Master's Thesis of Science in Agriculture

Effect of rumen undegradable protein ratio and feed intake level on productivity in Hanwoo steers for shortening feeding period

한우 거세우 단기 비육을 위한

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Woohyeong Hong

Department of International Agricultural Technology

Graduate School of International Agricultural Technology

Seoul National University

Effect of rumen undegradable protein ratio and feed intake level on productivity in Hanwoo steers for shortening feeding period

A thesis
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By
Woo Hyeong Hong

Supervised by
Prof. Kyoung Hoon Kim

Major of International Agricultural Technology
Department of International Agricultural Technology
Graduate School of International Agricultural Technology
Seoul National University

Approved as a qualified thesis
for the Degree of Master of Science in Agriculture
by the committee members

Chairman	**Jong Geun Kim, Ph.D.**	
Member	**Kyoung Hoon Kim, Ph.D.**	
Member	**Sang Kee Kang, Ph.D.**	

Abstract

Effect of rumen undegradable protein ratio and feed intake level on productivity in Hanwoo steers for shortening feeding period

Woo Hyeong Hong

Major of Applied Animal Science

Department of International Agricultural Technology

The Graduate School of International Agricultural Technology

Seoul National University

Shortening feeding period of Hanwoo steers would enable meat producers to save the production cost and reduce the emission of environmental pollutants. Two studies were performed to investigate the effect of different undegradable protein (RUP) ratios and feed intake levels on the growth performances and quality grade of carcasses slaughtered at 26 months of age. Total 32 Hanwoo steers (average 8 months of age) were blocked by body weight (BW) and randomly allocated two treatments (4 pens/each treatment) in both experiments.

Treatments were 37% RUP (SBM) and 47% RUP (FSBM) of CP in Exp. 1, and low (L-FSBM) and high (H-FSBM) intakes with same RUP in Exp. 2. Compared to SBM, FSMB significantly increased ($p < 0.01$) average daily gain (ADG) and feed conversion ratio (FCR) and tended to increase rib-eye area in Exp.1. However, there were no differences in quality traits, fatty acid compositions and gene expression involved in lipid metabolism of biopsy samples (17 months of age) and carcass between SBM and FSBM. In Exp.2, yield and quality traits in carcass samples and gene expressions in biopsy tissues tended to be higher ($p < 0.1$) for H-FSBM compared to L-FSBM. In both Experiments, final BW at 26 months of age for both FSBM diets were similar to the national average of BW in Hanwoo steers at 30 months of age. In conclusion, proper RUP ratio in diet and maximizing feed intake may increase daily gain in Hanwoo steers, which could shorten the feeding period by attaining the target BW earlier.

Keywords: Feed intake, Rumen undegradable protein, Shortening feeding period

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Contents

List of Table

List of Figure

List of Abbreviations

ACACA: Acetyl-CoA carboxylase

ADF: Acid detergent fiber

ADG: Average daily gain

ALP: alkaline-phosphatase

AST: aspartate aminotransferase

ATGL: Adipose triglyceride lipase

BCAA: Branched-chain amino acid

BCVFA: Branched-chain volatile fatty acid

BSCL2: Berardinelli-seip congenital lipodystrophy 2-seipin

BUN: blood urea nitrogen

CD36: Fatty acid translocase

CMS: condensed molasses soluble

CP: Crude protein

DDGS: distiller's dried grains with soluble

DGAT2: Diacylglycerol acyltransferase-2

DM: Dry matter

DMI: Dry matter intake

FABP4: Fatty acid binding protein 4

FASN: Fatty acid synthase

FCR: Feed conversion ratio

FFA: Free fatty acid

FID: Lame ionization detector

FSBM: Fermented soybean meal

GC: Gas chromatography

GGT: gamma-glutamyl transferase

GPAT1: Glycerol-3-phosphate acyltransferase-1

IACUC: Institutional animal care and use committee

LPL: Lipoprotein lipase

MCP: Microbial protein

MP: Metabolized protein

MUFA: Monounsaturated fatty acid

NDF: neutral detergent fiber;

