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Reduction in Omega-6 to Omega 3 ratio and improvement in blood lipid profiles in flaxseed fed Hanwoo steer and human

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Abstract

Aim : To determine the effect of feeding flaxseed on Omega-6 to Omega-3 ratio (n-6/n-3) in Korean native steers (Hanwoo) and effect of flaxseed-fed beef consumption on reducing blood lipid profile and glucose in normal human.

Methodology : A total of 60 Hanwoo steers (750 kg b.wt.) were assigned three treatments (20 per treatment). Each treatment group was divided into C (control, feeding basal diets without flaxseed for 40 days before slaughter), FS5 (feeding 5% flaxseed for 60 days before slaughter), and FS7.4 (feeding 7.4% flaxseed for 40 days before slaughter). Fatty acid composition from Hanwoow jugular vein and beef loin were analyzed. Clinical trials were carried out to investigate the effect of consumption of flaxseed-fed beef loin on blood lipid profile and glucose in twenty human subjects.

Results : n-6/n-3 ratio in the blood and beef loin of Hanwoo steers were lowered to 2.26-2.27 and 3.67-3.71 in the FS group, respectively, compared with the other groups. Oleic acid level in the blood and beef loin of Hanwoo steers increased to 40.12-42.01 and 52.27-52.79%, respectively, compared with other groups. Blood triacylglycerol, total cholesterol, and low-density lipoprotein-cholesterol levels in normal human fed with FS beef loin reduced by 25.35, 5.22, and 17.59%, compared to those before intake of beef loin. Blood high density lipoprotein-cholesterol (HDL-C) level in normal human fed with FS beef loin was increased by 6.07%. In human subjects fed with FS and C beef loin, blood glucose level was decreased by 6.42 and 11.82%, respectively.

Interpretation : The results demonstrated that feeding 5 and 7.4% flaxseed to Hanwoo steers for 40 to 60 days before slaughter could lower n-6 to n-3 ratio and enhance oleic acid in the blood and beef loin. Further, consumption of flaxseed-fed beef loin by human subjects could improve blood lipid profile.

Key words: Beef loin, Flaxseed, Hanwoo steers, Omega-6/omega-3 fatty acids

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Introduction

As eating habits has evolved into a catering industry or food service industry, including meat, fast food and instant food in Korea, mortality caused by cerebrovascular disease and cardiovascular disease (CVD) ranks second and third, following cancer in humans. As industrialization progresses and cooking oil industry flourishes, excess intake of omega-6 (n-6), a fatty acid, has emerged as a novel issue (Lands *et al.*, 2018). Excess intake of n-6 fatty acid with an increase of omega-6 to omega-3 fatty acid ratio (n-6/n-3) and their excess metabolites are linked to metabolic disorder, CVD, inflammation, carcinogenesis, obesity, type 2 diabetes, autoimmune diseases, and depressive disorder. It can also cause considerable harm to bones and general health conditions (Berger *et al.*, 2017). Due to the introduction of Western diet in Korea, intake of n-6 fatty acid has increased over time while the intake of n-3 fatty acid has largely decreased (Wijendran and Hayes, 2004), which has resulted in the current ratio of 15:1 to 30:1 (Simopoulos, 2009), against ideal ratio of 4:1 (Husted and Bouzinova, 2016).

Since n-6 and n-3 fatty acids tend to accumulate in the blood and tissues, it is critically important to maintain a balance between n-6 and n-3 in food to maintain proper health (Calder, 2012; Scafoli *et al.*, 2017). Although there is a minor difference between n-6 and n-3 in terms of molecular structure, biochemical metabolism and physiological function of these fatty acids are similar to each other. In addition, these two substances compete with each other for same enzyme in the metabolic pathway that features a non-reciprocal conversion (Gogos and Smith, 2010). In animal tissues, accumulation of n-3 fatty acids mainly rely on formulation of fatty acids of the diet (Bou *et al.*, 2005). n-6 fatty acid is rich in animals, and the grain-fed beef whose n-3 fatty acid is low can have a possibility of increased n-6/n-3 ratio with respect to those of the grass-fed beef (Legrand *et al.*, 2010).

