Effects of Rumen-Protected Methionine and Lysine or IGF-I Supplementation on Growth Performance and Carcass Characteristics of Hanwoo Steers

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반추위 보호 메티오닌과 라이신 혹은 IGF-I 첨가가 거세한우의 사양 성적 및 도체 특성에 미치는 영향

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Abstract This study aimed to investigate the effects of rumen-protected methionine and lysine (RPML) or insulin-like growth factor I (IGF-I) supplementation on the growth performance, plasma metabolite, and carcass characteristics of Hanwoo steers. Thirty-nine steers (264.4 \pm 60.7 kg, period : 11 \sim 28 months) were randomly assigned to the following groups: control group fed a basal diet (formula feed + rice straw), T1 group fed a basal diet + RPML, and T2 group fed a basal diet + IGF-I. The average daily gain (ADG) was slightly, but not significantly, higher in the T1 and T2 groups than in the control group. The concentrations of plasma metabolites were similar between the treatments. The carcass weight and marbling score tended to be higher in the T1 and T2 groups than in the control group, and the rib eye area was wider in the T2 group than in the control and T1 groups (p<0.01). RPML or IGF-I supplementation did not affect back fat thickness, meat color, or texture of Hanwoo steers. Thus, RPML or IGF-I supplementation had slightly positive effects on ADG, marbling score, and rib eye area. In particular, IGF-I supplementation had a positive effect on the rib eye area in Hanwoo steers.

요 약 본 연구는 반추위 보호 메티오닌과 라이신(RPML) 혹은 insulin-like growth factor I 첨가가 거세한우의 사양성적, 혈액성상 및 도체 특성에 미치는 영향을 규명하기 위해 수행되었다. 공시동물은 거세한우 39두(264.4 ± 60.7kg, 11.0 ± 0.9개월령)을 이용하였으며, 실험은 총 16개월 동안 수행하였다. 시험구는 기초사료(배합사료 + 볏짚)를 급여하는 대조구, T1구(기초사료 + RPML) 및 T2구(기초사료 + IGF-I)로 구분하여 실험을 수행하였다. 일당증체량은 대조구에 비해 T1구 및 T2구에서 높은 경향을 보였다. 처리구간 혈중 대사물질 농도는 유사한 수준이었다. 도체중과 근내지방도는 대조구에 비해 T1구 및 T2구에서 높은 경향을 보였으며, 등심단면적은 대조구 및 T1구에 비해 T2구에서 넓었다(р⟨0.01). RPML 혹은 IGF-I의 첨가가 등지방두께, 육색, 지방색, 조직감 혹은 성숙도에 미치는 영향은 없었다. 따라서 RPML 혹은 IGF-I의 첨가는 일당증체량, 근내지방도 및 등심단면적에 일부 긍정적인 영향을 미쳤으며, 특히 IGF-I의 첨가는 등심단면적 증가에 긍정적인 영향을 미치는 것으로 판단된다.

Keywords: Hanwoo Steers, Rumen-Protected Methionine and Lysine, Insulin Growth Factor-I, Average Daily Gain, Marbling Score, Rib Eye Area

This research was supported by a grant "Development of precise feeding program for the pregnancy, calf, growing and fattening stages of Hanwoo based on nutritional metabolic imprinting (PJ016226)" from the National Institute of Animal Science, Rural Development Administration, Republic of Korea

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Received April 29, 2024 Accepted July 5, 2024 Revised June 26, 2024 Published July 31, 2024