NEFA: non-esterified fatty acid

NH_3-N: Ammonia-nitrogen

PPARα: Peroxisome proliferator activated receptors-alpha

PPARβ/δ: Peroxisome proliferator activated receptors-beta/delta

PPARγ: Peroxisome proliferator activated receptors-gamma

PUFA: Polyunsaturated fatty acid

RDP: Rumen degradable protein

RUP: Rumen undegradable protein

SBM: Soybean meal

SCD: Stearoyl-CoA desaturase

SFA: Saturated fatty acid

SNAP23: Synaptosome-associated protein 23

SREBP: Sterol regulatory element-binding protein

TDN: Total digestible nutrients

TFA: Total fatty acid

TG: Triglyceride

TP: Total protein

TVFA: Total volatile fatty acid

VFA: Volatile fatty acid

VLCAD: Very long chain acyl-CoA dehydrogenase

VLDL: Very low-density lipoproteins

Zfp423: Zinc finger protein 423

1. Introduction

Hanwoo bull had been fed for 24 months to produce beef before 2000s. However, there were emerging evidences that intramuscular fat increased linearly from 200kg to 400kg in Angus steer research (Bruns et al., 2004), and energy accumulation ratio for Hanwoo steer also increased sharply from 21 months to 30 months of age (Kim et al., 2007a). Since then, feeding program for Hanwoo steer has been changed to rear until 30 months of age for producing sufficient intramuscular fat (Kim et al., 2005a, 2007a; Kwon et al., 2009; Li et al., 2010). In particular, the feed energy content was increased in the late fattening period and it caused dramatic changes in appearance rate of high-quality meat of grade from 16.7% in 2000 to 65.4% in 2021. On the other hand, there were concerns that the long–term fattening program can lead to reduce energy efficiency and increase inedible fat in beef. In addition, the long-term fattening program of Hanwoo has a risk of high production cost and low net profit due to an increase in international grain prices because feed ingredients relies entirely on imports from abroad. An economic analysis of long-term fattening programs of Hanwoo steers (Lee et al., 2013) also recommended slaughter at 28 months of age.

The research to shorten fattening period to 26 or 24 months was performed in Japan (Toshiyuki et al., 2013; Yoshimi et al., 2014). It was demonstrated that feeding steers to 24 months of age reduced total feed intake up to 1,000kg from 6,712kg to 5,666kg, even increase in 2 or 3% units in dietary crude protein (CP) and total digestible nutrients (TDN) contents through the whole period,

compared to the 28 months of conventional feeding program (Abe et al., 2018). In a research using Hanwoo steer (Reddy et al., 2018), increase in TDN and dietary CP contents for shortening feeding program from 30 to 28 months also was a positive effect on growth and carcass characteristics. However, there was no data showing the differences in total feed or nutrient intakes amount between the two feeding programs.

The nutrients intake for short-term feeding program should be increased in order to maintain the carcass weight and meat quality obtained from the long-term feeding program, However, there is lack of reference to develop novel short-term feeding program for Hanwoo steers. Therefore, this experiments were conducted to elucidate for the possibility of 26 months of feeding program composed of 2 stages, which is 4 months less than 30 months of the conventional feeding program. Two experiments were performed to investigate the effect of different rumen undegradable protein (RUP): rumen degradable protein (RDP) ratio of daily feed with isoenergetic and equal dietary CP contents (Exp.1) and the effect of different CP and TDN intakes by increasing feed intake (Exp.2) on the growth performance, yield and quality grade appearances and lipogenic gene expressions. In addition, the dry matter feed intake, CP and TDN intakes for the entire fattening period were calculated and compared with the 30-months of conventional feeding program.

2. Materials and Methods

2. 1. Experimental design, animal and diet

Both experiments (Exp. 1 and Exp. 2) and sample analysis were performed in the animal experiment farm and Lab of Animal Energy Metabolism, Seoul National University, Pyeongchang Campus, Republic of Korea. Experiments were conducted in correspondence with the Guidelines for Institutional Animal the Care and Use Committee (IACUC) of Experimental Animals of Seoul National University (SNU-210615-2).

Exp.1

A total of 32 Hanwoo steers (initial body weight of 217 ± 13kg) aged 8 months were randomly allocated two experimental groups (4 pens per each treatment): i) soybean meal-based concentrate with 37% RUP of CP (SBM) and ii) fermented soybean meal -based concentrate with 47% RUP of CP (FSBM) (Table 1, Figure 1 and 2). All steers were provided average of 5.3kg, as fed basis, for growing period (8 to 17 months of age) and 9.7kg for fattening period (18 to 26 months of age) of concentrates daily, which are consisted of 57% and 89% of total diet, respectively.

