

# Feeding Feedlot Steers Fish Oil Differentially Enhances the Fatty Acid Composition of Muscle Tissue<sup>1</sup>

T.J. Wistuba<sup>2,3</sup>, E.B. Kegley<sup>2</sup>, J.K. Apple<sup>2</sup>, and D.C. Rule<sup>4</sup>

## Story in Brief

Inclusion of fish oil, a source of omega-3 fatty acids, in ruminant diets may fortify the fatty acid composition of meats. Therefore, a 70-day study using 16 crossbred steers (970 ± 69.7 lb initial BW; four calves/pen; two pens/dietary treatment) was conducted. Dietary treatments consisted of: 1) control (75% corn, 11% soybean meal, and 10% cottonseed hull diet); and 2) the control diet with 3% fish oil replacing a portion of the corn. Calves were harvested on days 71 and 72. Collected tissue samples included the longissimus dorsi from the 6th to 7th rib section and ground tissue from the 10th to 12th rib. Total saturated fatty acids in both of the muscle tissue locations were increased ( $P < 0.01$ ) with the inclusion of fish oil in the diet. The proportion of monounsaturated fatty acids in muscle tissue was decreased ( $P < 0.01$ ) in the calves fed fish oil. Fish oil addition to the diet resulted in increased ( $P < 0.03$ ) proportions of total polyunsaturated fatty acids in the ground rib sections, however fish oil supplementation had no effect ( $P > 0.10$ ) on the proportion of total polyunsaturated fatty acids in the longissimus dorsi. Calves that consumed the fish oil diet had higher ( $P < 0.01$ ) levels of omega-3 fatty acids in both muscle tissues, resulting in the fish oil supplemented calves having a lower ( $P < 0.01$ ) omega-6:omega-3 ratio. Results indicate that fish oil may have a place in beef rations and a role in the niche marketing of beef provided there are no deleterious effects on consumer satisfaction.

## Introduction

In the future, there will be considerable emphasis on modification of fatty acid composition of beef. Recently, the dietary recommendation for humans of the highly unsaturated omega-3 fatty acids, specifically eicosapentaenoic acid (20:5) and docosahexanoic acid (22:6), was increased from 0.15 to 0.65 g/d. It has been demonstrated that intestinal supply and muscle tissue composition of fatty acids in beef cattle were affected by the fatty acid composition of the diet. Feeding lipids high in long chain polyunsaturated lipids can enhance the fatty acid concentrations found in meat from beef cattle and milk from dairy cattle. Limited research, however, is available regarding the effects of dietary manipulation on ruminant muscle and adipose tissue composition of long chain polyunsaturated fatty acids. Therefore, our objectives were to determine the effects of fish oil supplementation in the finishing diet on the fatty acid composition of muscle and adipose tissue of beef.

## Experimental Procedures

**Animals and feed.** Sixteen Angus crossbred steers (970 ± 69.7 lb initial BW) from the same maternal herd were obtained from the University of Arkansas Livestock and Forestry Branch Station in Batesville. Steers were blocked by weight and randomly assigned to pens with covered bunks and bunk aprons, such that four steers were maintained in each of four pens, for a total of 8 steers per dietary treatment. Dietary treatments (Table 1) consisted of: 1) control and 2) the control diet with 3% fish oil replacing corn on an equal weight basis. The diets were mixed at approximately weekly intervals. Steers were allowed ad libitum access to their respective diets and water for 70 d starting on October 24, 2000. Growth performance data are reported in Wistuba et al. (2002). Feed samples were taken on weigh days and analyzed.

**Tissue collection and sample preparation.** The cattle were stratified by treatment and harvested on d 71 and 72. Steers were stunned via captive bolt pistol and exsanguinated. After routine processing, carcasses were hung and chilled at 36°F for 24 h, at which time they were ribbed at the 12th rib. Left primal ribs from each carcass were removed and cut into three sections including the 6th to 7th, 8th to 9th, and 10th to 12th ribs at 96 h postmortem. The 6th to 12th rib sections were vacuum packaged in barrier bags. The vacuum-packaged muscles and carcasses were aged until 14 d postmortem in a cooler at 36 to 39°F. The muscles were removed from the vacuum bags after aging for the removal of two steaks that were 1-in-thick from the longissimus dorsi of the 6th to 7th rib section. The steaks were stripped of surrounding epimysium for the fatty acid analysis of the longissimus dorsi. Longissimus muscle from the 10th to 12th ribs was removed from the bone and ground for the determination of ground muscle fatty acid composition. Tissue samples were stored in airtight Whirl-Pak bags (Nasco) at -4°F. Prior to analysis, duplicate 30 g samples were pulverized in liquid nitrogen in a Waring blender, freeze dried, and stored at -4°F until fatty acid analysis.

