

Asian-Aust. J. Anim. Sci. Vol. 23, No. 8 : 1013 - 1021 August 2010

www.ajas.info

# Effects of Amino Acid-enriched Ruminally Protected Fatty Acids on Plasma Metabolites, Growth Performance and Carcass Characteristics of Hanwoo Steers

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**ABSTRACT :** This study was conducted to determine the effects of amino acid-enriched ruminally protected fatty acid (AARPFA) on plasma fatty acids and amino acids, growth performance and carcass characteristics of Korean native steers (Hanwoo) by simultaneous supply of fatty acids and limiting amino acids (methionine and lysine). Eighteen finishing Hanwoo steers, 18 months of age and weighing an average of 459.0±38.9 kg, were used for studies of the metabolism of plasma fatty acids and amino acids during supplementation of AARPFA. Also, 45 finishing Hanwoo steers, 16 months of age and weighing an average of 408.6±26.5 kg, were used for growth performance and carcass characteristics during supplemention of AARPFA. There were three treatments which comprised a basal diet supplemented with AARPFA at 0 g (T1), 50 g (T2) or 100 g (T3), respectively. Concentrations of saturated, unsaturated and total fatty acids in plasma were increased in T3 compared with other treatments (p<0.05). Concentrations of methionine and lysine in plasma were linearly increased with increasing levels of AARPFA (p<0.01). Average daily gain, dry matter intake and feed conversion ratio were not different among the treatments. Marbling score measured by ultra-sound scanning was higher in T3 than in T1 at 24 months of age (p<0.05). Rib eye area, back fat thickness, yield index and yield grade score were similar across the treatments. Marbling score and quality grade score were higher in T3 compared with other treatments (p<0.01). Thus, plasma fatty acids, methionine and lysine metabolism were affected by supplementing with 100 g of AARPFA which also had positive effects on marbling score and meat quality grade of finishing Hanwoo steers. (**Key Words :** Hanwoo Steers, Fatty Acids, Amino Acids, Marbling Score, Quality Grade)

# INTRODUCTION

Energy- and protein-rich feed resources have been used for beef cattle because productivity can be improved substantially by strategic supplementation with energy and amino acid. Ca salts of fatty acids have been reported to be insoluble at a pH range of 6-7 and have a net energy value of approximately three times that of corn (Andrew et al., 1990). When Ca salts of fatty acids from vegetable oils were included in beef cattle diets for additional energy supply, there were relatively few effects on rumen fermentation (Schauff and Clark, 1989). Ca salts of fatty acids are completely dissociated in the acidic conditions of the abomasum (Jenkins and Palmquist, 1984) and absorbed in the small intestine (Kowalski et al., 1997; Ramana Reddy et al., 2003). Fearon et al. (1994) also reported that duodenal input of fatty acid was increased by supplementing Ca salts of fatty acids.

In addition, inclusion of protected fatty acids in ruminant diets improves energy efficiency due to the lower ruminal production of methane and direct use of long-chain fatty acids in the metabolic pathways of fat synthesis,

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replacing the need for acetate and glucose (Doreau and Chilliard, 1997; Machmüller et al., 2000). Thus, protected fatty acids have improved intramuscular fat accumulation of finishing beef steers.

In recent years, productive diets for beef cattle have been used with various sources of undegradable protein, because production of microbial protein (amino acids) alone is insufficient to supply adequate amounts of amino acids for optimal production (Kung and Rode, 1996). However, most protein feeds are a poor source of at least one essential amino acid (Merchen and Titgemeyer, 1992), and methionine and lysine are generally the first limiting amino acids for production of beef cattle (Hussein and Berger, 1995). Supplementation of ruminally protected methionine and lysine improved body weight gain or feed efficiency of growing steers (Wright and Loerch, 1988). Especially, supplemental dietary methionine can be associated with an increase in intramuscular fat accumulation of finishing beef steers. This response is most likely related to the role of methionine as a methyl donor in transmethylation reactions occurring during lipid biosynthesis (Lehninger, 1977; Mayes, 1981).

Thus, the objective of the present study was to determine the effects of simultaneous supply of fatty acids and limiting amino acids (methionine and lysine) on plasma fatty acids and amino acids, growth performance and carcass characteristics of finishing Hanwoo steers.

