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Effects of polyunsaturated fatty acid supplementation on ruminal in situ forage degradability, performance, and physiological responses of feeder cattle[1]

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ABSTRACT: Two experiments were conducted to compare ruminal, physiological, and performance responses of forage-fed cattle consuming grain-based supplements without (NF) or with the inclusion (10%; DM basis) of a rumen-protected PUFA (PF) or SFA source (SF). Supplements were offered and consumed at 0.6% of BW/animal daily (DM basis). In Exp. 1, DMI and ruminal in situ forage degradability were evaluated in 3 Angus × Hereford cows fitted with ruminal cannulas and allocated to a 3 × 3 Latin square design. Within each experimental period, hay was offered in amounts to ensure ad libitum access from d 1 to 13, DMI was recorded from d 8 to 13, and cows were limited to receive 90% of their average hay DMI (d 1 to 13) from d 14 to 21. On d 16, polyester bags containing 4 g of ground hay (DM basis) were incubated within the rumen of each cow for 0, 4, 8, 12, 24, 36, 48, 72, and 96 h. Hay and total DMI were reduced ($P < 0.05$) in cows receiving PF compared with cows receiving SF and NF. No treatment effects were detected ($P > 0.48$) for ruminal disappearance rate and effective ruminal degradability of hay DM and NDF. In Exp. 2, preconditioning DMI, ADG, carcass traits, and plasma concentrations of cortisol, fatty acids, acute-phase proteins, and proinflammatory cytokines were assessed in 72 Angus × Hereford steers receiving supplement treatments during a 28-d preconditioning period. All steers were transported to a commercial growing lot after preconditioning (d 1) and were later moved to an adjacent commercial finishing yard (d 144), where they remained until slaughter. No treatment effects were detected ($P \geq 0.52$) for preconditioning ADG and G:F, but DMI tended ($P = 0.09$) to be reduced in steers receiving PF compared with those receiving NF and SF. Plasma PUFA concentrations were greater in steers receiving PF compared with those receiving NF and SF ($P = 0.01$). After transportation, concentration of tumor necrosis factor-α increased for steers receiving NF, did not change for steers receiving SF, but decreased for steers receiving PF (treatment × day interaction, $P < 0.01$). Steers fed PF had greater ($P = 0.02$) ADG compared with those fed NF during the growing phase. Carcass yield grade and marbling were greater ($P < 0.05$) for steers fed PF compared with those fed NF. In conclusion, PUFA supplementation did not affect ruminal forage degradability but did impair DMI in beef cows. Further, PUFA supplementation to steers during preconditioning reduced plasma concentrations of tumor necrosis factor-α after transportation, and benefited growing lot ADG and carcass marbling.

Key words: beef cattle, inflammation, performance, polyunsaturated fatty acid, ruminal in situ forage degradability, transportation

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INTRODUCTION

Stressful events, such as transportation and feedlot entry, stimulate the acute-phase response in cattle (Arthington et al., 2005). This immune response, although an important component of the innate system (Carroll and Forsberg, 2007), has been negatively associated with performance traits (Qiu et al., 2007; Araujo et al., 2010). Accordingly, management strategies that lessen the magnitude of the acute-phase response have been shown to benefit DMI, BW gain, and production

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efficiency parameters in beef cattle (Arthington et al., 2008).

Supplementation of rumen-protected PUFA to feeder heifers during preconditioning and after transportation decreased concentrations of haptoglobin after feedlot entry (Araujo et al., 2010). These results indicated that PUFA supplementation might be a strategy to alleviate the acute-phase response stimulated by these stressors. However, feedlot calves supplemented with rumen-protected PUFA experienced reduced DMI and consequent ADG compared with cohorts offered control diets (Araujo et al., 2010). These outcomes can be attributed to several factors, including reduced ruminal diet digestibility, given that ruminal biohydrogenation of rumen-protected PUFA supplements can be increased in cattle consuming typical feedlot diets (Allen, 2000; Araujo et al., 2010). Therefore, we hypothesized that PUFA supplementation to feeder calves during preconditioning only would alleviate the acute-phase response elicited by transport and feedlot entry without impairing feedlot DMI and consequent performance.

Two experiments were conducted to evaluate the inclusion of PUFA into beef cattle diets. Experiment 1 compared DMI and ruminal in situ forage degradability variables in mature beef cows receiving or not receiving a PUFA-enriched diet. Experiment 2 compared plasma concentrations of acute-phase proteins, fatty acids, cytokines, and cortisol, in addition to performance and carcass variables of feeder steers receiving or not receiving a PUFA-enriched preconditioning diet.

MATERIALS AND METHODS

All animals used were cared for in accordance with acceptable practices and experimental protocols reviewed and approved by the Oregon State University, Institutional Animal Care and Use Committee.

Experiment 1 was conducted from September to November 2009 at the Eastern Oregon Agricultural Research Station, Oregon State University, Burns. Experiment 2 was conducted from September 2009 to June 2010 and was divided into a preconditioning phase (d −28 to 0), a growing phase (d 1 to 144), and a finishing phase (d 144 to slaughter). The preconditioning phase was conducted at the Eastern Oregon Agricultural Research Station, Oregon State University, Burns, whereas the growing (Top Cut; Echo, OR) and finishing (Beef Northwest; Boardman, OR) phases were conducted at commercial feedyards. Treatment ingredients provided during Exp. 1 and 2 were originated from the same batch. Similarly, hay provided throughout Exp. 1 and during the preconditioning phase of Exp. 2 was harvested from the same field during June 2009.

Exp. 1

Animals. Three Angus × Hereford nonlactating, nonpregnant, mature cows (724 ± 39 kg of BW), housed in individual dry-lot pens (11 × 21 m) and fitted with ruminal cannulas, were allocated to a 3 × 3 Latin square design containing 3 periods of 21 d each, which may not have accounted for potential carryover treatment effects across periods and could not be balanced for residual effects. Treatments consisted of grain-based supplements (Table 1) without (**NF**) or with the inclusion of a rumen-protected PUFA source (**PF**; Megalac-R, Church & Dwight, Princeton, NJ) or a rumen-protected SFA source (**SF**; Megalac, Church & Dwight). The SF treatment was included to serve as an isolipidic, isocaloric, and isonitrogenous control treatment to PF.

Diets. Treatments were fed daily (0700 h) at a rate of 4.83, 4.42, and 4.42 kg of DM per cow for NF, PF, and SF, respectively. Treatment intakes (DM basis) corresponded to 0.66, 0.61, and 0.61% of initial full BW for NF, PF, and SF, respectively. Mixed alfalfa-grass hay was offered throughout the experiment. Treatments and hay were not mixed, and treatments were readily and completely consumed by cows. Samples of hay and treatment ingredients 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 ether extract (method 2003.05; AOAC 2006), CP (method 984.13; AOAC, 2006), ADF (method 973.18 modified for use in an Ankom 200 fiber analyzer, Ankom Technology Corp., Fairport, NY; AOAC, 2006), and NDF (Van Soest et al., 1991; modified for use in an Ankom 200 fiber analyzer, Ankom Technology Corp.). Calculations for TDN used the equation proposed by Weiss et al. (1992), whereas NE_m and NE_g were calculated with the equations proposed by the NRC (1996). Further, samples of hay and treatment ingredients were analyzed for fatty acid content using gas-liquid chromatography (Model 6890 Series II, Hewlett-Packard, Avondale, PA) according to the procedures described by Scholljegerdes et al. (2007). Composition and nutritional profiles of treatments are described in Tables 1 and 2, and were calculated from samples collected at the beginning of the study. Hay quality, calculated from samples collected weekly, was (DM basis) 54% TDN, 64% NDF, 43% ADF, 0.96 Mcal/kg of NE_m, 0.40 Mcal/kg of NE_g, 17.4% CP, and 1.5% ether extract. The hay fatty acid profile is described in Table 2. Water and a commercial mineral and vitamin mix (Cattleman's Choice, Performix Nutrition Systems, Nampa, ID), containing 14% Ca, 10% P, 16% NaCl, 1.5% Mg, 3,200 mg/kg of Cu, 65 mg/kg of I, 900 mg/kg of Mn, 140 mg/kg of Se, 6,000 mg/kg of Zn, 136,000 IU/kg of vitamin A, 13,000 IU/kg of vitamin D_3, and 50 IU/kg of vitamin E, were offered for ad libitum consumption throughout the experiment.