**Fatty acid analysis.** Triplicate 150-mg muscle tissue samples were subjected to direct transesterification by incubating in 2.0 mL of 0.2 M methanolic KOH according to the methods of Murrieta et al. (2003). Fatty acid methyl esters were transferred to gas liquid chromatograph vials that contained a 1.0-mm bed of anhydrous sodium sulfate. Separation of fatty acid methyl esters was achieved by gas liquid chromatography with a 100-m capillary column. Identification of peaks was accomplished using purified standards.

**Statistical analysis.** Tissue fatty acid data were subjected to analysis of variance using a general linear model (SAS Inst. Inc., Cary, NC). Data are presented as least-squares means. Separate analyses were conducted within each type of sampled tissue. The model included weight block and dietary treatment and calf was the experimental unit.

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<sup>2</sup> University of Arkansas, Fayetteville

<sup>3</sup> Current address: Morehead State University, Morehead, KY

<sup>4</sup> University of Wyoming, Laramie

## Results and Discussion

In ruminants as in other species, the degree of unsaturation of fat depends on anatomical location. In general, subcutaneous sites are most unsaturated followed by inter- and intramuscular fat and internal organ fat being most saturated. In addition, within subcutaneous locations, the most external layers of fat are the most unsaturated with saturation increasing with depth or distance from the exterior. Patterns of unsaturation are inversely related to temperature of the depot location, a concept that applies across many species.

*Fatty Acid Composition of Longissimus dorsi Muscle and Ground Rib Tissue.* Steers fed the fish oil diet had higher ( $P < 0.01$ ) levels of total saturated fatty acids and individual proportions of saturated fatty acids, except for the proportion of 18:0 which was not different ( $P > 0.10$ ) than the control calves in both ground rib and LD tissues (Tables 2 and 3). The findings in longissimus dorsi muscle and ground rib tissue are similar to others that reported that fish oil supplementation had no effect on the proportion of 18:0 in muscle phospholipids and triacylglycerol (Ashes et al., 1992). However, Scollan et al. (2001) reported that fish oil fed in a diet that was 60% forage and 40% grain reduced the proportion of 18:0 in muscle phospholipids and had no effect in neutral lipids and phospholipids in adipose tissue when compared to the control group. Rule et al. (1994) reported that increasing the proportion of 18:0 would be beneficial to the beef industry because this FA is hypocholesteremic in humans. Fish oil fed calves had greater ( $P < 0.01$ ) levels of 14:0, 15:0, 16:0, and 17:0 than the control fed calves. Because palmitic acid (16:0) is thought to be hyperlipidemic and may contribute to increasing serum cholesterol (Lough et al., 1992), increasing its proportion in beef would not be desired. In contrast to the current study, the proportion of 16:0 in muscle triacylglycerol and phospholipids was not affected by feeding fish oil in other studies (Ashes et al., 1992; Scollan et al., 2001).

Total monounsaturated fatty acids in the LD and ground rib were lower ( $P < 0.01$ ) in the fish oil fed calves, with the majority of this resulting from a decrease ( $P < 0.01$ ) in the proportion of 18:1<sup>cis-9</sup>. However, fish oil supplemented calves had greater ( $P < 0.08$ ) quantities of 18:1<sup>cis-11</sup>. Bolte et al. (2002) reported a similar reduction in 18:1<sup>cis-9</sup> with supplemental fish oil.

In the current study, fish oil addition to the diet had no effect ( $P > 0.10$ ) on the total proportion of PUFA in the longissimus dorsi. Although, in the ground muscle tissue, total proportions of PUFA in fish oil fed calves were greater ( $P < 0.01$ ). Fish oil fed calves did have higher ( $P < 0.09$ ) levels of 18:2<sup>cis-9, trans-11</sup>, 20:2, 20:4, and 22:5 in both tissues. However, in the current study the control supplemented calves had greater ( $P < 0.09$ ) concentrations of 18:2 in the LD and ground rib. Ashes et al. (1992) reported similar results in muscle phospholipids when feeding ruminally protected fish oil at 30% of the diet. However, fish oil had no effect on the proportions of 18:2 or 18:3 when it was fed at 20% of the diet. Ashes et al. (1992) also reported that fish oil supplementation increased the proportions of 20:3, 20:4, 20:5, and 22:6 in muscle when compared to control calves. In contrast, Scollan et al. (2001) suggested that fish oil supplementation increased the proportions of 20:3, 20:5, 22:5, and 22:6 but reduced the proportion of 20:4.