# **MATERIALS AND METHODS**

### Animals, experimental design, feeding and management

Eighteen finishing Hanwoo steers, 18 months of age and weighing an average of 459.0±38.9 kg, were used for studies of the metabolism of plasma fatty acids and amino acids during supplementation of amino acid-enriched ruminally protected fatty acid (AARPFA). The steers were randomly assigned to one of three treatment groups of six steers. The three treatments were a basal diet supplemented with AARPFA at 0 g (T1), 50 g (T2) and 100 g (T3), respectively.

The treatments were arranged in 3 pens with 6 steers per pen (10.6×10.6 m) each of which had a concreted floor with sawdust bedding. The pens were equipped with electronic head gates (Calan system, Seil Tech, Korea) to allow individual measurement of feed intake.

Experimental diets were offered twice daily in equal meals at 08:00 and 16:00 h. Animals were offered commercial concentrate *ad libitum*, and rice straw at 1.6 kg/animal/d. AARPFA was fed individually as a top dressing at the morning feeding. Steers had free access to fresh water and mineral block. The ingredients and chemical composition of the experimental diet are presented in Table 1.

**Table 1.** Ingredient and chemical composition of the experimental diet

uiet	Commercial	
Item		Rice straw
	concentrate	
Ingredient (DM, %)		
Corn, flaked	45.00	-
Barley	10.00	-
Soy-hull	15.00	-
Gluten feed	15.00	-
Wheat bran	7.99	-
Limestone	0.63	-
Lasalocid	0.02	-
VitMin. premix <sup>1</sup>	0.20	-
Salt	0.30	-
Molasses	3.00	-
Calcium phosphate	0.46	-
Sodium bicarbonate	0.40	-
Yeast culture	2.00	-
Total	100.00	-
Chemical composition (%)		
Dry matter	86.62±0.48	88.93±0.22
Crude protein	13.28±0.33	3.91±0.10
Ether extract	3.21±0.14	$1.29\pm0.06$
Crude fiber	6.18±0.56	31.50±0.20
Crude ash	4.92±0.10	10.50±0.56
Neutral detergent fiber	14.40±1.58	60.15±1.04
Acid detergent fiber	2.95±0.47	39.32±0.74

Means±standard deviation.

Ca salts of fatty acids were prepared by stirring and reacting a mixture of 424 units of palm oil and 59 units of calcium hydroxide. Powdered salts were selected by standard sieve (10 mesh) after pulverizing the Ca salts. 65 units of powdered Ca salts was mixed with 25 units of amino acids (methionine:lysine = 1:2) and 10 units of hydrogenated soybean oil. The mixture obtained was extruded and cut (3-4 mm length) for preparation of AARPFA.

Degradability of AARPFA was measured using three Hanwoo steers fitted with ruminal and duodenal cannulae. Nylon bags made from polyamide cloth (4 cm×3 cm, pore size 45  $\mu$ m) were used for gastrointestinal degradability of AARPFA. Chemical composition and degradability of AARPFA are presented in Table 2.

Forty five finishing Hanwoo steers, 16 months of age and weighing an average of 408.6±26.5 kg, were used for measurement of growth performance and carcass characteristics during supplementation with AARPFA. The experiment was conducted from 18 to 27 months of age

 $<sup>^1</sup>$  Contains the following: Vit. A, 2,650,000 IU; Vit. D<sub>3</sub>, 530,000 IU; Vit. E, 1,050 IU; BHT (butylated hydroxy toluene), 10,000 mg; Fe, 13,200 mg; Mn, 4,400 mg; Cu, 2,200 mg; co, 440 mg; I, 440 mg/kg.

