequate time for FA incorporation into circulation and tissues (Bilby et al., 2006; Farran et al., 2008). Also, no treatment effects $(P \geq 0.20)$ were detected if individual FA were calculated as a percentage of total FA (data not show). These outcomes were unexpected, given that the PUFA source was based on CSFA, and thus rumen-inert, and also due to differences in FA composition among treatments (Table 2). However, CSFA containing increased amounts of PUFA can be significantly dissociated into FFA and Ca in the rumen when ruminal pH is reduced (Sukhija and Palmquist, 1990), whereas FFA are highly susceptible to ruminal biohydrogenation (Harfoot and Hazlewood, 1988). In the present study, ruminal pH was not evaluated, but steers were offered grain-based diets (Table 1) that potentially maintained ruminal pH at reduced levels (Nagaraja and Titgemeyer, 2007), therefore preventing adequate PUFA absorption by the intestine and circulation.

In summary, PUFA supplementation during the feedlot receiving period did not affect plasma concentrations of acute-phase proteins, but decreased ADG and DMI of beef steers. However, treatments were offered without the proper adaptation period required to optimize intake and elicit the immunomodulatory effects of PUFA.

Exp. 2

No treatment effects (P = 0.98) were detected for ADG during the pretransport phase (Table 8). Similarly, no treatment effects (P = 0.23) were detected for posttransport ADG, total DMI, voluntary forage intake, and G:F (Table 8). Differently from Exp. 1, the

²EPA = eicosapentaenoic acid; DHA = docosahexaenoic acid.

³NF = grain-based concentrate without the addition of a supplemental fat source; SF = grain-based concentrate with the addition (2.1%; as-fed basis) of a rumen-protected SFA source (Energy Booster 100, MSC Co. Inc., Carpentersville, IL); PF = grain-based concentrate with the addition (2.5%; as-fed basis) of a rumen-protected PUFA source (Megalac-R, Church & Dwight Co. Inc., Princeton, NJ).

Table 8. Average daily gain and posttransport total DMI, forage intake, G:F, and plasma concentrations of cortisol and ceruloplasmin in heifers offered concentrates without (NF; n=3) or with the inclusion of a rumen-protected PUFA (PF; n=3) source from d-30 to 29 relative to transport and feedyard entry (d 0) in Exp. 2^1

Item	NF	PF	SEM	P-value
Pretransport phase				
Shrunk BW (d -30), kg	278	275	6.7	0.69
ADG , $^2 kg/d$	0.12	0.12	0.06	0.98
Posttransport phase				
Shrunk BW $(d-1)$, kg	282	276	7	0.51
Shrunk BW (d 30), kg	307	297	7	0.30
ADG , 3 kg/d	0.78	0.66	0.06	0.23
DMI, 4 % of BW	2.07	1.96	0.08	0.39
Voluntary forage DMI, % of BW	1.03	1.10	0.06	0.41
$G:F^5$	0.12	0.11	0.01	0.32
Cortisol, ng/mL	3.97	3.62	0.39	0.54
Ceruloplasmin, mg/dL	31.4	29.1	1.4	0.24

¹NF = grain-based concentrate without the addition of a supplemental fat source; PF = grain-based concentrate with the addition (150 g per heifer daily; as-fed basis) of a rumen-protected PUFA source (Megalac-R, Church & Dwight Co. Inc., Princeton, NJ).

PF treatment was offered to heifers for 30 d before transport. As previously stated, a period of adaptation to fat supplementation is essential to maintain optimal feed intake, especially when CSFA are supplemented (Zinn, 1988; Grummer et al., 1990). Therefore, heifers were already adapted to the PF treatment during the posttransport phase when DMI was evaluated, which may partially explain the differences in treatment effects on DMI between Exp.1 and 2. In addition, heifers were offered a forage-based diet with treatments provided through limit-fed concentrates, whereas steers from Exp. 1 had free-choice access to a high-concentrate diet. According to the DMI evaluation, the PF treatment from Exp. 2 provided, on average, 2.9% dietary fat (DM basis). Hess et al. (2008) indicated that inclusion of supplemental fat up to 3\% of diet DM is recommended if the goal is to maximize the use of forage-based diets and prevent impaired ruminal fiber digestibility and forage intake. Therefore, the reduced intake and fat content of the PF treatment in Exp. 2 compared with Exp. 1 may also explain the lack of treatment effects on DMI in Exp. 2, and consequently on ADG, because ADG and DMI were positively correlated (P = 0.03; Table 9).