Calves that consumed the fish oil diet had greater ( $P < 0.01$ ) levels of omega-3 fatty acids in the LD and ground rib tissues. However, there was no effect ( $P > 0.10$ ) due to dietary treatment on the level of omega-6 fatty acids. These findings resulted in the fish oil supplemented calves having a lower ( $P < 0.01$ ), more desirable, omega-6:omega-3 ratio. The significance of omega-3 fatty acids, particularly 20:5 and 22:6, to human health has been discovered within the past two decades (Ponnampalam et al., 2001). The recom-

mended level of consumption of 20:5 and 22:6 for adults ranges from 0.3 to 0.65 g/d. Based on the recommended levels, the weight percentages of 20:5 and 22:6 in longissimus dorsi muscle and ground rib tissue locations could supply an adult eating a 100 g portion of meat a day with a portion of their recommended daily allowance. In the current study, there was no difference in the polyunsaturated:saturated fatty acid ratio due to dietary treatment. Scollan et al. (2001) suggested that fish oil supplementation tended to decrease the polyunsaturated:saturated fatty acid ratio.

## Implications

The increase in the proportion of 18:2<sup>cis-9, trans-11</sup>, 20:5, and 22:6 that was observed in the tissues of fish oil supplemented steers may not supply adequate quantities of these fatty acids to meet the recommended daily allowances for humans. Inclusion of this beef in a balanced diet will aid in the improvement of the essential fatty acid status in the diet of humans.

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**Table 1. Ingredient and nutrient compositions (as-fed basis) of experimental diets.**

Item	Control	Fish oil
Ingredient		
		%
Corn, cracked	75.4	72.4
Cottonseed hulls	10	10
Soybean meal	11.2	11.2
Cane molasses	2	2
Dicalcium phosphate	0.4	0.4
Limestone, 38% Ca	0.85	0.85
Salt	0.15	0.15
Fish oil	0	3
Rumensin premix <sup>a</sup>	+	+
Vitamin premix <sup>b</sup>	+	+
Trace mineral premix <sup>c</sup>	+	+
Nutrient composition		
Dry matter	88.3	86.8
Crude protein	13.78	13.45
Acid detergent fiber	7.8	7.6
Neutral detergent fiber	11.5	11.4
Fat	3.44	6.66
NE <sub>m</sub> , Mcal/kg <sup>d</sup>	2.08	2.17
NE <sub>g</sub> , Mcal/kg <sup>d</sup>	1.32	1.39

<sup>a</sup> Premix supplied 22 ppm of monensin.

<sup>b</sup> Premix supplied per pound of diet: 225 IU of vitamin A, 75 IU of vitamin D<sub>3</sub>, and 0.15 IU vitamin E.

<sup>c</sup> Premix supplied: 20 ppm of Zn as ZnO, 10 ppm of Mn as MnO, 8 ppm of Cu as CuSO<sub>4</sub>, 0.10 ppm of Se as Na<sub>2</sub>SeO<sub>3</sub>, and 0.10 ppm of Co as CoCO<sub>3</sub>.

<sup>d</sup> Calculated.