Exp.2

A total of 32 Hanwoo steers with initial body weight of 217 ± 14kg (average age = 8 months) were arranged to 8 pens in completely randomize design (CRD) with 2 treatments. i) low intake of FSBM diet (L-FSBM; 5.3 and 9.7kg for

growing and fattening stage, respectively) and ii) high intake of FSBM diet (H-FSBM; 6.3kg and 10.2kg for growing and fattening stage, respectively) (Table 1, Figure 3 and 4).

The ingredient of concentrates for growing and fattening stages are shown in Table 1 and timothy hay and rice straw hay were offered for growing stage and fattening stages of steers, respectively, in both experiments. The concentrates for growing stage and fattening stage contained 72% and 74% TDN, and 18% and 16% CP, as-fed basis, respectively (Table 2). Experimental diets supplied to animals twice a day (09:00 and 17:00). And Steers were allowed *ab libitum* water intake and mineral block.

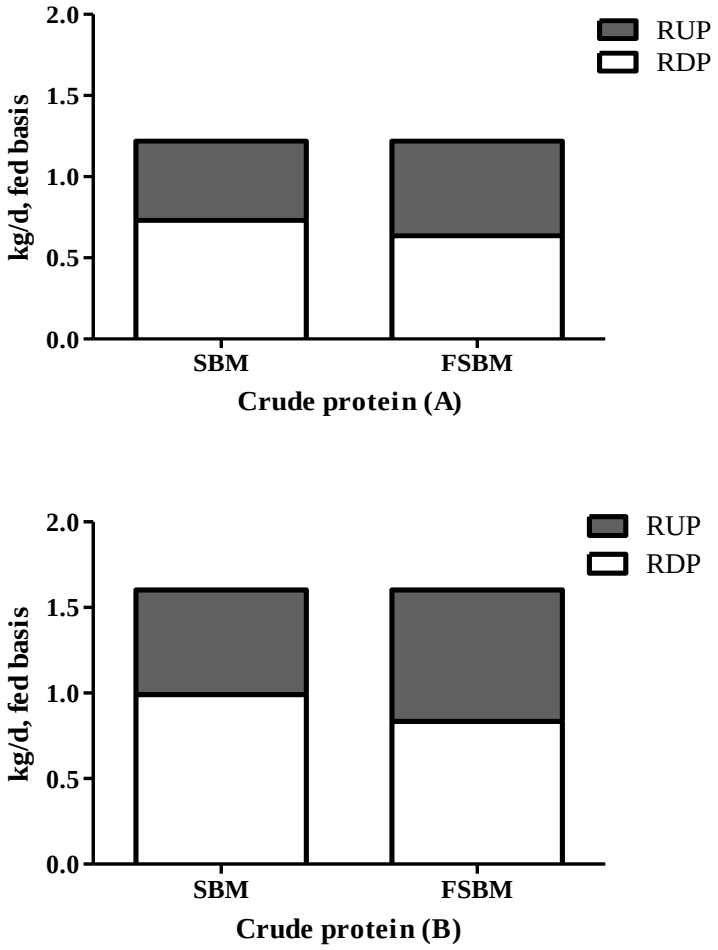


Figure 1. Dietary crude protein provision and RUP ratios (37% CP for SBM and 47% for FSBM) on growing stage (A) and fattening stage (B) in Exp. 1.

