

## Effect of Fish Oil Supplement on Growth Performance, Ruminal Metabolism and Fatty Acid Composition of Longissimus Muscle in Korean Cattle

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**ABSTRACT :** We investigated the effect of fish oil (FOS) on growth performance, ruminal metabolism and fatty acid composition and physical characteristics of longissimus muscle in 10 steers and 10 bulls of Korean cattle. Concentrates diet was supplemented with FOS at 5% of the diet. FOS contained 3.34% eicosapentaenoic acid (EPA) and 24.87% docosahexaenoic acid (DHA) of total fatty acids by weight. Average daily weight gain and feed efficiency were not affected ( $p > 0.871$ ) by FOS, but feed intake was decreased. FOS had lower ( $p < 0.003$ ) pH and higher ( $p < 0.001$ )  $\text{NH}_3\text{-N}$  than that of control. There was a treatment effect ( $p < 0.001$ ) for serum cholesterol concentrations. FOS increased ( $p < 0.009$ ) concentrations of n-3 fatty acids, including linolenic, EPA and DHA in longissimus muscle. Physical traits were significantly ( $p < 0.015$ ) changed by feeding FOS except for pH and lightness (L). We concluded that the fatty acid composition and physical properties of the muscle in fattening Korean cattle can be altered by feeding 5% FOS. (*Asian-Aust. J. Anim. Sci.* 2002, Vol 15, No. 1 : 66-71)

**Key Words :** Fish Oil , Korean Cattle, Fatty Acid Composition

### INTRODUCTION

The incorporation of lipids in ruminant diets is commonly practiced. Lipids are used in ruminant diets which contain high caloric value can be useful for overcoming limitations in energy supplies in high yielding ruminants. Researchers have also used fat supplementation in an attempt to alter fatty acid composition in ruminant muscle (Engle et al., 2000; Kiteessa et al., 2001; Scollan et al., 2001). Ashes et al. (1992) found that fish oil supplementation decreased stearic acid concentration in ruminant muscle. The long-chain polyunsaturated fatty acids of fish oil are known to be effective in reducing the risk of coronary heart disease as they are anti-thrombic and anti-arrhythmic. Longissimus muscle tissue from the steers given the feeds containing fish oil had a slightly higher content of fatty acid than the animals on the control feed (Scollan et al., 2001). This suggesting that fish oil could increase the marbling score of the muscle. However, the level of lipids in ruminant diets should be limited to avoid adverse effects on ruminal fermentation. Dietary lipids undergo rapid and extensive hydrolysis in the rumen to form free fatty acids and glycerol (Jenkins, 1993). Unsaturated fatty acids are then biohydrogenated to more saturated fatty acid, and glycerol is converted to propionate (Chalupa et al., 1986). However, it was demonstrated that ruminal microorganisms in vitro did not hydrogenate 20:5n-3 and 22:6n-3 to any significant extent (Ashes et al., 1992).

The objective of this experiment was to investigate the impact of feeding fish oil supplement (FOS) on growth performance, ruminal metabolism and fatty acid composition of longissimus muscle in Korean cattle.

### MATERIALS AND METHODS

The experiment were carried out with 10 early finishing bulls and 10 steers of the Korean cattle (average weight  $400 \pm 20$  kg). Animals were allocated to two groups consisted 5 bulls and 5 steers per one groups. Diet treatment (table 1) were control and 5% FOS. In control, corn grain was replaced with 5% FOS. Table 1 shows the formulation and chemical composition of the experimental diets. The FOS, Enerlac® provided by Chile, contained 55.0% ether extract, 3.34% EPA and 23.86% DHA (table 2).