Sampling. Cow full and shrunk BW were recorded 8 and 7 d, respectively, before the beginning of the experiment to calculate treatment intake. Within each experimental period, hay DMI was evaluated daily from d 1 to 13, whereas intake data from d 8 and 13 were used for treatment DMI comparison. From d 1 to 13, hay

Table 1. Ingredient composition, nutrient profile, and intake of treatments offered during Exp. 1 (n = 3 per treatment) and Exp. 2 (n = 6 per treatment)[1]

	Exp. 1			Exp. 2		
Item	NF	SF	PF	NF	SF	PF
Ingredient, % (as-fed basis)						
Cracked corn	89.0	74.6	74.6	86.3	72.5	72.5
Soybean meal	11.0	15.2	15.2	10.5	14.5	14.5
Megalac-R	0.0	0.0	10.2	0.0	0.0	9.4
Megalac	0.0	10.2	0.0	0.0	9.4	0.0
Mineral and vitamin mix[2]	0.0	0.0	0.0	3.2	3.6	3.6
Nutrient profile[3] (DM basis)						
NE_g,[4] Mcal/kg	1.46	1.72	1.72	1.40	1.63	1.64
NE_m,[4] Mcal/kg	2.21	2.58	2.59	2.05	2.38	2.40
CP, %	13.2	14.1	14.2	12.6	13.5	13.6
NDF, %	12.0	11.0	10.7	11.5	10.6	10.3
Ether extract, %	4.70	13.1	13.3	4.50	12.2	12.4
PUFA, %	2.69	3.09	5.22	2.58	2.87	4.86
Linoleic acid, %	2.60	2.95	4.85	2.49	2.75	4.52
Linolenic acid, %	0.08	0.10	0.31	0.08	0.09	0.29
SFA + MUFA, %	2.01	10.01	8.08	1.92	9.33	7.54
Ca, %	0.07	1.29	1.36	0.57	1.74	1.80
P, %	0.47	0.44	0.44	0.82	0.83	0.83
Daily intake[5]						
DM, kg	4.83	4.42	4.42	1.56	1.42	1.42
NE_g,[4] Mcal	7.05	7.60	7.60	2.20	2.31	2.33
NE_m,[4] Mcal	10.7	11.4	11.4	3.20	3.38	3.41
CP, kg	0.64	0.62	0.63	0.20	0.19	0.19
NDF, kg	0.58	0.48	0.47	0.18	0.15	0.14
Ether extract, g	227	579	588	70.2	173	176
PUFA, g	130	137	231	40.2	40.8	69.0
Linoleic acid, g	126	130	214	38.8	39.1	64.2
Linolenic acid, g	3.86	4.42	13.70	1.25	1.28	4.12
SFA + MUFA, g	97.1	442	357	30.0	132	107
Ca, g	3.40	57.0	60.1	9.05	24.8	25.7
P, g	22.9	19.6	19.6	12.8	11.8	11.8

[1]NF = grain-based concentrate without the addition of a supplemental fat source; SF = grain-based concentrate with the addition of a rumen-protected SFA source (Megalac, Church & Dwight Co. Inc., Princeton, NJ; PF = grain-based concentrate with the addition of a rumen-protected PUFA source (Megalac-R, Church & Dwight Co. Inc.).

[2]Cattleman's Choice (Performix Nutrition Systems, Nampa, ID); contained 14% Ca, 10% P, 16% NaCl, 1.5% Mg, 3,200 mg/kg of Cu, 65 mg/kg of I, 900 mg/kg of Mn, 140 mg/kg of Se, 6,000 mg/kg of Zn, 136,000 IU/kg of vitamin A, 13,000 IU/kg of vitamin D_3, and 50 IU/kg of vitamin E.

[3]Values obtained from a commercial laboratory wet chemistry analysis (Dairy One Forage Laboratory, Ithaca, NY). Fatty acid content was determined based on values in Table 2, and according to the procedures described by Scholljegerdes et al. (2007).

[4]Calculated with the following equations (NRC 1996): $NE_m = 1.37ME - 0.138ME^2 + 0.0105ME^3 - 1.12$; $NE_g = 1.42ME - 0.174ME^2 + 0.0122ME^3 - 0.165$, given that ME = 0.82 × DE, and 1 kg of TDN = 4.4 Mcal of DE.

[5]Estimated from the treatment consumption of the individual experimental unit.

Table 2. Fatty acid profile of feedstuffs offered to cattle during Exp. 1 and 2[1]

Fatty acid, %	Megalac[2]	Megalac-R[2]	Corn	Soybean meal	Hay
Palmitic acid (16:0)	52.9	31.6	13.9	16.4	25.0
Stearic acid (18:0)	4.6	4.4	1.8	4.2	3.2
Oleic acid (18:1)	31.6	28.2	24.8	11.1	3.7
Linoleic acid (18:2)	7.7	28.1	55.4	54.9	14.8
Linolenic acid (18:3)	0.3	2.5	1.6	9.0	21.2
Total PUFA	8.1	31.0	57.2	64.1	41.1
Total SFA + MUFA	91.9	69.0	42.8	35.9	58.9
Other	2.9	5.2	2.5	4.4	32.1

[1]As a percentage of total fatty acids. All feedstuffs were analyzed for fatty acid content according to the procedures described by Scholljegerdes et al. (2007).

[2]Church & Dwight Co. Inc. (Princeton, NJ). Megalac served as the SFA supplement, whereas Megalac-R served as the PUFA supplement offered to animals in Exp. 1 and 2.

was offered in amounts to ensure ad libitum intake, and orts were collected and weighed daily. Samples of the offered and nonconsumed hay were collected daily from each pen and dried for 96 h at 50°C in forced-air ovens for DM calculation. Initial shrunk BW was used for calculation of DMI as a percentage of BW. From d 14 to 21, cows were limited to receive 90% of their voluntary hay DMI determined from d 1 to 13. Immediately before treatments were provided on d 16, Dacron bags (50 ± 10 μm pore size; Ankom Technology Corp.) containing 4 g (DM basis) of ground dietary hay (2-mm screen; Wiley Mill, Model 4, Arthur H. Thomas, Philadelphia, PA) were suspended in the ventral rumen of each cow and incubated in triplicate for 0, 4, 8, 12, 24, 36, 48, 72, and 96 h. Before ruminal incubation, all bags were soaked in warm water (39°C) for 15 min. After ruminal incubation, bags were washed repeatedly with running water until the rinse water was colorless and were subsequently dried for 96 h at 50°C in forced-air ovens. The 0-h bags were not incubated in the rumen but were subjected to the same soaking, rinsing, and drying procedures applied to the ruminally incubated bags. Dried samples were weighed for residual DM determination, and then triplicates were combined and analyzed for NDF (Robertson and Van Soest, 1981) using procedures modified for use in an Ankom 200 fiber analyzer (Ankom Technology Corp.).