1. Introduction

The productivity of cattle can be improved through the strategic supply of amino acids because methionine and lysine are generally the first limiting amino acids for the production of cattle [1]. Methionine and lysine are important amino acids involved in the nutritional metabolism of beef cattle and are among the most deficient amino acids in livestock. In particular, methionine is a typical sulfur-containing amino acid that is an initiation codon and methyl group donor in protein synthesis and plays an important role in cell growth and metabolism [2]. Methionine has also been reported to have an important effect on fat deposition in muscle [3]. Lysine is an essential amino acid in protein metabolism and is known to increase the utilization efficiency of methionine and participate in the synthesis of carnitine, which is related to fat metabolism [3]. However, because unprotected amino acids are rapidly degraded by rumen microbes, rumenprotected methionine and lysine (RPML) are used in dairy and beef cattle. RPML has been used to increase the milk yield and protein content in lactating dairy cows [4]. Ahn et al. (2019) reported that RPML supplementation had slightly positive effects on productivity without any negative influence on the growth performance, carcass characteristics, or meat properties of Hanwoo steers [5]. Insulin-like growth factor I (IGF-I) is essential for skeletal muscle development, growth, and maintenance. IGF-I can increase the muscle volume by promoting the synthesis of muscle proteins [6]. The IGF-I gene, located on chromosome 5 of cattle, is located in the intramuscular fat and growth-related quantitative trait locus (QTL). Davis et al. (2000) reported the effects of IGF-I on live weight, carcass weight, milk production, and fat deposition in cattle [6]. Yoon et al. stated that the IGF-I gene acts at the center of the network pathway of candidate genes that have a significant correlation with

intramuscular adipose tissue and that the expression level of IGF-I increases by approximately 1.7 times in meat with a high marbling score (No. 9) than in meat with a low score (No. 2) [8]. The content of IGF-I has been reported to influence body weight, carcass weight, milk yield, and fat deposition in cows [9]. It is also widely recognized as an essential factor in the development, growth, maintenance and increase of skeletal muscle volume by assisting in the synthesis of muscle proteins [10], and most studies have been conducted on growth traits. However, for rumen-protected amino acids, studies have been actively conducted in dairy cows rather than in Hanwoo steers, and for IGF-I, research on the growth performance and carcass characteristics of Hanwoo steers is lacking because most studies have focused on gene expression. Therefore, this study was conducted to investigate the effects of supplementing RPML or IGF-I on the growth performance, plasma metabolite levels, and carcass characteristics of Hanwoo steers.

2. Materials and methods

2.1 Animals, treatments, and management

In this study, 39 Hanwoo steers (11.0 \pm 0.9 months of age, 264.4 ± 60.7 kg) were equally divided between the treatment groups with 13 steers/group based on a completely randomized block design. The experiment was conducted over a total duration of 16 months (484 days), from an average age of 11 months to 27 months. The steers were divided into one of three treatment groups: a control group fed formula feed + rice straw; a T1 group fed formula feed + rice straw + RPML, at 20 g/steer/day; and a T2 group fed formula feed + rice straw + IGF-I at 20 g/steer/day. RPML was composed of 15% methionine + 16% lysine + 69% calcium salt-protected fat, and the calcium salt-protected fat used for coating methionine and lysine was prepared through a mixture of palm oil and calcium. The fatty acid composition of RPML was $1.52 \pm 0.04\%$ myristic acid (C14), $55.69 \pm 0.52\%$ palmitic acid (C16), $3.58 \pm 0.11\%$ stearic acid (C18), $33.79 \pm 0.38\%$ oleic acid (C18:1), and $5.42 \pm 0.07\%$ linoleic acid (C18:2). To produce the IGF-I used in this study, was obtained by extracting growth-related genes from Periserrula leucophryna-Paik-1977, analysing the amino

Table 1. Ingredients and chemical composition of experimental diet(ad-fed basis)