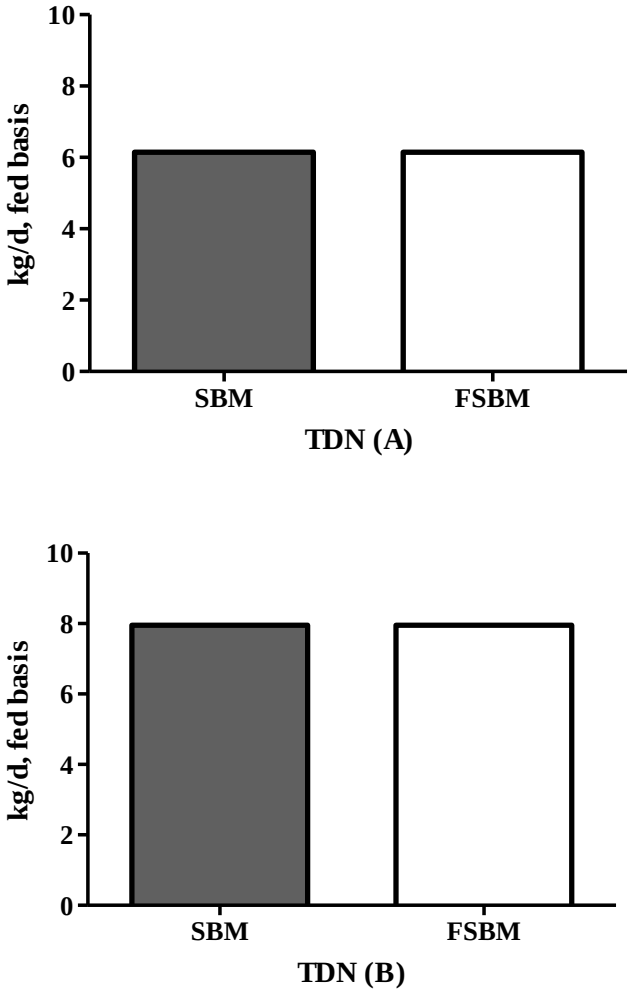


Figure 2. TDN provision of SBM (RUP 37% of CP) and FSBM (RUP 47% of CP) on growing stage (A) and fattening stage (B) in Exp. 1.

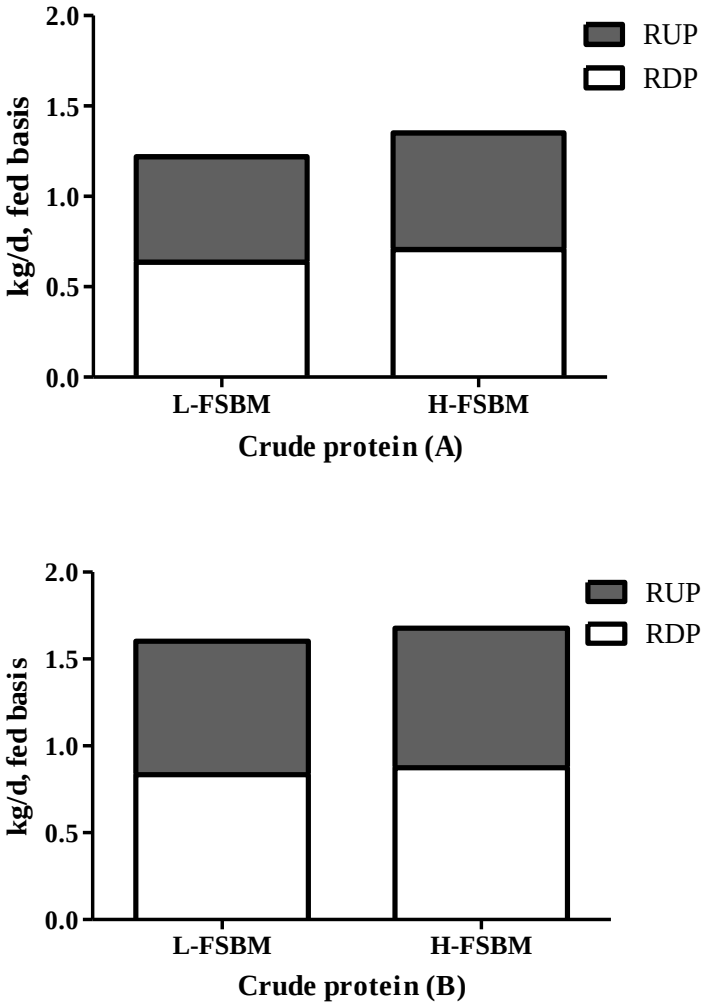
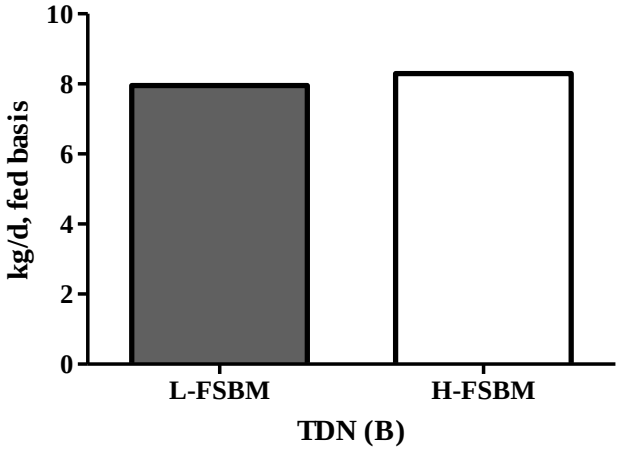


Figure 3. Dietary crude protein and RUP (47% for L-FSBM and H-FSBM) provision on growing stage (A) and fattening stage (B) in Exp. 2.