Some studies have reported methods to produce chicken (Ibrahim *et al.*, 2018), pork (Yang and Lien, 2016) and beef diets (McNiven *et al.*, 2011) by adjusting their n-6/n-3 ratio. However, little information is available on the production of Hanwoo beef (FS beef) with lower n-6/n-3 ratio through clinical trials (Legrand *et al.*, 2010). The n-6/n-3 ratio in corn is 46/1. The n-6/n-3 ratio in products of livestock fed with corn-centered feeds is also very high at 6:1 to 15/1 (Simopoulos, 2009). Since Korean dairy farmers who are engaged in the livestock industry mainly feed grain-centered assorted feed such as corn and soybean cake to produce livestock, n-6/n-3 ratios in these animals are similar to those fed with Western diet. It is well known that flaxseeds are rich in n-3 fatty acid and can lower blood lipids in both human and animals, thus reducing chances of CVD (Legrand *et al.*, 2010). The objective of this study was to determine the effect of feeding flaxseeds on n-6/n-3 ratio in blood and beef loin of Korean native steers (Hanwoo) and examine the effect of consumption of flaxseed-fed beef loin on blood lipid profiles and glucose in human subjects.

Materials and Methods

Experimental design and feeding management of animals: A total of 60 Hanwoo steers (750 kg b.wt.) that had attained the end of fattening period were assigned into three treatment groups (20 number per treatment group) using a completely randomized block design. Each treatment group was divided into C (control, feeding basal diets without flaxseed for 40 days before slaughter), FS5 (feeding 5% flaxseed for 60 days before slaughter) and FS7.4 (feeding 7.4% flaxseed for 40 days before slaughter). Hanwoo were fed with 8 kg of daily concentrates and 1 kg of rice straw as roughages. Concentrate as basal diets was fed a 4 kg each at 6:00 am and 5:00 pm. FS diets contained 5 or 7.4% flaxseed in basal diets. Compositions of fatty acids in basal diets, FS5 and FS7.4 experimental diets were: n-6/n-3 ratio, 12.23, 0.78 and 0.72; unsaturated fatty acids, 33.47, 53.54, and 55.43%; and saturated fatty acids, 66.53, 46.45 and 44.58%, respectively. This study was approved by Kangwon National University Animal Experimental Ethics Committee (Approval No. 016-017) and Bioethics Committee (Approval No. 016-35).

Collection of blood samples : Blood sample was randomly collected from Hanwoo of each treatment group on both the commencing date and end of experiment. Blood (3 ml) was collected from each subject animal from their jugular vein using a heparin-treated syringe. Plasma was separated from the collected blood after 15 min of centrifugation at 3,000 rpm and frozen immediately with liquid nitrogen at -196°C. These frozen plasma samples were stored at -20°C for biochemical analyses.

Fatty acid analysis: Beef loin was prepared from 10 Hanwoo per treatment group following slaughter and one kg per head was gathered. The loin was then cut into small pieces using a meat chopper. Three parts were randomly selected from chopped meat and used for fatty acid analysis (three repetitions for one loaf of loin). Lipid extracted from beef loin and plasma using chloroform and methanol mixture was saponified using 0.5 N methanolic NaOH solution. Methylation was carried out using BF₃-methanol. Then, 2 µl of supernatant was taken out and injected into a GC (model GC-15A, Shimadzu Corp., Kyoto, Japan) with a SPTM-2560 Capillary GC Column (L×I.D. 100 m×0.25 mm, df 0.20 µm Omegawax 320 capillary column, Sigma, USA). Supelco (37 component FAME Mix, Sigma-Aldrich Co., St. Louis, MO) was used as a standard solution and nonadecanoic acid (19:0) was used as an internal standard (Park *et al.*, 2010).

Clinical trials: Clinical trial was conducted on 20 college students. They were normal adults. Blood lipid profiles and glucose levels were measured using a Cholestech LDX® System (USA) in these human subjects empty stomach. After excluding 8 subjects who showed difference by a big margin in variation for their triacylglycerol and total cholesterol values, 12 out of these 20 subjects were finally selected. These subjects were informed of the study purpose and necessity of their cooperation before starting the experiment. These were allowed for their usual taste and dietary habits. They subjects were prohibited from eating

food that might affect their cholesterol level such as drinking or meat eating three days prior to measurement of blood lipid profiles with empty stomach, excluding each and every factor. This trial was repeatedly three times per treatment group (at an interval of 90 days). Blood lipid profiles and glucose levels were measured before and after consumption of beef loin, respectively. In order to measure fasting blood lipid profiles and glucose of subjects prior to consumption of beef loin, subjects were prohibited from eating anything from 12:00 of the night before the consumption to 10:00 in the next morning to keep them hungry. For each subject, 200 g of beef loin with basal diet was provided. Subjects consumed FS (flaxseed-fed beef, n-6/n-3 lowered Hanwoo beef), C (control beef, feeding basal diets without flaxseed) and GI beef loins (grain-fed imported beef), respectively.