Item	formula feed A ¹	$\begin{array}{c} \text{formula feed} \\ \text{B}^2 \end{array}$	Rice steaw						
Ingredient compositon (%)									
Corn grain	32.8	31.7	-						
Wheat grain	8.0	6.0	-						
Rice with hull	-	4.0	-						
Cane molasses	4.6	4.0	-						
Amino by product	2.0	-	-						
Wheat bran	3.0	7.0	-						
Corn gluten feed	23.0	23.0	-						
Rape seed meal	1.2	-	-						
Coconut meal	3.0	0.6	-						
Palm kernel meal	16.0	12.0	-						
DDGS ³ (Corn)	-	2.2	-						
DDGS(Wheat)	2.0	2.0	-						
Lupin	-	2.0	-						
Cottenseed	-	2.0	-						
Salt dehydrated	0.6	0.6	-						
Limestone	2.9	2.1	-						
Vitamin premix ⁴	0.1	0.1	-						
Mineral premix ⁵	0.1	0.1	-						
Feed additives	0.7	0.6	-						

----- Chemical compositoin (%) ----- 87.55 ± 0.77 51.67 ± 0.65 Dry matter 87.73±0.32 Crude protien 14.00±0.13 13.00±0.33 1.66 ± 0.28 Ether extract 3.21 ± 0.23 3.88 ± 0.27 1.04 ± 0.19 Crude ash 1.20±0.19 0.90 ± 0.36 3.75 ± 0.24 Crude fiber 6.71 ± 0.88 6.74 ± 0.81 15.25 ± 0.95 NDF^6 38.82±0.88 26.32 ± 0.92 25.10 ± 0.90 TDN^7 72.50 70.77

¹Formula feed A: Early fattening period; ²Formula feed B: Late fattening period; ³DDGS: dried distiller's grains with solubles; ⁴Vitamin premix: Mineral premix provided the following quantities of minerals per kilogram of diet: Fe, 5 mg; Cu, 7 mg; Zn, 30 mg; Mn, 24 mg; I, 0.6 mg; Co, 0.15 mg; Se, 0.15 mg; ⁵NDF: neutral detergent fiber; ⁶TDN: total digestible nutrients (calculated values)

acid sequence of IGF-I from bovine to obtain a construct, designing primers for vector cloning in Escherichia coli, and obtaining the peptide by transformation. IGF-I was made by peptide, citric acid, vitamin A, vitamin D3, and binding agents were used to prepare the peptide and evaluate its stability to heat and digestive enzymes. The animals were selected from cows of similar body weight and age, and animals were housed each pen accommodating three animals, and each pen measured 5×10 meters in size. The formula feed was fed twice daily (at 08:00 and 17:00). RPML or IGF-I were mixed and fed when feeding formula feed during the experimental period. Rice straw or water were freely available, and other feeding management practices were conducted in accordance with farm practices. The ingredients and chemical compositions of the experimental diets are listed in Table 1.

2.1.1 Growth performance and plasma metabolites

Body weight was measured using a cattle weight bridge. The average daily gain (ADG) was calculated by measuring body weight during the experimental period. Feed intake was measured daily by measuring the leftover feed present before the morning feeding. The feed conversion ratio (FCR) was calculated using dry matter intake (DMI) and ADG.

2.1.2 Plasma metabolites

For the analysis of plasma metabolites, blood samples (3 mL) were collected from the jugular vein of the experimental animals using an 18-gauge needle and a blood collection tube (Vacutainer, Becton-Dickinson, Franklin Lakes, NJ, USA) coated with heparin. Blood samples were centrifuged at $1,250 \times g$ for 10 min to separate the plasma and were analyzed using an automatic blood analyzer (Hitachi 7020, Hitachi, Tokyo, Japan). The analyses included measurements of albumin (ALB), alanine aminotransferase (ALT), blood urea nitrogen (BUN), calcium (Ca), cholesterol

(CHO), gamma-glutamyl transpeptidase (GGT), glucose (GLU), magnesium (Mg), non-esterified fatty acids (NEFA), inorganic phosphorus (IP), triglycerides (TG), and total protein (TP).

2.1.3 Ultrasonic measurement

Ultrasonic measurements were performed on all steers at 11, 13, and 26 months of age. To evaluate meat quality traits, 2 MHz real-time ultrasonic equipment was applied between the 13th rib and the lumbar vertebra interface (Honda-3100, Honda Electronics, Toyohashi, Japan). Back fat thickness, rib eye area, and marbling score were processed and analyzed using Image-Pro Express software (Version 4.1, Media Cybernetics, USA).