**Table 2.** Chemical composition of amino acid-enriched ruminally protected fatty acids and its dry matter (DM) degradability in the gastrointestinal tract of Hanwoo steers

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Item	AARPFA <sup>1</sup>
Chemical composition (%)	
Dry matter	99.05
Ether extract	51.17
Fatty acids	
Saturated fatty acid	16.07
Mono-unsaturated fatty acid	43.29
Poly-unsaturated fatty acid	40.63
Amino acid	
Methionine	9.09
Lysine	19.83
DM degradability (%)	
Rumen	25.10±1.75
Abomasum	4.53±1.10
Intestine	65.44±5.33
Total tract	95.07±7.09

Means±standard deviation.

after a two-month adaption period. The steers were randomly assigned to one of three treatment groups of fifteen steers. The three treatments were a basal diet supplemented with AARPFA at 0 g (T1), 50 g (T2) and 100 g (T3), respectively.

The treatments were arranged with 15 steers per pen in 3 pens (15.9×10.6 m) each of which had a concreted floor with sawdust bedding. The pens were equipped with electronic head gates (Calan system, Seil Tech, Korea) to allow individual measurement of feed intake.

Experimental diets were offered twice daily in equal meals at 08:00 and 16:00 h. Animals were offered commercial concentrate *ad libitum* until slaughtered and rice straw at 1.6 kg/animal/d at 18 months of age. The amount of rice straw offered was decreased with increasing age, and restricted to 0.6 kg/animal/d during the late finishing period. AARPFA was fed individually as a top dressing at morning feeding. Steers had free access to fresh water and mineral block during the whole period.

### Sampling, measurements and analyses

The diets used in this study were dried by forced-air oven (at 60°C, 48 h), ground by a Wiley mill (Thomas scientific, Model 4, USA) and analyzed for moisture, crude protein, ether extract, crude fiber and crude ash according to the procedures of the Association of Official Analytical Chemists (AOAC, 1990). The concentration of neutral detergent fiber corrected for residual ash was determined with heat-stable amylase and sodium sulphate according to the method of Van Soest et al. (1991), while the content of

acid detergent fiber corrected for residual ash was determined according to the procedure of AOAC (1990).

Jugular blood samples were collected into heparinized tubes (Becton Dickinson Co., USA) at 3 d before and 7 d after supplementing with AARPFA (at 6 h after morning feeding) to determine plasma fatty acid and amino acid concentrations. The tubes were immediately transferred onto ice and centrifuged (for 30 min at 3,000×g) to obtain plasma.

Total lipids were extracted from plasma using chloroform-methanol (2:1, vol/vol) after the method of Folch et al. (1957). The lipid extracts were saponified by the addition of 0.5 N KOH in methanol and methylated with boron trifluoride-methanol following a modification of the procedure of Morrison and Smith (1964). Fatty acid methyl esters were analyzed using gas chromatography (Shimadzu Model GC-17A Ver. 3, Japan).

Proteins in plasma were precipitated from 3 ml samples by addition of 90 mg of sulfosalicylic acid followed by centrifugation at 18,000×g for 20 min. The resulting plasma supernatant was analyzed for amino acid concentrations by high performance liquid chromatography (HPLC) equipment (Waters 2695, USA) according to the procedure of an AccQ-Tag amino acid analysis (AccQ-Fluo<sup>TM</sup> Reagent kit, Waters, WAT052880).

Steers were weighed every month during the experimental period. Dietary refusals were collected and weighed before morning feeding every day. Feed conversion ratio was expressed as average dry matter intake per average daily gain.

Back fat thickness, marbling score and rib eye area were predicted between the 13<sup>th</sup> thoracic and 1<sup>st</sup> lumbar vertebrae of steers using ultra sound scanning equipment (Aquila, 3.5 MHz, 18 cm linear probe, Pie Medical, Netherlands) at 18, 20, 22 and 24 months and before slaughter at 27 months of age.

Carcass characteristics such as yield and quality grades were assessed at 24 h post-mortem by a carcass grader of the Animal Products Grading Service (APGS, 2007), Korea. Quality (marbling score, meat color, fat color, texture and maturity) and yield (cold carcass weight, back fat thickness and rib eye area) characteristics were recorded. After a 24-h chill, cold carcass weights were measured and then the left side of each carcass was cut between the last rib and the first lumbar vertebrae to determine quality grade. The quality grade was determined by assessing the degree of marbling and firmness in the cut surface of the rib eye, in relation to the maturity, meat color and fat color of the carcass. Quality grades were classified as 1<sup>++</sup> (best), 1<sup>+</sup>, 1, 2 and 3 (worst) according to the Korean beef quality grading system. The rib eye area was measured from longissimus muscle taken at the 13th rib and back fat thickness was also measured at the 13th rib. Yield index was calculated as