Statistical analyses: The mean and standard deviation of data were computed using IBM SPSS statistics 22 (2013). To determine statistical significance, one-way analysis of variance (ANOVA) with Turkey's multiple range test was performed. Statistical significance of data was set at 95% level of confidence ($p < 0.05$).

Results and Discussion

Changes in fatty acid composition in plasma after feeding FS to Hanwoo are shown in Table 1. n-3 fatty acid in the plasma of FS group increased relative to control group at initial days prior to consumption of FS. However, n-6 fatty acid level significantly decreased ($p < 0.05$). The n-6/n-3 ratio in the FS group showed an

ideal ratio of less than 4:1 (2.26:1 to 2.78:1) ($p < 0.05$). Palmitoleic acid (16:1n-9) among monounsaturated fatty acids in the FS group significantly decreased compared to control group and at initial days prior to consumption of FS. However, oleic acid (18:1n-9) was found to increase significantly ($p < 0.05$). Saturated fatty acid (SFA) showed a decrease in the FS group during this period. However, unsaturated fatty acid (UFA) significantly increased with increase in UFA/SFA ($p < 0.05$). Activation of Δ -9 desaturase in biohydrogenation and adipose tissues of rumen microorganisms (Park, and Yu, 1994; Gogus and Smith, 2010) might be the probable reason why fatty acids in the blood and n-6/n-3 ratio decreased despite of high n-6/n-3 fatty acid and palmitoleic acid in the diet. It might also be the reason why oleic acid content in the blood increased despite a low figure of this substance in the diet. Rumen microorganisms can change *cis* unsaturated fatty acids into *trans* fats and saturated fatty acids via hardening *in vivo* and activation of Δ -9 desaturase could compose oleic acid from stearic acid (Raes *et al.*, 2001).

Changes in composition of fatty acids of beef loin after feeding FS to Hanwoo are shown in Table 2. n-3 fatty acid of beef loins in the FS group increased significantly compared to that in other groups ($p < 0.05$). However, n-6 fatty acid in beef loins of FS group was not significantly different from that in other groups. The n-6/n-3 ratio in the beef of FS group was significantly lower than that of grain-fed control and imported beef ($p < 0.05$). Oleic acid in the FS group was significantly higher than that in the grain-fed control and imported beef. The difference between control and

Table 1 : Effect of flax seed on fatty acid composition in plasma of Hanwoo steers according to feeding days (% of total fatty acids)

Common formula	Common name	Control group	FS groups ¹		
			Initial	40 days	60 days
8:0	Octanoic acid	0.52±0.24 ^{2c}	1.88±0.42 ^a	1.61±0.15 ^{ab}	1.33±0.21 ^b
10:0	Decanoic acid	1.78±0.33 ^b	1.32±0.11 ^c	1.58±0.27 ^{bc}	2.23±0.30 ^a
12:0	Lauric acid	2.05±0.16 ^b	1.81±0.01 ^b	3.18±0.06 ^a	3.26±0.12 ^a
14:0	Myristic acid	1.07±0.21 ^b	0.73±0.17 ^b	1.68±0.09 ^a	1.27±0.07 ^a
16:0	Palmitic acid	2.01±0.27 ^b	0.94±0.11 ^c	16.74±0.39 ^a	16.87±0.40 ^a
16:1n-9	Palmitoleic acid	15.14±0.46 ^b	17.34±0.81 ^a	3.46±0.15 ^c	2.26±0.31 ^d
18:0	Stearic acid	7.15±0.44 ^b	5.09±0.40 ^c	17.95±0.34 ^a	18.01±0.28 ^a
18:1n-9	Oleic acid	24.52±0.53 ^c	26.58±0.36 ^b	40.12±1.61 ^a	42.01±3.06 ^a
18:2n-6	Linoleic acid	33.00±0.61 ^a	29.07±0.10 ^b	8.46±0.60 ^c	7.94±0.46 ^c
18:3n-3	Linolenic acid	0.25±0.17 ^b	0.23±0.02 ^b	3.04±0.86 ^a	3.52±0.38 ^a
20:0	Arachidic acid	12.51±0.36 ^a	14.02±0.43 ^a	2.18±0.20 ^b	1.30±0.29 ^b
22:0	Behenic acid	-	-	-	-
22:1	Erucic acid	-	-	-	-
24:0	Lignoceric acid	-	-	-	-
SFA ³	-	47.09±0.78 ^a	45.78±1.07 ^b	43.92±0.63 ^b	44.27±0.84 ^b
UFA ⁴	-	52.91±0.55 ^b	54.22±0.25 ^a	55.08±0.51 ^a	55.73±0.61 ^a
n-6/n-3	-	132.7±0.52 ^a	126.4±0.37 ^b	2.78±0.05 ^c	2.26±0.09 ^c
UFA/SFA	-	1.12±0.02 ^b	1.18±0.05 ^b	1.25±0.07 ^a	1.26±0.02 ^a