2.1.4 Carcass characteristics

steers were sacrificed at a local slaughterhouse after the experimental period. The carcass yield grades (carcass weight, back fat thickness, rib eye area, and yield index) and quality grades (marbling score, meat color, fat color, texture, and maturity) were examined according to the criteria of the Korean carcass grading system [11]. The carcass was chilled for 24 h, and its weight was measured. Next, the left side of each carcass was cut between the thirteenth rib and the first lumbar vertebra and used to determine yield and quality traits. The rib eye area was measured from the *longissimus* muscle of the thirteenth rib. The back fat thickness was measured on the thirteenth rib. The yield index was calculated as follows: yield index = $[(11.06398 - 1.25149 \times back fat)]$ thickness (mm)] + $[0.28293 \times \text{rib eye area (cm}^2)]$ + [0.56781 × carcass weight (kg)]/[carcass weight (kg) × 100]. Yield grades were classified as grade A (best, yield index > 67.20), grade B (yield index 63.30 - 67.20), and grade C (worst, yield index \langle 63.30) as determined by the yield index. The yield grade scores were calculated as follows:

grade A = 3, grade B = 2, and grade C = 1. The quality grade was determined by assessing the degree of marbling on the cut surface of the rib eye based on the maturity, texture, meat color, and fat color of the carcass. Marbling scores were graded on a scale of 1 - 9, with higher numbers indicating better quality (1 = devoid and 9 = abundant). Additional scores included meat color (1 = bright red, 7 = dark red), fat color (1 = creamy white, 7 = yellowish), maturity (1 = youthful and 9 = old), and texture (1 = soft and 5 = firm). The quality grades were evaluated as follows: 1++ (excellent quality), 1+, 1, 2, and 3 (low quality). Quality grade scores were calculated as follows: $1^{++} = 5$, $1^{+} = 4$, 1 = 3, 2 = 12, 3 = 1. The auction price was calculated as the final successful bid price at the auction, based on quality grade and carcass yield [11].

2.2 Statistical analysis

For the statistical analysis of all results obtained in this study, the mean value and standard error were calculated using IBM SPSS V26 (Statistical Package for the Social Science, SPSS V26, Chicago, IL, USA), and the one-way arrangement for the mean value of each treatment group was tested at a significance level of 95% using Duncan's multiple test method through one-way ANOVA (p<0.05).

3. Result and discussion

Table 2 shows the effects of supplementation with RPML or IGF-I on the ADG, feed intake, and FCR of Hanwoo steers.

The initial body weight was similar between the treatment groups; however, the body weight at the end of the experimental period was slightly, but not significantly, higher in the T1 and T2 groups than in the control group. The ADG during the entire experimental period showed a higher trend in the T1 or T2 groups (0.92 kg/d) than in the control group (0.89 kg/d), but there was no statistically significant difference. ADG during the entire experimental period was numerically higher in the T1 or T2 groups than in the control group, and the FCR tended to be lower in the T1 or T2 groups than in the control group. Wright and Loerch reported that feeding