<sup>&</sup>lt;sup>1</sup> AARPFA = Amino acid-enriched ruminally protected fatty acids.

follows: Yield index: 68.184-(0.625×back fat thickness (mm))+(0.130×rib eye area (cm<sup>2</sup>))-(0.024×dressed weight amount (kg))+3.23. Yield grades were classified as A (best), B and C (worst) according to the Korean beef yield grading system as determined by yield index (grade A = more than 67.50, grade B = more than  $62.00 \sim less$  than 67.50 and grade C = less than 62.00). The degree of marbling was evaluated with the Korean Beef Marbling Standard, and the scores of meat color and fat color were made using the color standard (APGS, 2007). The scores for texture and maturity were made using the APGS reference index (APGS, 2007). The grading ranges were 1 to 9 for marbling score with higher numbers for better quality (1 = devoid, 9 = abundant); meat color (1 = brightly cherry red, 7 = extremely dark red); fat color (1 = white, 7 = dark yellow); texture (1 = soft, 3 = firm); maturity (1 = youthful, 9 = mature).

### Statistical analysis

The plasma concentrations of amino acids and fatty acids, growth performance, ultra sound scanning data (back fat thickness and rib eye area), carcass weight, rib eye area and back fat thickness were treated as continuous data and analyzed using PROC MIXED (SAS Inst. Inc., Cary, NC). Marbling score measured by ultra sound scanning, yield index, yield grade score, marbling score, meat color, fat color, texture, maturity and quality grade score were considered as categorical data and analyzed using the PROC GLIMMIX (SAS Inst. Inc.). Polynomial regressions were utilized to determine plasma concentration (amino acid and fatty acid), growth performance, ultra sound scanning (back fat thickness, rib eye area and marbling score) and carcass response to increasing levels of AARPFA.

# **RESULTS AND DISCUSSION**

# Plasma fatty acid and amino acid metabolism

Concentrations of myristic (C14:0), palmitoleic (C16:1 n-7), γ-linolenic (C18:3 n-6) and eicosenoic (C20:1 n-9) acid in plasma were increased by supplementing with AARPFA (Table 3), especially by 100 g of AARPFA (p<0.05). Concentrations of saturated fatty acid (SFA), unsaturated fatty acid (UFA) and total fatty acids in plasma were increased (p<0.05). Concentration of monounsaturated fatty acid (MUFA), and the ratios of UFA/SFA and MUFA/SFA were increased by supplementing with 100 g of AARPFA (p<0.05). However, AARPFA had no effect on the concentration of poly-unsaturated fatty acid (PUFA) in plasma.

In the present study, fatty acid concentrations in plasma increased linearly with dietary AARPFA level, indicating qualitatively that AARPFA was well protected from ruminal degradation and subsequently absorbed. Similar to the present results, Fearon et al. (1994) reported that duodenal input of fatty acid was increased by supplementing protected fatty acid. In addition, Sklan and Tinsky (1993) found that concentrations of free fatty acids and triglyceride in plasma were increased by supplementing with Ca salts of fatty acids in Israeli-Friesian cows.

Concentrations of methionine and lysine in plasma were similar among treatments before supplementing with AARPFA (Table 4), but concentrations of these amino acids in plasma increased linearly with increasing level of AARPFA (p<0.01). Supplementation of AARPFA had no effects on concentrations of essential, nonessential and total amino acids in plasma.

In the present study, concentrations of methionine and lysine in plasma were increased by AARPFA supplementation. These results were in agreement with those of previous authors (Wright and Loerch, 1988; Veira et al., 1991) who found a linear increase in concentrations of these amino acids in plasma with increasing levels of protected methionine and lysine.

Thus, the AARPFA was well protected from ruminal degradation and had positive effects on increasing the supply of fatty acids and amino acids in finishing Hanwoo steers.

### **Growth performance and carcass characteristics**

Final body weight and average daily gain (ADG) were not different among the treatments (Table 5). Concentrate, rice straw and dry matter intake (DMI) were similar in steers on different treatments. Also, feed conversion ratio was not affected by supplementing with AARPFA.