Statistical Analysis. All data were analyzed using cow as the experimental unit and were initially tested for normality with the Shapiro-Wilk test from the UNIVARIATE procedure (SAS Inst. Inc., Cary, NC). All data were normally distributed (W ≥0.90). Voluntary forage and total DMI were analyzed using the MIXED procedure of SAS and the Satterthwaite approximation to determine the denominator degrees of freedom for the tests of fixed effects. The model statement contained the effects of treatment, day, and their interaction, in addition to period as an independent variable. Data were analyzed using cow as the random variable. The specified term for the repeated statement was day, the subject was cow(period × treatment), and the covariance structure used was autoregressive, which provided the best fit for these analyses according to the Akaike information criterion. Kinetic parameters of hay DM and NDF disappearance were estimated using the nonlinear regression procedures of SAS, as described by Vendramini et al. (2008) but without inference for lag time attributable to the sampling schedule adopted herein (Fadel, 1992). Treatment effects on ruminal in situ forage degradability parameters were also analyzed using the MIXED procedure of SAS and the Satterthwaite approximation to determine the denominator degrees of freedom for the tests of fixed effects. The model statement contained the effects of treatment, in addition to period as an independent variable. Data were analyzed using cow as the random variable. Results are reported as least squares means and were separated using the PDIFF option of SAS. 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.

Exp. 2

Animals. Seventy-two Angus × Hereford steers (initial BW 207 ± 2.6 kg; initial age 183 ± 1.6 d), weaned at 7 mo of age (d −55), were ranked by BW on d −30 of the study and allocated to 18 dry-lot pens (8.5 × 21 m; 4 steers/pen). Pens were randomly assigned to receive NF, PF, or SF (Table 1; 6 pens/treatment) during the preconditioning phase (d −28 to 0). On the morning of d 0, steers were combined into 1 group, loaded into a commercial livestock trailer, and transported to the growing lot. The travel time was 10 h; however, steers were maintained in the trailer for a total of 24 h before being unloaded (d 1) to simulate the stress challenge of a long haul (Arthington et al., 2008). On d 144, steers were moved to an adjacent finishing yard, where they remained until slaughter. During both the growing and finishing phases, all steers were maintained in a common pen, managed similarly, and received the same diet, which did not contain any of the preconditioning treatments. Slaughter date was determined according to days on feed (**DOF**) in the finishing yard and availability at the commercial packing facility (Tyson Fresh Meats Inc., Pasco, WA). As a result, steers were slaughtered on 2 separate dates, 15 d apart, irrespective of the preconditioning treatment (d 244 and 259; 36 steers slaughtered at each date). Within treatments, average DOF at the finishing yard were 107 ± 2 for NF, 108 ± 2 for PF, and 108 ± 2 for SF.

All steers were administered Clostrishield 7 and Virashield 6 + Somnus (Novartis Animal Health, Bucyrus, KS) at approximately 30 d of age, and One Shot Ultra 7, Bovi-Shield Gold 5, TSV-2, and Dectomax (all from Pfizer Animal Health, New York, NY) at weaning (d −55). Steers received a booster of Bovi-Shield Gold 5, UltraChoice 7, and TSV-2 (all from Pfizer Animal Health) at the beginning of the study (d −28). On d 3 of the growing phase, all steers were again administered Bovi-Shield Gold 5 and Dectomax (both from Pfizer Animal Health) after blood samples were collected, thus preventing any confounding effects between stress and vaccination on the acute-phase measures evaluated herein (Stokka et al., 1994). At the beginning of the finishing phase, all steers were administered Pyramid 5 (Fort Dodge Animal Health, Overland Park, KS), Caliber 7 (Boehringer Ingelheim Vetmedica Inc., St. Joseph, MO), Valbazen (Pfizer Animal Health), and ProMectin (Ivermectin, Vedco Inc., St. Joseph, MO) and were implanted with Component TE-S (VetLife, West Des Moines, IA). No incidences of mortality or morbidity were observed during the entire experiment.

Diets. Treatments were fed daily during the preconditioning phase only (0800 h) at a rate of 1.56, 1.42, and 1.42 kg of DM per steer for NF, PF, and SF, respectively. Treatment intakes (DM basis) corresponded to

Table 3. Ingredient composition of growing and finishing diets offered to steers in Exp. 2

	Growing phase[1]				Finishing phase[2]				
Ingredient, % (as-fed basis)	A	B	C	D	A	B	C	D	E
Alfalfa hay	44.0	34.5	20.0	11.8	25.8	17.3	8.6	3.4	3.4
Wet distillers grain	15.0	15.0	20.0	20.0	25.0	21.1	17.9	15.5	15.5
Corn silage	10.0	15.0	20.0	20.0	0.0	0.0	0.0	0.0	0.0
High-moisture corn	6.0	10.0	6.0	12.0	0.0	0.0	10.0	11.3	11.3
Steam-flaked corn	0.0	0.0	0.0	0.0	40.2	39.3	29.4	29.9	29.9
Wheat screenings	10.0	12.5	6.0	5.0	0.0	0.0	0.0	0.0	0.0
Potato slurry	10.0	10.0	25.0	30.0	0.0	10.0	16.8	22.5	22.5
Culled french fries	0.0	0.0	0.0	0.0	0.0	5.0	10.0	14.1	14.1
Vegetable oil	0.0	0.0	0.0	0.0	0.0	0.3	0.3	0.3	0.3
Mineral and vitamin mix	5.0	3.0	3.0	3.0	9.0	7.0	3.0	3.0	3.0

[1]A = offered from d 1 to 11; B = offered from d 12 to 22; C = offered from d 23 to 93; D = offered from d 93 to 144.

[2]A = offered from d 145 to 155; B = offered from d 156 to 166; C = offered from d 167 to 177; D = offered from d 178 to 208; E = offered from d 209 to slaughter.

0.68, 0.62, and 0.62% of expected preconditioning average full BW (230 kg) for NF, PF, and SF, respectively. Mixed alfalfa-grass hay was offered in amounts to ensure ad libitum access during the preconditioning phase (d −28 to 0). Treatments and hay were not mixed, and treatments were readily and completely consumed by steers. Samples of hay and treatment ingredients fed during the preconditioning phase were analyzed for nutrient content as in Exp. 1 (Tables 1 and 2). Water was offered for ad libitum consumption throughout the preconditioning phase. All steers were offered the same diets during both the growing and finishing phases, according to the management procedures of the respective growing and finishing yards (Table 3). Diets offered to steers during the preconditioning, growing, and finishing phases were formulated to exceed NRC (1996) maintenance recommendations for growing and finishing cattle, and to meet NRC (1996) requirements for the growth rates described herein.

Sampling. Steer shrunk BW was collected at the beginning of the experiment (d −30) and in the finishing phase (d 144) after 16 h of feed and water restriction. Shrunk BW was also recorded on d 1 immediately after unloading at the feedyard. Final BW was calculated based on HCW adjusted to a 63% dressing percentage (Loza et al., 2010). Growth rates were determined using BW values obtained on d −30 and 1 (preconditioning ADG), d 1 and 144 (growing phase ADG), in addition to d 144 and final BW (finishing phase ADG).