Table 2. Changes in growth performance of Hanwoo steers

Item	Control ¹	T1 ²	T2 ³	SEM ⁴	P-value			
Early fattening period (0 - 205 days)								
Months of age	11.3	11.0	11.1	0.34	-			
Initial body weight (kg)	263.9	266.3	262.8	12.38	0.994			
Final body weight (kg)	461.3	465.9	467.0	12.12	0.981			
Total gain (kg)	197.3	199.6	204.2	2.93	0.639			
ADG ⁵ (kg)	0.96	0.97	1.00	0.01	0.639			
Feed intake (DM ⁶ kg/day/steer)	8.63	8.59	8.62	0.27	-			
Formula feed	5.81	5.77	5.80	0.20	-			
Rice straw	2.82	2.82	2.82	0.01	-			
FCR ⁷	9.03	8.86	8.68	0.69	0.613			
Late fattening period (206 - 484 days)								
Months of age	18.0	17.9	17.9	0.34	-			
Initial body weight (kg)	461.3	465.9	467.0	12.12	0.981			
Final body weight (kg)	694.3	710.4	709.0	8.94	0.735			
Total gain (kg)	233.0	244.4	242.0	9.70	0.894			
ADG (kg)	0.84	0.88	0.87	0.03	0.894			
Feed intake (DM kg/day/steer)	10.31	10.29	10.30	0.30	-			
Formula feed	8.58	8.56	8.57	0.25	-			
Rice straw	1.73	1.73	1.73	0.02	-			
FCR	12.43	12.06	12.72	2.32	0.862			
Whole	e period ((0 - 484	í days)					
Months of age	27.3	27.0	27.0	0.34	-			
Initial body weight (kg)	263.9	266.3	262.8	12.38	0.994			
Final body weight (kg)	694.3	710.4	709.0	8.94	0.735			
Total gain (kg)	430.3	444.1	446.2	11.13	0.852			
ADG (kg)	0.89	0.92	0.92	0.02	0.852			
Feed intake (DM kg/day/steer)	9.50	9.48	9.49	0.21	-			
Formula feed	7.41	7.39	7.40	0.17	-			
Rice straw	2.09	2.09	2.09	0.02	-			
FCR	10.74	10.43	10.55	1.25	0.889			

¹Control: fomula feed + rice straw: ²T1: formula feed + rice straw + rumen-protected methionine and lysine: ³T2: formula feed + rice straw + insulin like growth factor-I; ⁴SEM: standard error of mean: ⁵ADG: average daily gain: ⁶DM: dary matter; ⁷FCR: feed conversion ratio.

RPML improved average daily gain and feed efficiency of steers [12]. Sulfur-containing amino acids are essential amino acids in the livestock. Methionine deficiency inhibits livestock growth and causes liver disease. In this study, the tendency for ADG and FCR to improve in the T1 group compared to the control group was also thought to be the result of maintaining or improving liver function. IGF-I is mainly secreted by the liver, synthesized in tissues, and is involved in physiological functions related to growth, development, metabolism, and lactation in cattle [13]. This supports the results showing a tendency of increased ADG with the supplementation of IGF-I. Therefore, supplementation of rumenprotected amino acids or IGF-I did not affect the feed intake of Hanwoo steers but was thought to affect the improvement in ADG. Table 3 shows the effects of RPML or IGF-I supplementation on plasma metabolite concentrations in Hanwoo steers. Overall, the effects of RPML and IGF-I on plasma metabolite concentrations in Hanwoo steers were small. Plasma glucose and BUN concentrations were similar between the treatments in the early (13 months of age) and late (27 months of age) fattening periods. The concentrations of NEFA, TP, albumin, and creatinine in the serum were lower in the T1 group than in the control and T2 groups; however, the differences were not statistically significant. The plasma cholesterol concentration in the T2 group tended to be lower than that in the control and T1 groups during the entire experimental period, but the difference was not statistically significant. There was no consistent effect of supplementation with RPML or IGF-I on serum triglyceride, AST, ALT, or GGT concentrations. In this study, there were no differences in plasma glucose or BUN concentrations between the treatments during the entire experimental period. In contrast, the plasma NEFA concentration was low (160 - 210 μEq/L) in the 1st period (11 months of age) but high (400 - 500 μ Eq/L) in the 2nd (17 months of