¹FS groups: 40 days (7.4% flaxseed-fed), 60 days (5% flaxseed-fed). ²Standard deviation of mean values (n=10). ³SFA: saturated fatty acid. ⁴UFA: unsaturated fatty acid. ^{a,b,c}Mean values with different superscripts differ significantly ($p < 0.05$)

Table 2 : Fatty acid composition of flaxseed-fed beef (FS) beef loin (% of total fatty acids)¹

Common formula	Common name	Control beef	GI beef	FS5	FS7.4
8:0	Octanoic acid	0.75±0.062 ^a	0.13±0.02 ^b	0.19±0.03 ^b	-
10:0	Decanoic acid	0.76±0.03 ^a	0.12±0.05 ^b	0.12±0.08 ^b	0.21±0.18 ^b
12:0	Lauric acid	0.12±0.03	0.13±0.04	0.14±0.05	0.25±0.25
14:0	Myristic acid	3.86±0.71	3.83±0.27	3.27±1.16	3.41±0.55
16:0	Palmitic acid	27.13±0.84 ^a	27.62±1.71 ^a	26.30±0.70 ^b	25.07±1.31 ^c
16:1n-9	Palmitoleic acid	6.68±0.91 ^a	4.95±0.84 ^c	5.74±1.07 ^b	5.86±0.56 ^b
18:0	Stearic acid	9.45±0.73 ^b	13.26±0.30 ^a	9.32±1.90 ^b	9.07±1.67 ^b
18:1n-9	Oleic acid	50.67±0.84 ^b	47.97±0.36 ^c	52.27±1.30 ^a	52.79±1.54 ^a
18:2n-6	Linoleic acid	1.88±0.36	2.01±0.53	2.19±0.45	2.24±0.30
18:3n-3	Linolenic acid	0.08±0.12 ^b	0.09±0.03 ^b	0.59±0.35 ^a	0.61±0.13 ^a
20:0	Arachidic acid	0.05±0.01 ^c	0.01±0.00 ^c	0.56±0.14 ^b	0.71±0.11 ^a
22:0	Behenic acid	-	-	-	-
22:1	Erucic acid	-	-	-	-
24:0	Lignoceric acid	-	-	-	-
SFA ³	-	40.73±0.51 ^b	44.99±0.34 ^a	39.45±0.57 ^b	38.40±0.27 ^c
UFA ⁴	-	59.27±0.38 ^b	55.01±0.25 ^c	60.45±0.29 ^b	61.31±0.47 ^a
n-6/n-3	-	23.50±0.78 ^a	22.33±0.65 ^a	3.71±0.10 ^c	3.67±0.07 ^c
UFA/SFA	-	1.46±0.06a ^b	1.22±0.07 ^b	1.53±0.03 ^a	1.60±0.08 ^a

¹Control beef : grain-fed Hanwoo beef loin, GI beef: grain-fed imported beef loin, FS5: 5% flaxseed-fed beef, FS7.4: 7.4% flaxseed-fed beef. ²Standard deviation of the mean values (n=10). ³SFA: saturated fatty acid. ⁴UFA: unsaturated fatty acid. ^{a,b,c}Mean values with different superscripts differ significantly (p<0.05)

imported beef, both of which took in grain diet, was significant ($p < 0.05$). However, there was no statistical difference between FS5 and FS7.4. Saturated fatty acid content in imported beef was highest. It showed no significant difference between control beef and FS5, while it was significantly lower in FS7.4 ($p < 0.05$). Unsaturated fatty acid in FS7.4 was highest, followed by FS5 and control beef. The imported beef had significantly lower content of unsaturated fatty acid ($p < 0.05$), however, there was no significant difference between control beef and FS5.

The reason why n-3 fatty acid and oleic acid in the loin of FS groups were higher than control beef and the imported beef while their n-6/n-3 ratio was significantly lower due to high intake of n-3 fatty acid from flaxseeds and the action of rumen microorganisms, as well as distribution channel of imported beef. For control, beef produced with feed concentrates, it is known that n-6 fatty acid provided from the diet is high while n-3 fatty acid is low (Raes *et al.*, 2001). For imported beef, a livestock product that is also produced from concentrates, n-6 fatty acid might also be higher than its n-3 fatty acid. Because the rate of reduction of n-3 fatty acid is much more accelerated than that of n-6 fatty acid due to long distribution channel of import and longer duration (Raes *et al.*, 2001). With regard to both n-3 fatty acid and oleic acid accumulated in the loin of Grades 1 to 5 beef (that is, 1⁺⁺, 1⁺, 1, 2, and 3 of Hanwoo beef), the values obtained in this study were higher than those reported by Lee *et al.* (2010). The contents of n-3 fatty acid in the study of Lee *et al.* (2010) in these five grades were 0.14, 0.07, 0.05, 0.30 and 0.03% and those of oleic acid were 53.10, 51.54, 51.85, 49.79, and 47.40%, respectively. It is