During the preconditioning phase, pen voluntary hay intake was recorded daily. Hay was offered in amounts to ensure ad libitum intake and orts were collected and weighed daily. Samples of the offered and nonconsumed hay were collected daily from each pen and dried for 96 h at 50°C in forced-air ovens for DM calculation. Estimated duodenal flow of linoleic and linolenic acids during the preconditioning phase were calculated based on the treatment and hay intake of each pen, feed nutritional analysis, and 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), which has been shown to estimate intestinal fatty acid flow adequately in cattle (Moate et al., 2004). Average shrunk BW during the preconditioning phase (values obtained on d −30 and 1) was used for calculation of DMI as a percentage of BW. Total BW gain achieved during the preconditioning phase was divided by total DM consumed during the same period for G:F calculation. During the growing and finishing phases, DMI and G:F were not evaluated. Blood samples were collected on d −30, 0, 1, and 3. All samples were analyzed for concentrations of cortisol, ceruloplasmin, haptoglobin, and fatty acids. Samples collected on d 0, 1, and 3 were also analyzed for concentrations of IL-1β, IL-6, and tumor necrosis factor (**TNF**)-α. Steer rectal temperature was assessed with a digital thermometer (GLA M750 digital thermometer, GLA Agricultural Electronics, San Luis Obispo, CA) concurrently with each blood collection. Hot carcass weight was collected at slaughter. After a 24-h chill, trained personnel assessed carcass backfat thickness at the 12th-rib and LM area, whereas all other carcass measures were recorded from a USDA grader.

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, and were immediately placed on ice. Samples were centrifuged at 2,500 × *g* for 30 min at 4°C for plasma collection and stored at −80°C on the same day of collection.

Concentrations of cortisol were determined using a bovine-specific commercial ELISA kit (Endocrine Technologies Inc., Newark, CA). Concentrations of ceruloplasmin and haptoglobin were determined according to procedures described previously (Demetriou et al., 1974; Makimura and Suzuki, 1982). All samples were analyzed in duplicate. For all assays, each plate was

balanced for treatment, whereas repeated samples from each calf were analyzed within the same plate. The intra- and interassay CV were, respectively, 2.9 and 5.6% for haptoglobin, 12.9 and 14.6% for ceruloplasmin, and 7.6 and 11.9% for cortisol. Concentrations of IL-1β, IL-6, interferon-γ, and TNF-α were determined by a multiplex bovine-specific ELISA (SearchLight, Aushon Biosystems Inc., Billerica, MA).The intra- and interassay CV for cytokines were less than 15%. Plasma samples were also analyzed for fatty acid content using gas-liquid chromatography (Model 6890 Series II, Hewlett-Packard) according to the procedures described by Scholljegerdes et al. (2007)

Statistical Analysis. All data were analyzed using pen as the experimental unit and were initially tested for normality with the Shapiro-Wilk test from the UNIVARIATE procedure of SAS. Only cytokine data were not normally distributed (W $\leq$0.69); therefore, they were log-transformed to achieve normality (W $\geq$0.90). Performance and physiological data were analyzed using the MIXED procedure of SAS and the Satterthwaite approximation to determine the denominator degrees of freedom for the tests of fixed effects. The model statement used for plasma measurements (d 0, 1, and 3 only) and DMI contained the effects of treatment, day, and their interaction. Data were analyzed using pen(treatment), in addition to calf(pen) for plasma measurements only, as random variables. The specified term for the repeated statement was day, the subject was calf(pen) for plasma measurements and pen(treatment) for DMI, and the covariance structure used was autoregressive, which provided the best fit for these analyses according to the Akaike information criterion. The model statement used for analysis of plasma measurements on d −30, nutrient intake, estimated duodenal flow of linoleic and linolenic acids, ADG, G:F, and DOF contained only the effects of treatment, whereas the random variable was pen(treatment). The model statement used for carcass trait analysis contained the effects of treatment, whereas DOF was included as a covariate. The random variable was pen(treatment). The GLIMMIX procedure of SAS was also used to evaluate the proportion of carcasses grading choice. The model statement contained the effects of treatment, with DOF included as a covariate, whereas the random variable was pen(treatment). All results are reported as least squares means and were separated using the PDIFF option of SAS. 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 treatment effects if no interactions were significant, or according to the highest-order interaction detected.

RESULTS AND DISCUSSION

Exp. 1

This experiment was conducted to evaluate the effects of PUFA supplementation on ruminal in situ forage degradability parameters in beef cattle receiving treatments similar to those in Araujo et al. (2010) and Exp. 2, and to determine whether reduced DMI in PUFA-supplemented cattle was due to impaired ruminal forage degradability (Araujo et al., 2010).

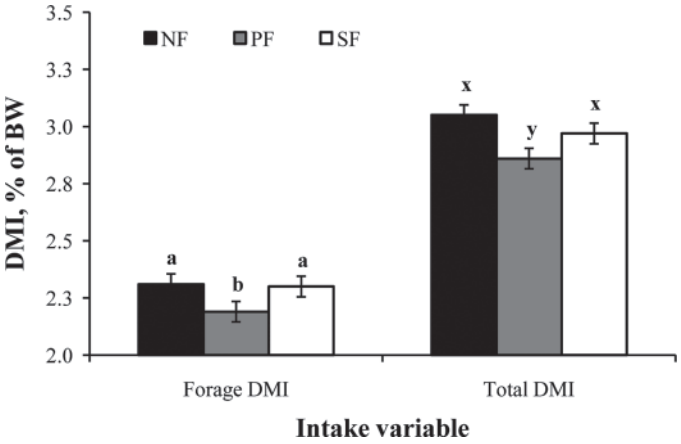

Figure 1. Mean (±SEM) forage and total DMI, as a percentage of BW, of mature cows offered forage-based diets without (NF; n = 3) or with the inclusion (500 g/cow daily; as-fed basis) of a rumen-protected SFA (SF; Megalac, Church & Dwight Co. Inc., Princeton, NJ; n = 3) or a PUFA (PF; Megalac-R, Church & Dwight Co. Inc.; n = 3) in Exp. 1. A treatment effect was detected ($P = 0.05$ and 0.01 for forage and total DMI, respectively). Within variable, values bearing a different letter differ at $P = 0.05$ for the forage DMI comparison (a, b), and at $P = 0.04$ (PF vs. SF) and $P = 0.01$ (PF vs. NF) for the total DMI comparison (x, y).

Cows receiving PF had decreased voluntary forage intake and total DMI compared with cows receiving SF ($P = 0.05$) and NF ($P = 0.04$ and 0.01, respectively), whereas no differences were detected between cows receiving SF and NF (Figure 1). These results support previous efforts indicating that rumen-protected PUFA supplementation reduced DMI in cattle, even when compared with rumen-protected SFA sources (Araujo et al., 2010). One could speculate that the reduced DMI in cows fed PF was due to impaired forage digestibility (Allen, 2000). However, in the present study, no treatment effects were detected for ruminal disappearance rates of hay DM and NDF (Table 4). Similarly, no treatment effects were detected for effective ruminal degradability of hay DM and NDF (Table 4).