age) and 3rd (27 months of age) experimental periods. When feed intake or energy is insufficient, NEFA is released through the hydrolysis of adipose tissue, and its concentration in the plasma increases owing to incomplete oxidation of lipids because of decreased liver function [14]. In general, BUN concentration is increased when protein and nitrogen compound intake is high [15]. Total plasma proteins, such as raw materials for immune antibodies, blood coagulation, maintenance of osmotic pressure, maintenance of the cellular environment, and metabolites. play important roles in the body of livestock [16]. In the present study, there was no difference in the amount of protein supplied based on the supplementation of RPML or IGF-I; therefore, there was no difference in BUN and TP concentrations in the plasm. Plasma cholesterol concentration is decreased because of a lack of fat intake from decreased liver function or insufficient feed intake [17]. In our results, there no difference in plasma cholesterol concentrations between the treatments, which

may result from similar energy (fat) intake between the treatments. Plasma triglycerides are the major components of muscle fat and are present in the blood in the form of lipoproteins that bind to plasma proteins [18]. The lack of difference in triglyceride concentration between the treatments, similar to the cholesterol concentration, is considered to be related to the results (Table 2), in which body weight and feed intake were similar between the treatments. Plasma creatinine concentration is an indicator of kidney function and muscle mass, and although it varies according to growth, sex, weight, and disease, the differences are not significant [19]. In the present study, feed intake was similar between the treatments, and the difference in body weight was small; therefore, the plasma creatinine concentration showed similar results. In general, the concentrations of AST, ALT, and GGT in the plasma mainly depend on factors such as excessive feeding of formula feed, mycotoxins, and an increase/decrease in rumen ammonia concentration [20,21]. In the

Table 3. Changes in plasma metabolites concentrations of Hanwoo steers

Item		GLU ⁵ (mg/dl)	NEFA ⁶ (uEq/ℓ)	BUN ⁷ (mg/dl)	TP ⁸ (g/dl)	ALB ⁹ (g/dl)	CHOL ¹⁰ (mg/dl)	TG ¹¹ (mg/dl)	CREA ¹² (mg/dl)	AST ¹³ (U/ l)	ALT ¹⁴ (U/ ℓ)	GGT ¹⁵ (U/ℓ)
	Control ¹	131.13	213.63	25.13	9.53	5.24	193.63	33.25	1.46	128.88	37.50	46.25
	$T1^2$	126.50	161.00	22.01	9.01	4.94	188.63	39.75	1.35	113.25	36.50	33.63
At 11 months of age (1st	$T2^{3}$	128.63	168.00	23.71	9.23	5.14	180.38	29.38	1.50	109.25	37.50	32.50
	SEM^4	3.826	19.107	0.960	0.218	0.122	10.475	2.111	0.036	7.031	1.187	3.460
	P-value	0.894	0.495	0.434	0.648	0.612	0.883	0.127	0.215	0.505	0.930	0.202
At 18 months of age (2 nd)	Control	82.00	446.75	21.70	7.04	3.71	162.38	26.50	1.04	102.50	27.00	23.50
	T1	78.75	416.38	22.31	6.86	3.69	168.25	24.13	0.96	110.88	28.00	28.25
	T2	80.25	564.50	22.53	7.11	3.80	155.88	19.88	1.05	97.75	25.88	21.38
	SEM	1.206	31.807	0.583	0.060	0.039	2.873	1.636	0.021	4.679	0.641	2.388
	P-value	0.566	0.132	0.847	0.222	0.491	0.219	0.255	0.174	0.531	0.418	0.505
At 27 months of age (3 rd)	Control	79.50	427.38	21.40	6.96	3.76	171.13	26.38	1.45	86.88	24.75	26.38
	T1	73.38	408.00	21.33	6.50	3.56	169.38	23.50	1.35	94.50	26.63	31.50
) T2	77.38	528.00	22.58	6.74	3.73	158.63	19.38	1.48	90.63	26.00	24.25
	SEM	1.262	27.321	0.538	0.087	0.052	3.627	1.586	0.032	2.308	0.507	2.231
	P-value	0.131	0.158	0.588	0.090	0.258	0.327	0.198	0.249	0.421	0.320	0.412