**Table 2. Influence of fish oil supplementation on the fatty acid composition of longissimus dorsi muscle tissue.**

Fatty acid <sup>a</sup>	Control	Fish oil	SEM	P<
Saturated fatty acids	45.77	51.13	0.90	0.01
14:0	2.72	3.67	0.21	0.01
15:0	0.33	0.55	0.03	0.01
16:0	26.02	30.05	0.51	0.01
17:0	1.11	1.62	0.05	0.01
18:0	15.55	14.99	0.58	0.51
22:0	0.02	0.18	0.02	0.01
Monounsaturated fatty acids	47.2	38.98	0.85	0.01
14:1 <sup>cis-9</sup>	0.43	0.42	0.06	0.88
16:1	2.98	2.62	0.18	0.18
17:1	0.7	0.62	0.04	0.21
18:1 <sup>cis-9</sup>	39.01	25.93	0.83	0.01
18:1 <sup>cis-11</sup>	1.42	1.81	0.07	0.01
18:1 <sup>trans-9</sup>	0.11	0.10	0.02	0.70
18:1 <sup>trans-11</sup>	2.55	7.48	0.31	0.01
Polyunsaturated fatty acids	3.71	4.01	0.39	0.60
18:2	2.54	1.93	0.24	0.09
18:2 <sup>cis-9, trans-11</sup>	0.21	0.26	0.02	0.03
18:3	0.19	0.32	0.02	0.01
20:2	0.17	0.28	0.03	0.04
20:3	0.41	0.49	0.11	0.63
20:4	0.04	0.35	0.05	0.01
20:5	0.15	0.24	0.04	0.18
22:5	0.00	0.15	0.03	0.01
22:6	0.00	0.003	0.002	0.36
Omega-3 <sup>b</sup>	0.34	0.71	0.06	0.01
Omega-6 <sup>c</sup>	3.00	2.76	0.33	0.63
Omega-6:omega-3	9.81	3.92	1.15	0.01
Polyunsaturated:saturated	0.08	0.08	0.008	0.86

<sup>a</sup> Weight percentage values are relative proportions of all peaks observed by gas liquid chromatography.

<sup>b</sup> Omega-3 fatty acids included 18:3<sup>cis-9,12,15</sup>, 20:5<sup>cis-5,8,11,14,17</sup>, 22:5<sup>cis-7,10,13,16,19</sup>, and 22:6<sup>cis-4,7,10,13,16,19</sup>.

<sup>c</sup> Omega-6 fatty acids included 18:2<sup>cis-9,12</sup>, 20:3<sup>cis-8,11,14</sup>, and 20:4<sup>cis-5,8,11,14</sup>.

**Table 3. Influence of fish oil supplementation on the fatty acid composition of ground muscle tissue.**

Fatty acid <sup>a</sup>	Control	Fish oil	SEM	P<
Saturated fatty acids	45.85	53.88	1.04	0.01
14:0	2.80	4.40	0.16	0.01
15:0	0.41	0.68	0.02	0.01
16:0	25.26	30.83	0.66	0.01
17:0	1.18	1.59	0.04	0.01
18:0	16.15	16.28	0.69	0.90
22:0	0.01	0.04	0.005	0.01
Monounsaturated fatty acids	48.09	36.69	0.86	0.01
14:1 <sup>cis-9</sup>	0.54	0.54	0.06	0.94
16:1	3.00	2.74	0.16	0.27
17:1	0.74	0.58	0.03	0.01
18:1 <sup>cis-9</sup>	38.75	21.81	0.59	0.01
18:1 <sup>cis-11</sup>	1.40	1.59	0.07	0.08
18:1 <sup>trans-9</sup>	3.54	9.29	0.38	0.01
Polyunsaturated fatty acids	2.31	2.69	0.09	0.01
18:2	1.60	1.13	0.05	0.01
18:2 <sup>cis-9, trans-11</sup>	0.025	0.052	0.002	0.01
18:3	0.42	0.39	0.03	0.46
20:2	0.04	0.09	0.005	0.01
20:3	0.08	0.25	0.02	0.01
20:4	0.07	0.42	0.02	0.01
20:5	0.03	0.25	0.016	0.01
22:5	0.04	0.09	0.01	0.01
22:6	0.00	0.01	0.003	0.03
Omega-3 <sup>b</sup>	0.48	0.75	0.04	0.01
Omega-6 <sup>c</sup>	1.76	1.80	0.07	0.69
Omega-6:omega-3	3.71	2.45	0.19	0.01
Polyunsaturated:saturated	0.05	0.05	0.003	0.98

<sup>a</sup> Weight percentage values are relative proportions of all peaks observed by gas liquid chromatography.

<sup>b</sup> Omega-3 fatty acids included 18:3<sup>cis-9,12,15</sup>, 20:5<sup>cis-5,8,11,14,17</sup>, 22:5<sup>cis-7,10,13,16,19</sup>, and 22:6<sup>cis-4,7,10,13,16,19</sup>.

<sup>c</sup> Omega-6 fatty acids included 18:2<sup>cis-9,12</sup>, 20:3<sup>cis-8,11,14</sup>, and 20:4<sup>cis-5,8,11,14</sup>.