The present results were supported by previous studies, such as those of Gilbert et al. (2003) who found no differences in DMI, ADG and feed conversion ratio of Brangus steers by supplementing with protected canola lipid, and Ngidi et al. (1990) who reported that DMI, ADG and feed conversion ratio of Angus-Hereford crossbred steers fed high concentrate diets were not affected by supplementing with 2% Ca salts of fatty acids. Moreover, previous works suggested that protected fatty acids had no negative effects on DMI, ADG and feed efficiency (Haaland et al., 1981; Ramana Reddy et al., 2003).

In experiments with growing steers, supplementation of ruminally protected methionine and lysine was found to improve ADG or feed conversion ratio without affecting the DMI (Wright and Loerch, 1988; Veira et al., 1991). However, because most previous trials evaluated supplementation of ruminally protected methionine and lysine during the growing phase, the present study was conducted to provide such evaluation during finishing. Therefore, there were no positive effects on ADG, DMI and feed conversion ratio of finishing Hanwoo steers in the present study. These results were in agreement with those of

Table 3. Changes of fatty acid concentrations (mg/ml) in plasma 3 days before and 7 days after start of feeding amino acid-enriched ruminally protected fatty acids

Itam		$T1^1$	$T2^2$	T3 <sup>3</sup>	SEM	p-value	
Item		11				Linear	Quadratic
3 days before	C14:0	5.14	5.36	5.17	0.254	0.731	0.731
	C16:0	0.72	1.02	0.98	0.079	0.177	0.301
	C16:1 n-7	7.15	7.83	7.21	0.385	0.473	0.466
	C18:0	4.57	4.77	4.54	0.277	0.759	0.743
	C18:1 n-9	15.25	12.66	14.08	0.895	0.280	0.324
	C18:2 n-6	0.29	0.32	0.33	0.028	0.721	0.828
	C18:3 n-3	0.88	0.86	1.23	0.097	0.603	0.342
	C18:3 n-6	0.24	0.21	0.23	0.016	0.482	0.505
	C20:1 n-9	1.12	1.09	1.27	0.086	0.074	0.106
	Other	3.71	4.19	3.60	0.426	0.627	0.593
	$SFA^4$	10.43	11.15	10.70	0.558	0.627	0.649
	UFA <sup>5</sup>	24.93	22.97	24.35	1.358	0.581	0.597
	MUFA <sup>6</sup>	23.52	21.59	22.56	1.257	0.575	0.616
	PUFA <sup>7</sup>	1.41	1.39	1.78	0.131	0.703	0.473
	TFA <sup>8</sup>	39.07	38.31	38.64	2.209	0.904	0.916
	UFA/SFA	2.42	2.06	2.27	0.078	0.075	0.100
	MUFA/SFA	2.28	1.94	2.10	0.076	0.084	0.123
	PUFA/SFA	0.06	0.06	0.07	0.004	0.956	0.564
7 days after	C14:0	5.05 <sup>b</sup>	6.47 <sup>a</sup>	$6.60^{a}$	0.239	0.012	0.081
	C16:0	0.95	1.59	1.12	0.115	0.016	0.018
	C16:1 n-7	6.82°	$8.50^{b}$	9.88 <sup>a</sup>	0.391	0.083	0.758
	C18:0	4.53 <sup>b</sup>	$6.48^{a}$	5.97 <sup>a</sup>	0.318	0.013	0.038
	C18:1 n-9	15.25	15.92	20.22	0.975	0.765	0.329
	C18:2 n-6	0.33	0.40	0.39	0.028	0.445	0.566
	C18:3 n-3	1.02	1.25	1.47	0.096	0.573	0.996
	C18:3 n-6	$0.22^{b}$	$0.27^{ab}$	$0.33^{a}$	0.018	0.406	0.975
	C20:1 n-9	1.28 <sup>b</sup>	1.44 <sup>b</sup>	1.85 <sup>a</sup>	0.085	0.281	0.119
	Other	4.74	5.75	5.81	0.290	0.251	0.437
	SFA	10.53 <sup>b</sup>	14.54 <sup>a</sup>	13.69 <sup>a</sup>	0.613	0.006	0.022
	UFA	24.92 <sup>b</sup>	27.79 <sup>b</sup>	34.14 <sup>a</sup>	1.411	0.813	0.445
	MUFA	23.35 <sup>b</sup>	25.87 <sup>b</sup>	31.95 <sup>a</sup>	1.302	0.864	0.390
	PUFA	1.57	1.92	2.19	0.128	0.474	0.882
	TFA	40.19 <sup>b</sup>	$48.07^{a}$	53.64 <sup>a</sup>	1.938	0.158	0.196
	UFA/SFA	2.39 <sup>ab</sup>	1.93 <sup>b</sup>	$2.50^{a}$	0.104	0.025	0.015
	MUFA/SFA	2.23 <sup>b</sup>	$1.80^{b}$	$2.34^{a}$	0.096	0.057	0.493
	PUFA/SFA	0.06	0.07	0.06	0.002	0.224	0.249