These results indicate that PF did not affect ruminal in situ forage degradability, but did decrease forage and total DMI in beef cows. Hess et al. (2008) suggested that inclusion of supplemental fat up to 3% of dietary DM is recommended to maximize the use of forage-based diets and to prevent impaired forage digestibility and intake. In the present study, according to the DMI evaluation and feed nutritional analysis, supplemental fat was included at 1.3, 3.5, and 3.6% of dietary DM for cows fed NF, SF and PF, respectively. Given that forage and total DMI were similar between cows fed SF and NF, the SF and PF treatments provided similar amounts of supplemental fat, and ruminal in situ forage degradability variables were similar among treatments, the treatment differences detected herein for forage and

Table 4. Ruminal in situ DM and NDF disappearance kinetics and effective degradability of mixed alfalfa-grass hay incubated in mature cows offered forage-based diets without (NF; n = 3) or with the inclusion of a rumen-protected SFA (SF; n = 3) or PUFA (PF; n = 3) source in Exp. 1[1]

	Treatment				*P*-value			
Item	NF	SF	PF	SEM	Main[2]	NF vs. SF[3]	NF vs. PF[3]	SF vs. PF[3]
Ruminal disappearance rate, %/h								
DM	6.86	6.81	7.49	0.40	0.48	0.91	0.24	0.27
NDF	6.05	6.17	6.43	0.30	0.69	0.77	0.42	0.57
Effective degradability[4]								
DM, % of total DM	56.8	57.0	57.4	0.53	0.76	0.76	0.49	0.66
NDF, % of total NDF	65.1	65.3	65.8	0.42	0.60	0.73	0.35	0.53

[1]NF = grain-based concentrate without the addition of a supplemental fat source; SF = grain-based concentrate with the addition (500 g/cow daily; as-fed basis) of a rumen-protected SFA source (Megalac, Church & Dwight Co. Inc., Princeton, NJ); PF = grain-based concentrate with the addition (500 g/cow daily; as-fed basis) of a rumen-protected PUFA source (Megalac-R, Church & Dwight Co. Inc.).

[2]Main treatment effects.

[3]Calculated using the PDIFF option (SAS Inst. Inc., Cary, NC) for means separation.

[4]Calculated by fixing the ruminal passage rate at 0.046/h (Poore et al., 1990) and using the model proposed by Ørskov and McDonald (1979).

total DMI should not be attributed to PUFA inclusion rate. Accordingly, Leupp et al. (2006) reported that ruminal forage digestibility was not affected in forage-fed steers offered supplemental PUFA at 4% of dietary DM. Further, the PUFA and SFA sources used herein were based on Ca-soaps of fatty acids, whereas the majority of fat sources included in the review by Hess et al. (2008) were not rumen protected. Rumen-protected fatty acid sources yield reduced amounts of FFA available for ruminal biohydrogenation compared with nonprotected fats (Jenkins, 1993); therefore, dietary inclusion of rumen-protected fats is likely greater than the limits proposed by Hess et al. (2008), Nevertheless, optimal inclusion rates of rumen-protected fat supplements into forage-based beef cattle diets are yet to be determined.

However, Ca-soaps of fatty acids rich in PUFA can be dissociated into FFA and Ca in the rumen to a greater extent than Ca-soaps rich in SFA when ruminal pH is reduced (Sukhija and Palmquist, 1990), whereas FFA are highly susceptible to ruminal biohydrogenation and can impair ruminal digestibility parameters (Harfoot and Hazlewood, 1988). In the present study, however, ruminal pH was not evaluated, but cows were offered high-fiber diets (Table 1) that likely prevented reduced ruminal pH (Nagaraja and Titgemeyer, 2007) and consequent dissociation and biohydrogenation of Ca-soaps of both fatty acid sources (Sukhija and Palmquist, 1990). Treatment effects detected for intake variables should also not be associated with the chemical composition of the PUFA source (Ngidi et al., 1990; Simas et al., 1995; Bateman et al., 1996) or with dietary palatability (Grummer et al., 1990), given that both SF and PF sources were based on Ca-soaps of fatty acids, whereas treatments were offered separately from hay and were readily as well as completely consumed by cows. On the other hand, PUFA supplementation can impair DMI by other means, such as reducing gut motility and enhancing cholecystokinin release, even when compared with SFA (Drackley et al., 1992; Allen, 2000). These physiological mechanisms may help explain why the PUFA source offered herein and by Araujo et al. (2010) was detrimental to feed intake; therefore, further research should be conducted to address this subject.

In summary, inclusion of a rumen-protected PUFA source in forage-based diets reduced forage and DMI in beef cows; however, ruminal in situ forage degradability variables were not affected. Therefore, additional research is required to determine the negative effects of supplemental PUFA as Ca-soaps of fatty acids on feed intake of forage-fed beef cattle, including assessment of physiological mechanisms associated with digestive function.

Exp. 2

No treatment effects were detected (data not shown) for rectal temperatures or for plasma concentrations of cortisol ($P = 0.98$), ceruloplasmin ($P = 0.72$), haptoglobin ($P = 0.73$), and total and individual fatty acids ($P = 0.30$ and ≥ 0.24) on d −30. This outcome illustrates the similar management condition by which the steers were maintained before the beginning of the experiment. Therefore, all physiological measurements obtained on d −30 were excluded from subsequent analysis.

No treatment effects were detected on calf preconditioning ADG and G:F (Table 5). However, similar to Exp. 1, mean DMI during preconditioning tended ($P = 0.09$) to be reduced for calves fed PF compared with those fed SF and NF (Table 5). Nevertheless, differences detected in DMI were not substantial enough to affect calf preconditioning performance (Table 5). Supporting this rationale, based on the hay and treatment consumption of each pen, no treatment effects were detected (data not shown) for steer average daily NE_m intake (8.07, 8.07, and 8.31 Mcal/steer for NF, PF, and SF, respectively; $P = 0.37$; SEM = 0.13), NE_g intake (4.24, 4.26, and 4.36 Mcal/steer for NF, PF, and

Table 5. Performance and physiological responses of steers offered forage-based preconditioning diets without (NF; n = 6) or with the inclusion of a rumen-protected SFA (SF; n = 6) or PUFA (PF; n = 6) source in Exp. 2[1]

	Treatment				*P*-value			
Item	NF	SF	PF	SEM	Main[2]	NF vs. SF[3]	NF vs. PF[3]	SF vs. PF[3]
Performance trait								
Shrunk BW (d −32), kg	208	208	207	5	0.98	0.92	0.91	0.83
Shrunk BW (d 1), kg	234	236	234	5	0.93	0.79	0.93	0.73
Preconditioning phase ADG,[4] kg	0.83	0.87	0.84	0.06	0.85	0.61	0.94	0.66
Preconditioning phase G:F,[5] kg/kg	0.148	0.153	0.134	0.011	0.52	0.77	0.42	0.28
Preconditioning DMI, % of BW	3.07	3.06	2.94	0.05	0.10	0.88	0.09	0.09
Shrunk BW (d 144), kg	402	409	413	7	0.53	0.46	0.27	0.70
Growing phase ADG,[6] kg	1.17	1.20	1.25	0.02	0.05	0.27	0.02	0.19
Final BW,[7] kg	626	629	637	9	0.67	0.80	0.38	0.53
Finishing phase ADG,[8] kg	2.10	2.05	2.09	0.05	0.80	0.52	0.86	0.65
Days on feed	107	108	108	2	0.85	0.78	0.56	0.76
Physiological response[9]								
Rectal temperature, °C	39.7	39.7	39.8	0.05	0.63	0.81	0.50	0.36
Haptoglobin, 450 nm × 100	3.99	4.43	3.65	0.50	0.55	0.53	0.64	0.29
Ceruloplasmin, mg/dL	26.0	26.5	27.5	0.95	0.52	0.72	0.27	0.45
Cortisol, ng/mL	36.7	36.7	28.7	4.0	0.29	0.99	0.18	0.18
IL-6, pg/mL (log)	0.88	0.56	0.79	0.26	0.68	0.39	0.70	0.63
IL-1β, pg/mL (log)	1.51	1.15	1.46	0.15	0.19	0.11	0.81	0.15

[1]NF = grain-based concentrate without the addition of a supplemental fat source; SF = grain-based concentrate with the addition (150 g/steer daily; as-fed basis) of a rumen-protected SFA source (Megalac, Church & Dwight Co. Inc., Princeton, NJ); PF = grain-based concentrate with the addition (150 g/steer daily; as-fed basis) of a rumen-protected PUFA source (Megalac-R, Church & Dwight Co. Inc.). Treatments were offered during the preconditioning phase only (d −28 to 0). All steers were transported for 24 h to a commercial growing lot on d 0, where none of the preconditioning treatments was offered.