¹Control: formula feed + rice straw: ²T1: formula feed + rice straw + rumen-protected methionine and lysine: ³T2: formula feed + rice straw + insulin like growth factor- I; ⁴SEM: standard error of mean: ⁵GLU: glucose; ⁶NEFA: non esterified fatty acid: ⁷BUN: blood urea nitrogen; ⁸TP: total protein: ⁹ALB: albumin: ¹⁰CHOL: cholesterol; ¹¹TG: triglycerides; ¹²CREA: creatinine; ¹³AST: aspartate-amino-transferase: ¹⁴ALT: alanine-amino-transaminase; ¹⁵GGT: γ-glutamyl-transferase.

present study, we found that there was no difference in the concentrations of AST, ALT, and GGT in the plasma between the treatments because the nutritional level (protein, etc.) and feeding amount of the formula feed for each treatment were the same as the external environmental factors (such as fungal toxins). Therefore, in our results, unlike the prediction (hypothesis) that plasma energy, protein, or liver function-related metabolite concentrations would be affected by supplementing RPML or IGF-I during the initial experimental design process (hypothesis), these substances were considered to have little effect on the concentration of metabolites in the plasma of Hanwoo steers. Table 4 shows the effects of supplementation with RPML or IGF-1 on the characteristics of Hanwoo steers as measured using ultrasonic. There were no differences between treatments in marbling score, rib eye area, or back fat thickness measured using ultrasound at 11 months of age. In the case of the marbling score at 27 months of age, the T1 and T2 groups showed a higher tendency than the control group, and in the case of the rib eye area, the T1 group had a wider area than the control group (P (0.05). Although the difference was not statistically significant, back fat thickness was thicker in the T1 and T2 groups than in the control group. In the present study,

Table 4. Changes in ultrasonic measurements of Hawnoo steers

Item	Control ¹	$T1^2$	$T2^3$	SEM ⁴	P-value
At 11 months of age					
Marbling score	1.00	1.00	1.00	0.000	-
Rib eye area (cm²)	52.29	54.71	53.00	0.817	0.483
Back fat thickness (mm)	1.79	1.57	1.21	0.214	0.569
At 27 months of age					
Marbling score	4.14	5.14	4.29	0.412	0.587
Rib eye area (cm²)	90.44^{b}	95.03 ^a	92.51^{ab}	0.918	0.030
Back fat thickness (mm)	14.71	16.47	15.04	1.459	0.223

¹Control: formula feed + rice straw; ²T1: formula feed + rice straw + rumen-protected methionine and lysine; ³T2: formula feed + rice straw + insulin like growth factor-I

compared to the control group, the marbling score, rib eye area, and back fat thickness tended to increase with supplementation of RPML compared to the control group. There is a positive correlation between marbling score and back fat thickness in Hanwoo carcasses, which is greatly influenced by the nutrients consumed, especially energy [22,23].

Fat is the easiest way to increase energy in feed, except in cases where the fat content is excessively high, and an improvement in the marbling score has been reported in previous

Table 5. Changes in carcass characteristics of Hanwoo steers

Items	$Control^1$	$T1^2$	$T2^3$	SEM ⁴	P-value
Yield traits ⁵					
Carcass weight (kg)	418.4	423.8	425.8	6.42	0.894
Rib eye area (cm²)	90.08^{b}	93.38 ^{ab}	95.85 ^a	0.80	0.009
Back fat thickness (mm)	14.46	15.77	14.31	0.53	0.476
Yield index	61.96	61.85	62.36	0.18	0.508
Yield grade (A:B:C, %)	46:39:15	31:46:23	46:54:0	-	-
Yield grade score ⁶	2.31	2.08	2.46	0.11	0.365
Quality traits ⁷					
Marbling score	5.00	6.00	5.60	0.22	0.501
Meat color	4.88	5.00	5.00	0.04	1.000
Fat color	2.88	3.00	3.00	0.04	1.000
Texture	1.13	1.17	1.00	0.05	0.357
Maturity	3.00	3.00	3.00	0.00	-
Quality grade (1 ⁺⁺ :1 ⁺ :1)	0:61:38	23:30:46	8:46:46	-	-
Quality grade score ⁸	3.62	3.77	3.62	0.11	0.800
Auction price (won/kg)	19,952	19,954	20,133	196.41	0.915