a,b Least squares means with different superscripts in the same row significantly differ (p<0.05).

<sup>1,2,3</sup> In this and all other tables, T1 = Feeding group of basal diet (concentrate feed and rice straw); T2 = Feeding group of basal diet+50 g/d of amino acidenriched ruminally protected fatty acids; T3 = Feeding group of basal diet+100 g/d of amino acid-enriched ruminally protected fatty acids. 

<sup>4</sup> SFA = Saturated fatty acid. 

<sup>5</sup> UFA = Unsaturated fatty acid. 

<sup>6</sup> MUFA = Mono-unsaturated fatty acid.

<sup>&</sup>lt;sup>7</sup> PUFA = Poly-unsaturated fatty acid. <sup>8</sup> TFA = Total fatty acid.

<b>Table 4.</b> Changes of amino acid concentrations	(μg/ml) in plasma 3	3 days before and 7	7 days after start of fee	eding amino acid-enriched
ruminally protected fatty acids				

Item		T1	T2	Т3	SEM	p-v	p-value	
item		11	12	13	SEM	Linear	Quadratic	
3 days before	Methionine	8.11	7.84	8.10	0.44	0.802	0.796	
	Lysine	18.73	18.10	18.72	1.02	0.801	0.794	
	$EAA^1$	162.73	161.31	170.80	5.99	0.811	0.693	
NEAA <sup>2</sup>	$NEAA^2$	108.67	109.54	108.27	3.46	0.908	0.895	
	Total	279.51	278.69	279.07	9.09	0.974	0.978	
7 days after	Methionine	8.19 <sup>c</sup>	$9.40^{b}$	10.09 <sup>a</sup>	0.23	0.001	0.134	
	Lysine	18.89 <sup>c</sup>	$21.70^{b}$	23.29 <sup>a</sup>	0.54	0.001	0.132	
	EAA	184.49	182.34	183.88	3.50	0.823	0.830	
	NEAA	117.25	118.42	120.91	2.52	0.967	0.914	
	Total	303.31	307.79	314.88	5.71	0.910	0.924	

a,b,c Least squares means with different superscripts in the same row significantly differ (p<0.05).

Table 5. Effect of amino acid-enriched ruminally protected fatty acids on body weight gain, intake and feed conversion ratio of Hanwoo steers

Item	T1	TO	т2	CEM	p-v	p-value	
	11	T2	Т3	SEM	Linear	Quadratic	
Initial body weight (kg)	461.6	470.4	460.9	4.58	0.39	0.36	
Final body weight (kg)	652.4	645.9	656.2	5.15	0.52	0.45	
Average daily gain (kg/d)	0.77	0.72	0.80	0.02	0.19	0.11	
Intake (DM kg/d)							
Concentrate	8.94	8.52	8.83	0.08	0.03	0.03	
Rice straw	0.77	0.77	0.77	0.02	1.00	1.00	
Dry matter intake	8.88	8.50	8.78	0.08	0.05	0.05	
Feed conversion ratio	13.90	13.26	13.03	0.32	0.56	0.77	

Oke et al. (1986) and Strasia et al. (1986) who found a positive growth response to ruminally protected methionine and lysine during the growing phase but not during the finishing phase.