No treatment effects $(P \ge 0.24)$ were detected for plasma concentrations of ceruloplasmin and cortisol (Table 8). Ceruloplasmin peaked after transport and feedlot entry (day effect, P < 0.01). Cortisol concentrations peaked on d 3 (day effect, P < 0.01; data not shown), indicating a stress response to transport and feedlot entry (Crookshank et al., 1979; Sapolsky et al., 2000). A treatment × day interaction was detected (P < 0.01) for haptoglobin analysis (Figure 2) because PF-fed heifers had reduced haptoglobin concentrations compared with NF-fed heifers during the initial 8 d after transport. These results suggest that PF-fed heifers had an alleviated acute-phase protein response compared with NF cohorts, whereas the similar cortisol concentrations

Table 9. Pearson correlation coefficients among posttransport ADG, plasma measurements, and DMI of heifers from Exp. 2^1

Item	ADG	Ceruloplasmin	Cortisol	DMI
Ceruloplasmin	-0.39			
·	0.05			
Cortisol	-0.18	0.40		
	0.39	0.05		
DMI	0.44	-0.06	0.17	
	0.03	0.75	0.42	
Haptoglobin	-0.02	0.31	0.11	-0.03
	0.90	0.13	0.61	0.89

¹Within an item, upper row = correlation coefficient; lower row = P-value.

²Calculated using shrunk BW obtained on d -30 and 1.

³Calculated using shrunk BW obtained on d 1 and 30.

⁴Based on average full BW obtained during the posttransport phase (310 and 316 kg of BW for PF and NF, respectively).

 $^{^5}$ Calculated by dividing the total DM consumed from d 2 to 28 into the total BW gain achieved over this time period.

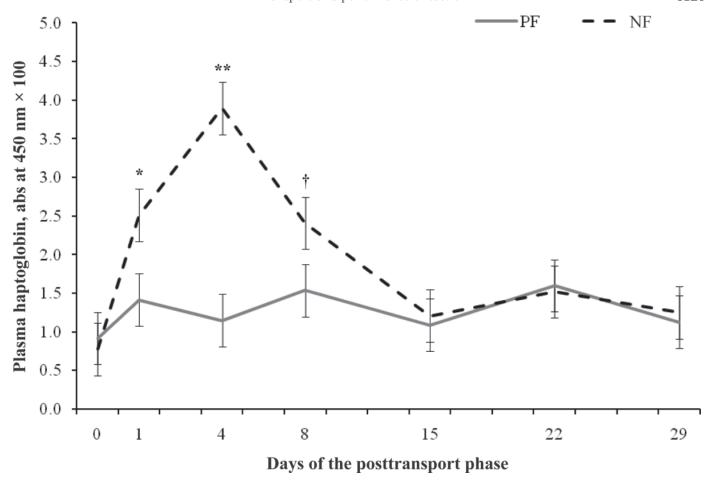


Figure 2. Plasma haptoglobin concentrations of heifers offered concentrates without (NF; n = 3) or with the inclusion (150 g per heifer daily; as-fed basis) of a rumen-protected PUFA [PF (Church & Dwight Co. Inc., Princeton, NJ); n = 3] source from d -30 to 29 relative to transport and feedyard entry (d 0) in Exp. 2. A treatment \times day interaction was detected (P < 0.01). Treatment comparison within days: $\dagger P < 0.10$, *P < 0.05, and **P < 0.01.

suggest that heifers from both treatments experienced a similar stress challenge due to transport (Crookshank et al., 1979; Sapolsky et al., 2000). Plasma concentrations of ceruloplasmin and haptoglobin are typically correlated (Cooke et al., 2009). The lack of treatment effects on ceruloplasmin can be explained, at least partially, by the increased plasma concentrations of this acute-phase protein in heifers from both treatments. According to The Merck Veterinary Manual (Merck, 1997), normal concentrations of ceruloplasmin in cattle range from 16.8 to 34.2 mg/dL. In the present study, mean ceruloplasmin concentrations of heifers from both treatments approached the maximum value (Table 8), whereas values above the normal range were detected immediately after transport and feedlot entry (Figure 3). Plasma ceruloplasmin concentrations are highly modulated by Cu intake (Arthington et al., 1996), and a mineral supplement containing 0.15% of Cu was offered for ad libitum consumption throughout the experiment. Therefore, perhaps dietary Cu concentrations maintained ceruloplasmin concentrations at increased concentrations and overrode any potential treatment effects similar to those detected for haptoglobin. Nevertheless, independent of treatments, a negative correlation was detected (P=0.05) between ceruloplasmin and ADG, and a positive correlation was detected (P=0.05) between ceruloplasmin and cortisol (Table 9). These outcomes support previous data from our research group indicating that increased concentrations of ceruloplasmin are associated with reduced growth rates (Cooke et al., 2009), and that stress challenges that stimulate cortisol production are positively associated with the acute-phase protein response in cattle (Crookshank et al., 1979; Arthington et al., 2003; Cooke et al., 2009).