[2]Main treatment effects.

[3]Calculated using the PDIFF option (SAS Inst. Inc., Cary, NC) for means separation.

[4]Calculated using shrunk BW obtained on d −32 and 1.

[5]Calculated by dividing the total DM consumed from d −28 to 0 into the total shrunk BW gain achieved over this time period.

[6]Calculated using shrunk BW obtained on d 1 and 144.

[7]HCW adjusted to a 63% dressing percentage (Loza et al., 2010).

[8]Calculated using shrunk BW obtained on d 144 and final BW.

[9]Measurements obtained on d 0 (before loading), d 1 (immediately after unloading), and d 3. No treatment × day interaction was detected ($P \geq 0.35$) for any of the variables reported; therefore, values are presented as least squares means across sampling days.

SF, respectively; $P = 0.30$; SEM = 0.06), and CP intake (1.07, 1.04, and 1.09 kg/steer for NF, PF, and SF, respectively; $P = 0.41$; SEM = 0.10).

A treatment effect was detected ($P < 0.05$; Table 6) for plasma concentrations of stearic acid, oleic acid, CLA, arachidonic acid, eicosapentaenoic acid, docosapentaenoic acid, docosahexaenoic acid, and MUFA. A treatment × day interaction was detected ($P < 0.05$; Table 7) for plasma concentrations of total fatty acids, palmitic acid, linoleic acid, linolenic acid, SFA, PUFA, and n-6 fatty acids. Supporting our findings, previous research reported that fat supplementation increased total fatty acids, whereas PUFA supplementation increased total fatty acids and PUFA concentrations in plasma of forage-fed beef cattle (Lake et al., 2007; Scholljegerdes et al., 2007). Within plasma PUFA, steers fed PF had greater ($P < 0.05$) concentrations of linoleic acid, CLA, and total n-6 fatty acids compared with those fed SF and NF, and had greater ($P \leq 0.04$) concentrations of arachidonic, docosapentaenoic, and docosahexaenoic acids compared with steers fed NF only (Tables 6 and 7). On the other hand, steers fed PF had reduced ($P < 0.05$) plasma concentrations of linolenic and eicosapentaenoic acids and tended ($P \leq 0.07$) to have reduced total n-3 fatty acids compared with those fed NF and SF (Tables 6 and 7). According to the treatment and hay intake of each pen, feed nutritional analysis, and the CPM-Dairy model (version 3.08.01), the average daily duodenal flow of linoleic and linolenic acids per steer was greater ($P < 0.01$; data not shown) for PF compared with NF and SF (23.85, 6.75, and 10.58 g/d of linoleic acid, SEM = 0.22; and 2.22, 0.67, and 0.88 g/d of linolenic acid, SEM = 0.03; respectively), and was also greater ($P < 0.01$; data not shown) for SF compared with NF. Others have suggested that the plasma fatty acid profile typically reflects duodenal fatty acid flow (Archibeque et al., 2005; Scholljegerdes et al., 2007; Hess et al., 2008). Upon absorption, linoleic acid serves as a precursor of arachidonic acid, whereas linolenic acid serves as a precursor of eicosapentaenoic and docosahexaenoic acids (Yaqoob and Calder, 2007). Therefore, it was expected that steers fed PF would have greater plasma concentrations of linoleic and linolenic acids and their respective derivates compared with their cohorts fed NF and SF. However, other physiological mechanisms that may alter the plasma fatty

Table 6. Plasma fatty acid concentrations (mg/g of dried plasma) of steers offered forage-based preconditioning diets without (NF; n = 6) or with the inclusion of a rumen-protected SFA (SF; n = 6) or PUFA (PF; n = 6) source in Exp. 2[1,2]

	Treatment				*P*-value			
Item	NF	SF	PF	SEM	Main[3]	NF vs. SF[4]	NF vs. PF[4]	SF vs. PF[4]
Stearic acid (18:0)	3.65	4.50	4.74	0.09	<0.01	<0.01	<0.01	0.10
Oleic acid (18:1 *cis*-9)	2.22	3.18	2.08	0.07	<0.01	<0.01	0.18	<0.01
CLA (18:2 *cis*-9, *trans*-11)	0.035	0.043	0.057	0.003	<0.01	0.08	<0.01	0.01
Arachidonic acid (20:4n-6)	0.615	0.799	0.797	0.021	<0.01	<0.01	<0.01	0.95
Eicosapentaenoic acid (20:5n-3)	0.302	0.307	0.250	0.013	0.02	0.77	0.01	<0.01
Docosapentaenoic acid (22:5n-3)	0.374	0.407	0.415	0.012	0.05	0.05	0.03	0.66
Docosahexaenoic acid (22:6n-3)	0.127	0.138	0.145	0.006	0.10	0.15	0.04	0.44
Total MUFA	3.67	4.68	3.45	0.10	<0.01	<0.01	0.17	<0.01
Total n-3 fatty acids	1.86	1.86	1.69	0.06	0.10	0.96	0.06	0.07

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

[2]Treatments were offered during the preconditioning phase only (d −30 to 0). All steers were transported for 24 h to a commercial growing lot, where none of the preconditioning treatments were offered. Blood samples were collected on d 0 (before loading), d 1 (immediately after unloading), and d 3.

[3]Main treatment effects.

[4]Calculated using the PDIFF option (SAS Inst. Inc., Cary, NC) for means separation.

acid profile, such as tissue incorporation and enzymatic activity (Archibeque et al., 2005; Scholljegerdes et al., 2007), were not evaluated in the present experiment. It is also important to note that plasma PUFA concentrations were greater in steers fed PF compared with those fed SF and NF on d 3, approximately 72 h after treatments were withdrawn, indicating that circulating availability and perhaps tissue concentrations of these fatty acids were increased in steers fed PF during transport and in the initial days of the growing phase.