Back fat thickness and rib eye area measured by ultra sound scanning were not affected by supplementing with AARPFA (Table 6). Although marbling score measured by ultra sound scanning was not different among the treatments from 18 to 22 months of age, it was higher in T3 than in T1 at 24 months of age (p<0.05).

Similar to the present results, Salinas et al. (2006) reported that back fat thickness and rib eye area measured using ultra sound scanning were not affected by supplementing with Ca salts of tallow. However, marbling score measured using ultra sound scanning was increased by supplementing with 100 g of AARPFA. The results showed that increased marbling score was related to interaction of fatty acids and amino acids when fatty acid accumulation was age dependent and intramuscular fat was the last to be accumulated during growth (Salinas et al., 2006).

In carcass yield traits, rib eye area, back fat thickness,

yield index and yield grade were similar across the treatments (Table 7). In the yield grades of T1, incidence of A, B and C grades was 40%, 47% and 13%, respectively. The A, B and C grades in T2 were 40%, 53% and 7%, respectively, whereas in T3 were three grades were 67%, 33% and 0%, respectively.

In carcass quality traits, meat color, fat color, texture and maturity were similar in steers on the different treatments. Marbling score and quality grade score were higher in T3 compared with other treatments (p<0.01). The appearances of desirable high quality grade (1<sup>++</sup>, 1<sup>+</sup> and 1) of beef based on consumers demand were 73%, 87% and 100% in T1, T2 and T3, respectively. The best quality grade (1<sup>++</sup>) incidence was 0%, 7% and 20% in T1, T2 and T3, respectively.

In the present study, marbling score and quality grade score were increased by supplementing of AARPFA. It was thought that inclusion of protected fatty acids in ruminant diets improves energy efficiency due to the lower ruminal production of methane and direct use of long-chain fatty acids in the metabolic pathways of fat synthesis, precluding the need for acetate and glucose (Doreau and Chilliard,

<sup>&</sup>lt;sup>1</sup> EAA = Essential amino acid. <sup>2</sup> NEAA = Nonessential amino acid.

**Table 6.** Effect of amino acid-enriched ruminally protected fatty acids on back fat thickness, rib eye area and marbling score measured using ultra sound scanning of Hanwoo steers

Item	Months of ago	T1	T2	Т3	SEM	p-value	
	Months of age					Linear	Quadratic
Back fat thickness (mm) <sup>1</sup>	18 (Initial)	4.50	4.69	4.47	0.21	0.66	0.65
	20	5.30	5.70	5.53	0.23	0.54	0.57
	22	6.80	7.20	6.67	0.23	0.40	0.36
	24	8.48	8.60	7.97	0.37	0.75	0.64
	27 (Final)	9.97	10.47	9.07	0.48	0.42	0.36
Rib eye area (cm <sup>2</sup> ) <sup>1</sup>	18	63.44	64.97	61.49	0.78	0.18	0.13
	20	68.97	71.41	67.27	0.79	0.07	0.05
	22	75.97	76.25	72.97	0.64	0.29	0.18
	24	80.39	81.87	78.10	0.57	0.05	0.03
	27	87.44	87.60	85.97	0.87	0.71	0.63
Marbling score <sup>1,2</sup>	18	1.13	1.20	1.40	0.07	0.83	0.66
	20	2.00	2.07	2.33	0.10	0.78	0.63
	22	2.53	3.07	3.13	0.12	0.23	0.36
	24	3.67 <sup>b</sup>	$4.27^{ab}$	$4.80^{a}$	0.18	0.64	0.93
	27	5.13 <sup>b</sup>	5.73 <sup>b</sup>	$6.80^{a}$	0.23	0.96	0.61

a,b Least squares means with different superscripts in the same row significantly differ (p<0.05).