The animals allocated were group-fed on each concentrate diet and had free access to fresh water and rice straw as a roughage source. Body weights were measured on two consecutive days at the beginning and end of the experiment. Feed samples were analyzed in accordance with AOAC (1996) to determine DM, ash, CP, and ether extract. Neutral detergent fibers (NDF) was determined according to the procedures described by Goering and Van Soest (1970). Fatty acid composition in feed samples was determined by gas chromatography (GC Star 3400, Varian, Sugarland, USA). Fatty acid composition was expressed as the percentages of total fatty acid by weight (table 2). Blood samples were taken from the jugular vein within 30 min before slaughter of cattle, placed on ice, and transported to the laboratory; serum was isolated by centrifugation at  $1,500 \times g$  at  $4^\circ\text{C}$  for 15 min. Lipids in longissimus muscle were extracted using chloroform-method (Folch et al., 1957). This extract, containing approximately 45 mg of

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**Table 1.** Ingredient and composition of experimental diets

Ingredient <sup>a</sup>	Control	FOS <sup>b</sup>
Corn grain	67.24	62.24
Wheat bran	15.00	15.00
Corn gluten feed	5.00	5.00
Cane molasses	5.00	5.00
Rapeseed meal	3.00	3.00
Urea	0.32	0.32
Tallow	0.73	0.73
FOS <sup>b</sup>	-	5.00
Limestone (1 mm)	2.40	2.40
Salt	0.50	0.50
Calcium sulfate	0.10	0.10
Tricalciumphosphate	0.71	0.71
Vit. min. premix <sup>c</sup>	0.10	0.10
Total	100.00	100.00

<sup>a</sup>Percentage of diet on a DM basis.

<sup>b</sup>Fish oil supplement.

<sup>c</sup>Vitamin A, 4,000,000 IU; Vitamin D3, 400,000 IU; Vitamin E, 20,000 IU; Fe, 50,000 mg; Co, 100 mg; Cu, 5,000 mg; Mn, 20,000 mg; Zn, 20,000 mg; I, 290 mg; Se, 100 mg; Antioxidant, 6,000 mg.

meat. was converted to fatty acid methyl esters using a mixture of boron trifluoride (35%), methanol (35%), and hexane (30%) by heating for 45 min at 95°C (Morrison and Smith, 1964). The fatty acid methyl ester was separated by gas chromatography using an Carbowax (Stabilwax-DA) analytical capillary column under the following conditions: injector at 210°C, detector at 240°C and column oven temperature programmed from 160°C to 240°C at 3°C/min. Individual fatty acid ester was identified by retention time of known compounds (Sigma Chemical Co. St. Louis, MO).

Meat color coordinates (L, a, b) of longissimus muscle was recorded using a Chroma Meter CR-10 (Minolta Cameras, Osaka, Japan). pH, cooking loss and shear force of longissimus muscle were conducted according to Kim et al. (1999). Samples from each carcass were analyzed for percentage extractable fat according to AOAC (1996) procedures. The longissimus muscle samples (about 1 g each) were placed into prefold oven-dried filter paper, weighed and placed in a drying oven (100°C) for 24 h. After removal from the oven, samples were reweighed and fat content was determined using the Goldfish diethyl ether extraction method.

Rumen fluid was collected via stomach tube from bulls and steers 0, 2, 4, and 8 h after feeding on d 120. Samples were frozen in dry ice immediately after determining pH. The frozen samples were stored at -20°C for further analysis. Samples were thawed, and 5 ml of each sample was treated with 1 ml of 25% meta-phosphoric acid and centrifuged for VFA assay. A portion of each sample was centrifuged (10,000×g for 15 min at 4°C) and analyzed for NH<sub>3</sub>-N with the phenol-hypochlorite colorimetric procedure (Chaney and Marbach, 1962).

Statistical analysis of data was performed by analysis of variance for a completely randomized design using a GLM procedure of SAS (SAS Inst. Inc., Cary, NC). Differences among means of treatment were determined with orthogonal contrasts (Steele and Torrie, 1980).