known that oleic acid can increase the taste and flavor of beef by increasing its marbling attribute. As a consequence, it can function as a fatty acid that can lead to premium meat because it can increase preference of consumers and simultaneously lower blood lipids (Anderson *et al.*, 1975; Lunt and Smith, 1991; Raes *et al.*, 2001). Park and Yu (1994) reported that the qualitative superiority of Hanwoo beef is due to significantly higher oleic acid compared to imported beef. Such excellence is also attributable to the extension of distribution channel while imported beef involves long distribution days (Park and Yu, 1994).

In the present study, the Hanwoo steers fed with FS had lower n-6/n-3 but higher oleic acid in its loin, which is likely due to the action of Δ -9 desaturase. Cow's adipose tissues can synthesize oleic acids from stearic acid (18:0) via unsaturation of Δ -9 desaturase by stearyl Co-A desaturase genes and accumulation of oleic acids in its loin to strengthen the marbling of beef, thus producing high-quality premium meat (May *et al.*, 1993; Smith *et al.*, 2006). As shown in Table 1, despite a high level of stearic acid, stearic acid accumulated in Hanwoo beef loin decreased while oleic acid was increased. Due to the metabolism of Δ -9 desaturase mentioned above. It is well known that Japanese Wagyu beef and American Aberdeen Angus beef contain high levels of oleic acid accumulated in their loins. Changes in blood lipid profile and glucose levels in human subjects after consumption of FS (flaxseed-fed beef, n-6/n-3 lowered Hanwoo beef), C (control beef fed basal diets without flaxseed) or GI (grain-fed imported beef) loins are illustrated in Fig. 1. Blood triacylglycerol (TAG) level significantly reduced

Fig. 1 : Effects of flaxseed-fed beef loin (FS) on changes of blood lipid profiles and glucose in human subjects by clinical trials. Control beef : grain-fed Hanwoo beef loin, GI beef : grain-fed imported beef loin, FS beef : flaxseed-fed beef loin (n-6/n-3 lowered Hanwoo), TAG: triacylglycerol, TC: total cholesterol, LDL-C: low density lipoprotein-cholesterol, HDL-C: high density lipoprotein-cholesterol.

(25.35%, $p < 0.05$) in subjects fed with FS beef loin. However, those fed GI beef loin, it was further increased by 9.05%. Consumption of control beef loin did not change TAG level significantly. Total cholesterol level decreased by 5.22% in human subjects fed with FS beef loin while subjects fed GI beef loin was significantly increased by 8.21% ($p < 0.05$). Control beef loin did not lead to significant difference in total cholesterol level. Low density lipoprotein-cholesterol (LDL-C) significantly decreased by 17.59% in subjects fed with FS beef loin. However, it increased by 21.70% in subjects fed with GI beef loin ($p < 0.05$). It showed no different appreciable change in subjects fed with control beef loin. High density lipoprotein-cholesterol (HDL-C) increased by 6.07% in subjects fed with FS beef loin. However, in human subjects fed with GI beef and control beef, it significantly decreased by 14.46 and 11.46%, respectively ($p < 0.05$). Blood glucose levels in human subjects fed FS beef and control beef were significantly decreased by 6.42 and 11.82%, respectively. However, subjects fed with GI beef, it was increased by 15.19% ($p < 0.05$).

Intake of food high in n-6/n-3 ratio could raise the level of LDL-C in the blood and lead to CVD (Simopoulos, 2000; German and Dillard, 2004). LDL is the most important carrier that transports cholesterol from liver to blood. Cholesterol then accumulates in arteries and cells of epithelial tissues of the body. On the contrary, HDL-C can reverse transport cholesterol from the blood to the liver and release it into excrements after biligenesis. Cholesterol then passes through the digestive tract, eventually being removed from the blood (Daley et al., 2010; Riediger et al., 2009). The finding of this study is very important for producing beef with lower n-6 / n-3 ratio while enhancing oleic acid by feeding flaxseed to Hanwoo steers to reduce blood lipid profiles and glucose in humans after consuming flaxseed-fed beef.

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