No treatment effects were detected for rectal temperatures and for plasma concentrations of cortisol, ceruloplasmin, haptoglobin, IL-1β, and IL-6 (Table 5). Day effects ($P < 0.05$; Table 8) were detected for all these measurements. Cortisol and ceruloplasmin concentrations peaked on d 1, whereas concentrations of haptoglobin, IL-1β, and IL-6 and rectal temperature peaked on d 3 (Table 8). A treatment × day interaction was detected ($P = 0.04$; Figure 2) for plasma concentrations of TNF-α. After transportation, concentration of TNF-α increased for steers fed NF (day effect, $P = 0.08$), did not change for steers fed SF (day effect, $P = 0.47$), but decreased for steers fed PF (day effect, $P = 0.05$). As a consequence, steers fed PF had reduced ($P = 0.03$) plasma TNF-α concentrations compared with those fed NF on d 3 (Figure 2). These results indicate that, independently of treatment, steers experienced an acute-phase response characterized by an increased rectal temperature and circulating concentrations of acute-phase proteins and proinflammatory cytokines (Carroll and Forsberg, 2007; Arthington et al., 2008). To our knowledge, this is the first study demonstrating a proinflammatory cytokine response after transportation and feedlot entry. Further, treatment effects detected on plasma concentrations of TNF-α suggest that PUFA supplementation during preconditioning alleviated, at least partially, the inflammatory response elicited by transportation and feedlot entry, whereas similar cortisol concentrations indicated that steers from all treatments experienced a similar stress challenge during the same period (Crookshank et al., 1979; Sapolsky et al., 2000). Araujo et al. (2010) reported that feeder heifers offered preconditioning and receiving forage-based diets enriched with PUFA had reduced concentrations of haptoglobin compared with control cohorts during the initial 8 d after transport. Other studies using a similar transportation model as the one herein evaluated plasma concentrations of acute-phase proteins up to 28 d after transport, and reported treatment responses on or after 3 d after transport (Arthington et al., 2005, 2008). Therefore, if in the present study blood samples had been collected beyond d 3, perhaps treatment effects on haptoglobin and ceruloplasmin would have been detected, particularly because TNF-α has been shown to stimulate hepatic synthesis of acute-phase proteins individually (Yoshioka et al., 2002). The reason for the lack of treatment effects on plasma IL-1β and IL-6 is unknown, given that these cytokines and TNF-α are the main components of the T-helper 1 inflammatory response (Carroll and Forsberg, 2007). However, the biological activity of these cytokines is highly pleiotropic, redundant, and complex (Ozaki and Leonard, 2002), whereas other authors have also reported different individual responses of these cytokines to PUFA supplementation (Rezamand et al., 2009). Given the lack of research studies evaluating the proinflammatory cytokine response on transportation and feedlot entry, and the substantial role of these cytokines on cattle health and performance (Klasing and Korver, 1997), additional research is warranted to further investigate

Table 7. Plasma fatty acid concentrations (mg/g of dried plasma) of steers offered forage-based preconditioning diets without (NF; n = 6) or with the inclusion of a rumen-protected SFA (SF; n = 6) or PUFA (PF; n = 6) source in Exp. 2[1,2]

	Treatment				*P*-value			
Item	NF	SF	PF	SEM	Interaction[3]	NF vs. SF[4]	NF vs. PF[4]	SF vs. PF[4]
Palmitic acid (16:0)				0.09				
d 0	2.74	4.65	4.16			<0.01	<0.01	<0.01
d 1	2.52	3.73	3.57		<0.01	<0.01	<0.01	0.25
d 3	2.71	3.56	3.37			<0.01	<0.01	0.15
Linoleic acid (18:2)				0.29				
d 0	6.41	12.37	14.37			<0.01	<0.01	<0.01
d 1	4.99	9.25	12.03		<0.01	<0.01	<0.01	<0.01
d 3	5.33	8.98	10.81			<0.01	<0.01	<0.01
Linolenic acid (18:3)				0.04				
d 0	1.25	1.14	0.97			0.10	<0.01	0.02
d 1	0.91	0.89	0.83		0.01	0.71	0.22	0.38
d 3	1.02	0.99	0.84			0.68	0.01	0.03
Total SFA				0.22				
d 0	7.90	10.77	10.48			<0.01	<0.01	0.37
d 1	6.04	8.12	8.33		0.01	<0.01	<0.01	0.52
d 3	6.37	7.86	7.82			<0.01	<0.01	0.88
Total PUFA				0.37				
d 0	9.21	15.50	17.13			<0.01	<0.01	<0.01
d 1	7.81	12.48	15.29		<0.01	<0.01	<0.01	<0.01
d 3	8.34	12.26	14.08			<0.01	<0.01	<0.01
Total n-6 fatty acids				0.32				
d 0	7.31	13.64	15.55			<0.01	<0.01	<0.01
d 1	6.02	10.65	13.48		<0.01	<0.01	<0.01	<0.01
d 3	6.44	10.36	12.37			<0.01	<0.01	<0.01
Total fatty acids				0.66				
d 0	21.93	32.53	32.54			<0.01	<0.01	0.98
d 1	17.89	25.75	27.47		<0.01	<0.01	<0.01	0.08
d 3	18.31	24.42	25.17			<0.01	<0.01	0.44

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

[2]Treatments were offered during the preconditioning phase only (d −30 to 0). All steers were transported for 24 h to a commercial growing lot, where none of the preconditioning treatments were offered. Blood samples were collected on d 0 (before loading), d 1 (immediately after unloading), and d 3.

[3]Treatment × day interactions.

[4]Calculated using the PDIFF option (SAS Inst. Inc., Cary, NC) for means separation.

the effects of stressful management procedures on pro-inflammatory cytokines, and to develop strategies to modulate this response.

During the growing phase, steers fed PF had greater ($P = 0.02$) ADG compared with those fed NF, but had ADG similar to those fed SF (Table 5). No differences

Table 8. Day effects on rectal temperatures, and plasma concentrations of acute-phase proteins, cytokines, and cortisol of transported feeder steers (n = 72) in Exp. 2[1]

	Day of study				*P*-value			
Item	0	1	3	SEM	Main[2]	0 vs. 1[3]	0 vs. 3[3]	1 vs. 3[3]
Rectal temperature, °C	39.76	39.49	39.97	0.04	<0.01	<0.01	<0.01	<0.01
Haptoglobin, 450 nm × 100	3.77	3.85	4.45	0.32	0.04	0.76	0.02	0.02
Ceruloplasmin, mg/dL	24.9	28.6	26.5	0.6	<0.01	<0.01	<0.01	<0.01
Cortisol, ng/mL	27.3	43.1	31.7	2.5	<0.01	<0.01	<0.01	<0.01
IL-6, pg/mL (log)	0.50	0.77	0.91	0.17	0.05	0.12	0.04	0.42
IL-1β, pg/mL (log)	1.26	1.34	1.51	0.09	<0.01	0.15	<0.01	<0.01

[1]Steers were loaded into a commercial livestock trailer on d 0, and transported to a commercial feedyard. The total travel time was 10 h, but steers were maintained in the truck for a total of 24 h before being unloaded (d 1). All measurements were obtained on d 0 (before loading), d 1 (immediately after unloading), and d 3.

[2]Main day effects.

[3]Calculated using the PDIFF option (SAS Inst. Inc., Cary, NC) for means separation.