## RESULTS AND DISCUSSION

### Growth performance, ruminal metabolism and serum metabolites

FOS did not influence on animal growth although there

**Table 2.** Chemical composition and fatty acids profile of experimental diets, fish oil supplement (FOS) and rice straw

Items	Diets		FOS	Rice straw
	Control	FOS <sup>a</sup>		
	----- Chemical composition (%) -----			
DM	87.49	87.30	80.50	88.68
CP	12.54	12.14	0.49	4.82
Ether extract	2.73	3.65	55.00	1.54
NDF	18.53	17.00	0.00	67.28
Ash	6.78	5.52	3.20	10.08
	----- Fatty acid profile <sup>b</sup> (%) -----			
16:0	19.97	11.85	6.90	-
16:1	-	7.57	17.20	-
18:0	4.28	3.72	6.19	-
18:1	30.42	36.12	39.28	-
18:2	43.85	32.77	2.22	-
18:3	1.48	1.17	1.01	-
20:5	-	2.0	3.34	-
22:6	-	4.80	23.86	-
Total	100	100	100	

<sup>a</sup>FOS was supplemented by 5% in substitution of corn grain. <sup>b</sup>Percentage of total fatty acids by weight.

was significant effect by group (table 3). On the other hand, feed intake was significantly ( $p < 0.016$ ) decreased by the FOS. There, feed efficiency was increased in bulls by FOS ( $p < 0.010$ ) due to no supplemental effect in growth. Scollan et al. (2001) reported that fish oil diet tend to reduce feed intake. Chillard and Doreau (1997) also reported a reduction of dry matter intake when dairy cows fed on maize and concentrates were supplemented with fish oil. It is thought that these effects of the rumen biohydrogenation rather than a negative effect of the fatty acids of fish oil on rumen function, since the fish oil does not disturb ruminal fiber digestion (Wonsil et al., 1994). Bulls grew faster ( $p < 0.001$ ) and ate more ( $p < 0.009$ ) than that of steers.

FOS had lower ( $p < 0.003$ ) pH than control (table 4). However, no significant effect ( $p < 0.07$ ) was found in total VFA production. Krysl et al. (1991) reported that infusion of soybean oil intraruminally did not affect total VFA. A lower tendency of molar proportions of acetate was found in FOS fed animals whereas molar proportions of isobutyrate and isovalerate were slightly lower in control animals. Differences in minor acids are consistent with our  $\text{NH}_3\text{-N}$  data and suggest greater fermentation of protein

because branched-chain VFA were derived from the fermentation of branched-chain amino acids (Russell and Sniffen, 1984). Acetate: propionate (A/P) ratio was not different because propionate proportion was not changed. This result was quite opposite from Krysl et al. (1991) who reported that a big shift in A/P ratio with increasing dietary soybean oil. Five percent supplement of FOS attributed only to less than 1% lipid, in resulting no effect to a reduction in fiber digestion. Chalupa et al. (1986) noted that the hydrolysis of triglycerides by bacterial lipase provides glycerol, which is then readily converted to propionate. Thus, the maintenance in molar proportion of propionate for FOS fed animals may be attributed to bacterial metabolism of glycerol.

There was a treatment effect ( $p < 0.010$ ) on serum cholesterol concentrations. Thomas et al. (1997) observed higher concentration of total cholesterol in cattle fed 4% soybean oil in diet. Likewise, the inclusion of lipid supplementation in the diet of ruminants normally increases both cholesterol and triacylglycerol (Cant et al., 1993). Although serum glucose concentration did not changed by FOS, the pattern of increased glucose concentration was

**Table 3.** Effect of fish oil supplement (FOS) on growth performance and feed efficiency of Korean cattle

Treatments	Bulls		Steers		SEM <sup>a</sup>	P		
	Control	FOS	Control	FOS		Groups	Treatment	Groups * Treatment
Initial live weight, kg	382.0	403.0	386.7	398.3	16.70	1.000	0.325	0.772
Final live weight, kg	642.0	662.3	565.0	547.3	6.02	0.006	0.960	0.483
Total weight gain, kg	260.0	259.3	178.3	149.0	4.43	0.001	0.378	0.399
Daily weight gain, kg/d	1.02	1.01	0.76	0.63	0.01	0.001	0.357	0.383
Feed intake, kg/d	10.9	8.47	8.27	7.93	0.36	0.009	0.016	0.051
Feed efficiency	0.09	0.12	0.09	0.08	0.02	0.006	0.187	0.006