The main hypothesis of the present study was that supplementing PUFA before and during the receiving period would alleviate the acute-phase protein response triggered by transport and feedyard entry, and thus benefit heifer performance. Unlike Exp. 1, treatments were provided before transport to ensure that PF-fed heifers were adapted to the fat supplement and had the opportunity for incorporating dietary PUFA into their tissues and circulation before entering the receiving period. Further, a third treatment containing a SF-source was not included because of similar responses detected in NF- and SF-fed steers in Exp. 1. Nevertheless, treatment intakes in the present experiment were isocaloric

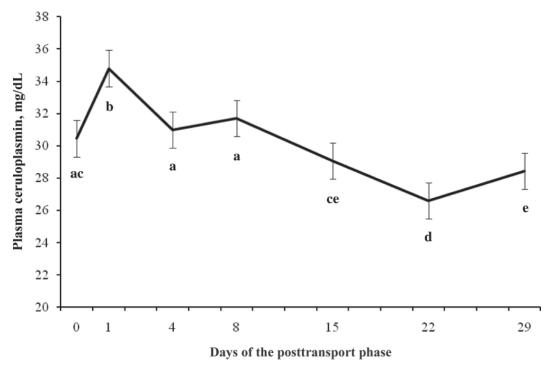


Figure 3. Plasma ceruloplasmin concentrations, pooled across treatments, of heifers offered concentrates without (NF; n = 3) or with the inclusion (150 g per heifer daily; as-fed basis) of a rumen-protected PUFA [PF (Church & Dwight Co. Inc., Princeton, NJ); n = 3] source from d -30 to 29 relative to transport and feedyard entry (d 0) in Exp. 2. A day effect was detected (P < 0.01). ^{a-e}Days not bearing a common letter differ (P < 0.05).

and isonitrogenous. Plasma FA content was not evaluated in this experiment to determine PUFA incorporation into circulation. However, unlike Exp. 1, heifers from both treatments were offered high-fiber diets, which likely prevented reduced ruminal pH and consequent dissociation and biohydrogenation of supplemental PUFA (Harfoot and Hazlewood, 1988; Sukhija and Palmquist, 1990; Nagaraja and Titgemeyer, 2007). Supporting our hypothesis, posttransport DMI was not different between treatment groups. Plasma concentrations of haptoglobin were reduced in PF-fed heifers, suggesting an alleviated acute-phase protein response in these heifers during the receiving period. However, the mechanisms involved in this process are not yet understood, but PUFA are believed to modulate the immune system by altering the inflammatory reaction (Miles and Calder, 1998).

As previously cited, linolenic acid promotes the synthesis of eicosanoids that do not elicit an inflammatory response, such as PGE_3 , and also stimulate synthesis of T_{H2} anti-inflammatory cytokines such as IL-4, IL-10, and IL-13. Conversely, linoleic acid promotes the synthesis of PGE_2 , which is a potent stimulator of the acute-phase protein response, and the T_{H1} pro-inflammatory cytokines (IL-1, IL-6, and $TNF-\alpha$) that trigger hepatic synthesis of acute-phase proteins (Carroll and Forsberg, 2007; Yaqoob and Calder, 2007; Schmitz and Ecker, 2008). The rumen-protected PUFA source offered to heifers in the present study contained linoleic and linolenic acids (Table 2), although linoleic concen-

trations were greater compared with linolenic acid concentrations (28.5 and 3.0%, respectively; DM basis). However, cattle requirements for linoleic and linolenic acids are still unknown. According to the CPM-Dairy model (Cornell-Penn-Miner Dairy; version 3.08.01; University of Pennsylvania, Kennett Square; Cornell University, Ithaca, NY; and William H. Miner Agricultural Research Institute, Chazy, NY), the PF treatment provided 6.17 g/d of linoleic and 0.95 g/d of linolenic acid that were absorbable by the duodenum of treated cows, whereas the NF treatment provided, respectively, 0.94 and 0.21 g/d of absorbable linoleic and linolenic acid. Further, circulating concentrations of eicosanoids and cytokines were not evaluated in the present study. Therefore, it cannot be concluded if PF-fed heifers had reduced haptoglobin concentrations because of an altered cytokine profile caused by the additional supply of linolenic acid, linoleic acid, or both. Further research is warranted to determine the dietary levels of linoleic and linolenic acids required to trigger a pro- or antiinflammatory response, respectively, in cattle.

In summary, heifers supplemented with PUFA before and after transport and feedyard entry had reduced posttransport haptoglobin concentrations compared with nonsupplemented cohorts. However, no treatment effects were detected for posttransport performance measures. Therefore, additional research is required to further investigate the effects of PUFA supplementation on performance and acute-phase protein response in transport-stressed feeder calves.

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