¹Control: formula feed + rice straw; ²T1: formula feed + rice straw + rumen-protected methionine and lysine; ³T2: formula feed + rice straw + insulin like growth factor-I;

⁴SEM: standard error of mean; ⁵Area was measured from longissmus muscle taken at 13th rib and back fat thickness was also measured at 13th 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)]l + 3.23. Carcass yield grades from C (low yield) to A (high yield); ⁶A grade = 3, B grade = 2, C grade = 1; ⁷Grading ranges are 1 to 9 for marbling score with higher numbers for better quality (1 = devoid, 9 = abundant); meat color (1 = bright red, 7 = dark red); fat color (1 = creamy white, 7 = yellowish). texture (1 = soft, 3 = firm); quality grades from 3 (low quality) to 1 (very high quality); ⁸1++ grade = 5, 1+ grade = 4, 1 grade = 3, 2 grade = 2, 3 grade = 1.

^{; &}lt;sup>4</sup>SEM: standard error of mean

studies [22,23]. Although without statistical significance, the marbling score tended to improve owing to the supplementation of rumen-protected fat, which is consistent with the results of previous studies. In the present study, the rib eye area increased when RPML or IGF-I were supplemented. This is consistent with the results of increased rib eye area owing to additional supplementation of amino acids and an increase in IGF reported in previous studies [22-24].

Table 5 shows the effects of supplementation with RPML or IGF-I on the carcass characteristics of Hanwoo steers. The carcass weight was slightly, but not significantly, higher in the T1 and T2 groups than in the control group, which was considered to be related to higher live weight (Table 2). The rib eye area of Hanwoo steers increased after supplementation with RPML or IGF-I (P $\langle 0.05 \rangle$ in this study. This result is consistent with the results of previous studies [22–24] and the result of ultrasound measurements at 27 months of age (Table 4).

Marbling score was slightly, but not significantly, higher in the T1 and T2 groups than in the control group. The appearance of meat quality grade 1^{++} appeared in the order T1 \rangle T2 \rangle control groups. In addition, the carcass auction price tended to be higher in the T1 and T2 groups than in the control group, with the carcass auction price in the T2 group showing the highest value. In previous studies [22-24], RPML supplementation provides the amounts of methionine required for the transmethylation reaction during lipid transport and biosynthesis, improving the intramuscular fat content of Hanwoo steers. This is associated with the role of methyl donors. Park et al. reported similar results in that rib eye area and intramuscular fat content were increased by feeding rumen-protected amino acids to Hanwoo steers [22,23]. The concentration of IGF-I in the plasma of cattle affects body weight, milk production, and fat

deposition [7]. IGF-I is essential for maintaining and increasing the body volume [6]. Yoon et al. reported that the expression of IGF-I tended to increase as the marbling score increased and that the expression of IGF-I is closely correlated with the marbling score [25]. Unlike in previous studies, supplementation with IGF-I had a greater effect on muscle protein synthesis than on intramuscular fat. Therefore, in this study, supplementation with RPML had a positive effect on the marbling score of Hanwoo steers, and IGF-I had a positive effect on the rib area.

4. Conculsion

In this study, added the IGF-1 had a low effect on ADG and no negative effect on plasma metabolite concentrations. rib eye area was wider in the T2 group than in the control and T1 groups (p<0.01). The results of this study indicate that supplementation with RPML or IGF-I have some positive effect on improving the growth performance and carcass characteristics of Hanwoo steers. The supplementation of IGF-1 significantly contributed to the increase in rib eye area of castrated steers. Therefore, further research is recommended to explore the effects of IGF-1 supplementation on growth performance and slaughter outcomes in these animals.

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