<sup>a</sup> Standard error of mean

**Table 4.** Effect of fish oil supplement (FOS) on ruminal pH, VFA and  $\text{NH}_3\text{-N}$  concentration in rumen fluid of Korean cattle

Treatments	Bulls		Steers		SEM <sup>a</sup>	P		
	Control	FOS	Control	FOS		Groups	Treatment	Groups* Treatment
pH	6.71	6.46	6.70	6.49	0.01	0.726	0.003	0.907
Total VFA, mM	49.86	70.20	71.41	79.04	37.07	0.050	0.074	0.088
Molar proportion, mol/100 mol								
Acetate (A)	40.77	39.12	48.98	44.68	16.61	0.070	0.248	1.000
Propionate (P)	22.35	25.44	28.83	29.13	10.13	0.091	0.977	0.435
Isobutyrate	2.11	2.16	1.64	1.70	0.13	0.169	0.651	1.000
Butyrate	21.80	21.71	12.71	17.48	11.58	0.046	0.242	1.000
Isovalerate	7.88	7.56	3.72	4.73	0.69	0.002	0.181	1.000
Valerate	5.11	4.01	3.12	2.30	0.39	0.016	0.277	0.183
A/P ratio	1.85	2.16	1.68	1.58	0.08	0.074	0.132	0.128
$\text{NH}_3\text{-N}$ , mg/100 ml	50.72	78.62	46.39	69.31	29.45	0.138	0.001	0.561

<sup>a</sup> Standard error of mean

**Table 5.** Effect of fish oil supplement (FOS) on serum metabolites of Korean cattle (mg/dl)

Treatments	Bulls		Steers		SEM <sup>a</sup>	P		
	Control	FOS	Control	FOS		Groups	Treatment	Groups* Treatment
Fatty acids								
Glucose	66.0	73.0	84.3	93.3	25.61	0.003	0.116	0.831
Serum Urea Nitrogen	13.33	16.33	14.00	14.00	4.62	0.616	0.376	0.376
Creatinine	1.47	1.50	0.83	1.07	0.02	0.001	0.236	0.365
Cholesterol	125.67	171.33	98.33	164.00	48.28	0.330	0.010	0.566
Ca	10.20	10.40	8.83	9.67	0.27	0.028	0.223	0.442
P	7.23	7.50	6.20	6.40	0.73	0.139	0.728	0.960

<sup>a</sup>Standard error of mean.

**Table 6.** Effect of fish oil supplement (FOS) on intramuscle fat content and fatty acid composition (%) of longissimus muscle in Korean cattle

Treatments	Bulls		Steers		SEM <sup>a</sup>	P		
	Control	FOS	Control	FOS		Groups	Treatment	Groups* Treatment
Fatty acids								
Intramuscle fat. %	8.2	9.1	13.9	19.1	1.66	0.001	0.094	0.028
14:0	3.16	2.96	3.46	3.45	0.20	0.271	0.119	0.533
14:1	0.20	0.20	1.55	1.00	0.14	0.001	0.579	0.579
16:0	27.86	28.44	26.10	23.69	0.61	0.077	0.213	0.564
16:1	4.29	3.75	5.73	5.88	0.24	0.001	0.555	0.774
18:0	13.51	13.47	11.35	11.06	1.32	0.180	0.516	0.779
18:1	45.66	45.85	45.88	46.26	4.64	0.725	0.751	0.990
18:2	5.32	5.33	3.71	3.79	0.05	0.007	0.923	0.935
18:3	0.27	0.28	0.53	1.21	0.13	0.002	0.009	0.010
20:5	0.23	0.57	0.56	1.22	0.11	0.001	0.001	0.148
22:6	0.46	1.15	0.13	2.45	0.07	0.122	0.001	0.008
Total	100	100	100	100				

<sup>a</sup>Standard error of mean.