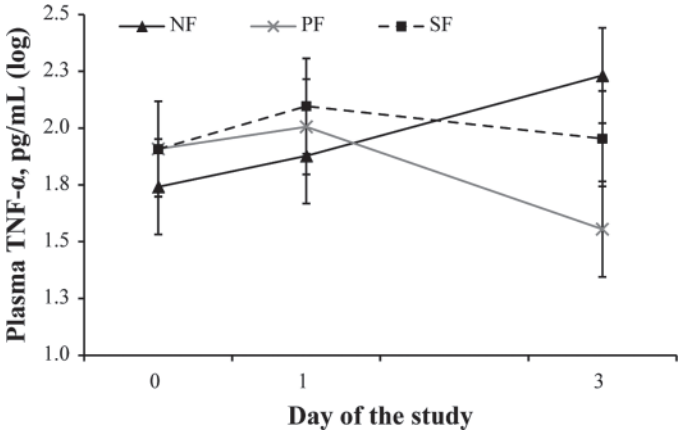

Figure 2. Plasma tumor necrosis factor (TNF)-α concentrations (±SEM) of steers offered forage-based preconditioning diets without (NF; n = 6) or with the inclusion of a rumen-protected SFA (SF; 150 g/steer daily, as-fed basis; Megalac, Church & Dwight Co. Inc., Princeton, NJ; n = 6) or PUFA (PF; 150 g/steer daily, as-fed basis; Megalac-R, Church & Dwight Co. Inc.; n = 6) source from d −28 to 0 relative to transport (d 0) and feedyard entry (d 1) in Exp. 2. A treatment effect was not observed ($P = 0.79$). However, a treatment × day interaction was detected ($P = 0.04$) because steers fed PF had reduced plasma TNF-α concentrations compared with steers fed NF ($P = 0.03$) on d 3.

were detected for growing phase ADG between steers fed PF and SF. During the finishing phase, no treatment effects were detected for ADG as well as DOF (Table 5). However, steers fed PF had increased ($P = 0.01$) marbling and tended ($P = 0.07$) to have a greater percentage of carcasses grading Choice compared with steers fed NF only (Table 9). In contrast, steers fed NF had improved carcass yield and retail product percentage compared with steers fed PF and SF (Table 9). The greater growing phase ADG of steers fed PF compared with their cohorts fed NF can be attributed, at least partially, to the alleviated TNF-α response after transport and growing lot entry. Proinflammatory cytokines have detrimental effects on animal performance via several mechanisms, such as increasing maintenance requirements, reducing muscle and bone development, and impairing hepatic metabolism and the somatotropic axis (Elsasser et al., 1997; Johnson, 1997). However, reduced feed intake is the major mechanism by which proinflammatory cytokines impair growth rates (Klasing and Korver, 1997). In the present study, DMI and feed efficiency measures were not evaluated during the growing phase. Thus, the potential mechanisms by which reduced TNF-α benefited growing-phase ADG in steers fed PF cannot be addressed. Treatment effects on carcass traits may be due, at least partially, to the greater ADG of steers fed PF during the growing phase. Supporting this rationale, others have suggested that enhanced early feedlot growth results in accelerated fat deposition, thus greater marbling and reduced carcass yield in cattle (Owens et al., 1993; Drager et al., 2004; McCurdy et al., 2010).

The main hypothesis of the present experiment, based on the results reported by Araujo et al. (2010), was that PUFA supplementation during preconditioning only would be an alternative to expose feeder steers to the immunological benefits of PUFA during transport and feedlot entry without impairing feedlot performance. The mechanisms by which PUFA modulate the immune system are associated with synthesis of eicosanoids and cytokines (Miles and Calder, 1998). Conversely, SFA does not substantially affect the immune and acute-phase responses (Miles and Calder, 1998; Farran et al., 2008). However, energy and protein intake modulates the immune system (Carroll and Forsberg, 2007). Therefore, the major goal of the present experiment was not to evaluate the effects of SFA per se, but

Table 9. Carcass traits of steers offered forage-based preconditioning diets without (NF; n = 6) or with the inclusion of a rumen-protected SFA (SF; n = 6) or PUFA (PF; n = 6) source in Exp. 2[1]

	Treatment				*P*-value			
Item	NF	SF	PF	SEM	Main[2]	NF vs. SF[3]	NF vs. PF[3]	SF vs. PF[3]
HCW, kg	394	396	402	6	0.58	0.76	0.31	0.48
Fat,[4] cm	1.55	1.69	1.63	0.06	0.29	0.12	0.38	0.51
LM area, cm^2	94.7	92.6	92.0	1.6	0.44	0.34	0.23	0.78
KPH, %	2.55	2.77	2.69	0.10	0.29	0.13	0.32	0.59
Marbling[5]	444	473	515	18	0.05	0.28	0.01	0.13
Yield grade[6]	3.16	3.46	3.48	0.10	0.04	0.04	0.04	0.89
Retail product,[7] %	49.4	48.7	48.7	0.2	0.05	0.04	0.04	0.95
Choice, %	70.4	83.3	91.3	8.0	0.19	0.25	0.07	0.48

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

[2]Main treatment effects.

[3]Calculated using the PDIFF option (SAS Inst. Inc., Cary, NC) for means separation.

[4]Backfat thickness measured at the 12th rib.

[5]Marbling score: 400 = Small^{00}, 500 = Modest^{00}.

[6]Calculated as reported by Lawrence et al. (2010).

[7]USDA retail yield equation = 51.34 − (5.78 × backfat) − (0.0093 × HCW) − (0.462 × KPH) + (0.74 × LM area).

to use the SF treatment as an isolipidic, isocaloric, and isonitrogenous control treatment relative to PF (Table 1). Supporting our hypothesis, PUFA supplementation was detrimental to preconditioning DMI, but it reduced the TNF-α response after transport and feedlot entry, and it benefited growing lot ADG and carcass marbling compared with NF. As expected, steers fed PF had greater plasma total PUFA concentrations compared with steers in the other treatments. Further, treatment effects detected in plasma total PUFA concentrations can be associated with treatment differences in estimated duodenal flow of linoleic and linolenic acids. Although plasma concentrations of total PUFA in steers fed SF were reduced compared with those fed PF, these were substantial and greater compared with steers fed NF. This outcome may be one of the reasons why PUFA supplementation did not have immunological and performance advantages compared with SFA supplementation herein. Further, as noted previously, the PUFA source offered contained both linoleic and linolenic acids (Table 2), although linoleic acid concentrations were greater than linolenic acid concentrations (28.1 and 2.5%, respectively; DM basis). Linolenic acid and its n-3 derivates promote synthesis of eicosanoids that do not elicit the acute-phase protein response, such as $PGE_{3,}$ and also stimulate synthesis of T-helper 2 anti-inflammatory cytokines such as IL-4, IL-10, and IL-13. Linoleic acid and its n-6 derivates promote the synthesis of PGE_2, a potent stimulator of the acute-phase and T-helper 1 proinflammatory cytokine (IL-1, IL-6, and TNF-α) responses (Yaqoob and Calder, 2007; Carroll and Forsberg, 2007; Schmitz and Ecker, 2008). In the present experiment, steers fed PF had greater plasma concentrations of all n-6 fatty acids, in addition to docosapentaenoic and docosahexaenoic acids (n-3 fatty acids) compared with steers fed NF. On the other hand, steers fed NF had increased plasma concentrations of linolenic and eicosapentaenoic acids compared with their cohorts fed PF. Given that cattle requirements for linoleic and linolenic acids and their respective fatty acid derivates are still unknown, whereas tissue fatty acids and circulating concentrations of eicosanoids were not evaluated, it cannot be concluded herein whether steers fed PF had reduced TNF-α concentrations and, consequently, had improved performance compared with their cohorts fed NF resulting rom the additional supply of linolenic acid, linoleic acid, or both. Thus, further research is warranted to determine the dietary content of linoleic and linolenic acids required to trigger a pro- or anti-inflammatory response, respectively, in cattle.

In summary, inclusion of a rumen-protected PUFA source in preconditioning diets reduced the TNF-α response triggered by transport and feedyard entry, and benefited feedlot performance and carcass marbling of feeder steers. Therefore, PUFA supplementation during preconditioning might be a feasible alternative to enhance immunological and performance variables in feeder cattle.

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