Table 7. Effects of amino acid-enriched ruminally protected fatty acids on carcass characteristics of Hanwoo steers

Item	T1	T2	Т3	SEM	p-value		
item	11				Linear	Quadratic	
Carcass weight (kg)	408.3	401.9	403.1	4.98	0.65	0.73	
Yield traits <sup>1</sup>							
Rib eye area (cm <sup>2</sup> )	84.67	88.00	88.27	0.87	0.20	0.40	
Back fat thickness (mm)	10.80	12.13	9.13	0.64	0.21	0.11	
Yield index (%)	65.87	65.64	67.50	0.50	0.57	0.33	
Yield grade (A:B:C, %)	40:47:13	40:53:7	67:33:0	-	-	-	
Yield grade score <sup>2</sup>	2.27	2.33	2.67	0.09	0.87	0.49	
Quality traits <sup>3</sup>							
Marbling score	5.07 <sup>b</sup>	5.73 <sup>b</sup>	6.93 <sup>a</sup>	0.24	0.68	0.57	
Meat color	4.67	4.47	4.53	0.07	0.32	0.41	
Fat color	3.00	3.00	3.00	-	-	-	
Texture	1.27	1.40	1.13	0.07	0.26	0.16	
Maturity	2.40	2.27	2.13	0.07	0.65	1.00	
Quality grade (1 <sup>++</sup> :1 <sup>+</sup> :1:2:3, %)	0:33:40:27:0	7:40:40:13:0	20:60:20:0:0	-	-	-	
Quality grade score <sup>4</sup>	3.07 <sup>b</sup>	$3.40^{b}$	$4.00^{a}$	0.13	0.69	0.58	

<sup>&</sup>lt;sup>a,b</sup> Least squares means with different superscripts in the same row significantly differ (p<0.05).

 $<sup>^{1}</sup>$  Months of age (p<0.01). Treatment×months of age (p<0.01).

Area was measured from *longissmus* muscle taken as 13<sup>th</sup> rib and back fat thickness were also measured at 13<sup>th</sup> rib; Yield index was calculated using the following equation: 68.184-(0.625×back fat thickness (mm))+(0.130×rib eye area (cm²))-(0.024×dressed weight amount (kg)); Carcass yield grades from C (low yield) to A (high yield).

<sup>&</sup>lt;sup>2</sup> Yield grade score: A = 3, B = 2 and C = 1.

<sup>&</sup>lt;sup>3</sup> Grading ranges are 1 to 9 for marbling score with higher numbers for better quality (1 = devoid, 9 = abundant); meat color (1 = brightly cherry red, 7 = extremely dark red); fat color (1 = white, 7 = dark yellow); texture (1 = soft, 3 = firm); maturity (1 = youthful, 9 = mature); quality grades from 3 (low quality) to 1<sup>++</sup>(very high quality).

<sup>&</sup>lt;sup>4</sup> Quality grade score:  $1^{++} = 5$ ,  $1^{+} = 4$ , 1 = 3, 2 = 2 and 3 = 1.

1997; Machmüller et al., 2000). In addition, methionine of AARPFA can be associated with increase in marbling score and quality grade score. This response is most likely related to the role of methionine as a methyl donor in transmethylation reactions occurring during lipid biosynthesis (Lehninger, 1977; Mayes, 1981).

Results of the present study agree with those of Ngidi et al. (1990) and Zinn et al. (2000) who fed Ca soaps of fatty acids to heifers and steers, respectively, and found no effects on back fat thickness and rib eye area. Moreover, Gillbert et al. (2003) reported that supplementation of protected canola lipid improved yield grade, marbling score and quality grade without affecting carcass weight, rib eye area and back fat thickness.

Thus, the present results indicated that supplementation with 100 g of AARPFA had positive effects on marbling score and meat quality grade due to increasing supply of fatty acids and limiting amino acids (methionine and lysine) in finishing Hanwoo steers.

# **CONCLUSION**

The present findings indicated that plasma fatty acids and methionine and lysine metabolism were affected by supplementing with AARPFA, which had positive effects on marbling score and meat quality grade of finishing Hanwoo steers. However, AARPFA had no positive effects on growth performance. Ca salts of palm oil fatty acids could be used as a means of protecting limiting amino acids (methionine and lysine) against degradation in the rumen, and this combination (AARPFA) could supply finishing Hanwoo steers with additional energy and absorbable amino acids.

### **ACKNOWLEDGMENTS**

This study was supported by 2007 Post Doctoral Course Program of National Institute of Animal Science, Rural Development Administration, Republic of Korea.

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