almost coincides with greater production of the glucogenic precursor, propionate (table 4). Yelich et al. (1995) observed higher concentration of glucose concentrations in heifers fed to gain 0.68 and 1.36 kg/d (83±2 mg/dl and 79.2±2 mg/dl, respectively). Their serum glucose concentrations seemed to reflect the supply of nutrients necessary to achieve gains similar to those of cattle in our study. Changes in ruminal NH<sub>3</sub>-N (table 4) were not sufficient to elicit changes in serum urea nitrogen by treatments (p>0.376). Hall et al. (1995) have reported that serum urea nitrogen concentrations reflect differences in the diet. Diets in this experiment were formulated to be isonitrogenous. Therefore, serum nitrogen concentrations were not expected to differ by treatments.

#### The fat and fatty acid composition and physical characteristics of longissimus muscle

Intramuscular fat content was not significantly different by FOS with varying 8.2 to 9.1% and 13.9 to 19.1% in bulls and steers, respectively (table 6). Fatty acid composition in the present trial was similar to those reported by Scollan et

al. (2001) and Scheeder et al. (2001) for steers and bulls fed oil supplemented diet, respectively. Fatty acids as a percentage of total fat ranged from 100.00 to 99.28% across treatment and groups. All the individual fatty acids were not affected by treatment. However, the proportion of palmitic acid seems to be increased by FOS in both bulls and steers. Because palmitic acid is thought to be hyperlipidemic and may contribute to increasing serum cholesterol (Solomon et al., 1992). This increased serum cholesterol concentration with FOS was quite coincide with this data. Compared with fat from control diet, the fat from animals fed the FOS had a higher proportion of palmitic acid and lower proportions of stearic acid as shown in the result of Scollan et al. (2001). The concentration and proportion of linolenic acid was much higher in the steers given the FOS with an average proportion of 1.21 compared with 0.53. However, this phenomenon could not be explained, because the linolenic acid content of FOS was not higher than control. Feeding FOS resulted in significant increases in their deposition in muscle lipid. Ashes et al. (1992) found that ruminal microorganisms could not hydrogenate 20 and 22 (n-3) fatty

**Table 7.** Effect of fish oil supplement (FOS) on physical characteristics of longissimus muscle of Korean cattle

Treatments	Bulls		Steers		SEM <sup>a</sup>	P		
	Control	FOS	Control	FOS		Groups	Treatment	Groups * Treatment
pH	5.52	5.53	5.51	5.52	0.00	0.200	0.006	0.009
Meat color								
Lightness (L)	38.9	39.3	38.6	39.2	1.09	0.000	0.820	0.102
Redness (a)	15.5	13.3	15.7	13.5	2.60	0.987	0.139	0.327
Yellowness (b)	4.1	2.7	5.1	2.8	0.82	0.001	0.094	0.028
Cooking loss, %	24.18	23.83	22.50	21.80	0.34	0.007	0.288	0.337
Shear force, kg/cm <sup>2</sup>	14.7	10.7	9.5	8.2	8.78	0.001	0.001	0.015

<sup>a</sup> Standard error of the mean.

acids to any significant extent. However, the increases were much less than those obtained when formaldehyde-treated fish oil was fed (McDonald and Scott, 1977). This illustrates well the extent to which polyunsaturated fatty acids of ruminant are amenable to manipulate if they escape rumen biohydrogenation. The pH values were in normal range for both control and treatment groups. On the other hand, redness (a) ( $p < 0.007$ ) and yellowness (b) ( $p < 0.006$ ) of meat color were significantly changed with FOS. Cooking loss ( $p < 0.015$ ) and shear force ( $p < 0.001$ ) also were greatly improved by FOS. In a previous investigation (Casutt et al., 1999), cooking loss and shear force were altered due to the varying fatty acid composition. The present results indicate that fatty acid composition and physical properties of the muscle fat of fattening Korean cattle can be altered by feeding to 5% FOS because fish oil is not well hydrogenated in the rumen but is incorporated into muscle. However, no differences between groups were found in almost of growth performance and ruminal metabolism data.

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