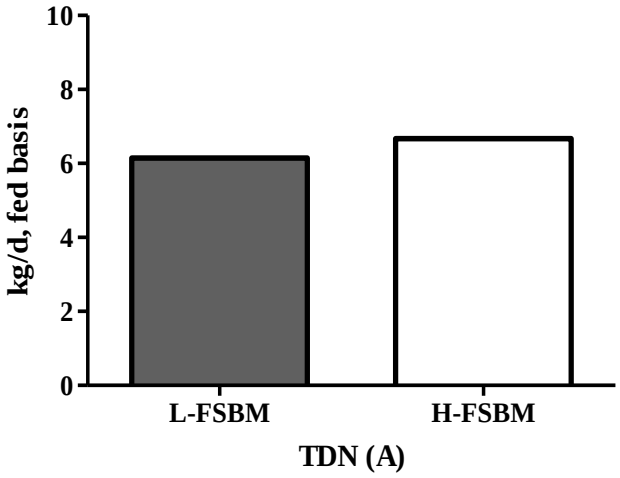


Figure 4. TDN provision of L-FSBM (low intake level of FSBM) and H-FSBM (high intake level of FSBM) on growing stage (A) and fattening stage (B) in Exp. 2.

Table 1. Ingredients composition of concentrates for Exp.1 and Exp.2

	Growing stage[1]		Fattening stage[2]	
Ingredients (%)	SBM	FSBM[3]	SBM	FSBM[3]
Corn flaked			25.01	30.07
Wheat fine	14.00	12.50	17.00	15.00
Corn gluten feed	14.00	16.40	15.50	6.72
Fermented soybean meal	-	4.00	-	2.50
Soybean Meal	6.70			
Corn DDGS	15.00	22.40	12.32	17.16
Corn fine	10.50	13.70	7.95	2.00
Soy hulls	11.50	3.18		
Oat straw ground			7.00	
molasses mixer	5.00	1.80	4.50	2.00
Palm Kernel Meal	13.00	16.00	2.40	14.00
Rice bran	2.50	5.50	2.00	3.00
Corn cobs			2.00	
Limestone	3.00	3.40	1.30	2.80
Alfalfa pellet	4.00			
Bentonite			1.00	
Buffer mix			0.80	0.60
Urea			0.62	
Salt	0.40	1.00	0.20	0.30
Mineral premix	0.30	0.10	0.19	0.10
Ammonium chloride			0.15	0.10
Vitamin premix	0.10	0.02	0.06	0.05
Molasses				1.00
Yeast fermentation product				0.10
CMS lysine by product				1.90
Corn small coarse				0.60

SBM, low RUP ratio diet (37% CP); FSBM, high RUP ratio diet (47% CP); DDGS, distiller's dried grains with soluble; CMS, condensed molasses soluble. [1]Growing stage = from 8 to 17 months of age. [2]Fattening stage = from 18 to 26 months of age. [3]FSBM = basal concentrate for Exp. 2

Table 2. Chemical composition of experimental diet for Exp.1 and Exp.2

	Growing stage[1]			Fattening stage[2]		
Composition (% DM)	Timothy	SBM	FSBM[3]	Rice straw	SBM	FSBM[3]
OM, %	88.1	88.4	87.4	84.2	86.5	87.1
CP, %	4.5	20.4	21.0	3.8	20.1	17.8
Ether extract, %	0.6	5.8	6.9	0.9	3.7	5.3
Ash, %	4.0	7.8	9.6	11.4	9.5	8.3
NDF, %	69.4	34.5	30.7	65.2	25.3	24.2
ADF, %	42.4	15.5	13.5	38.8	9.7	11.5
RDP, % of CP	49.9	62.8	53.8	16.8	63.0	53.0
RDP, %	2.3	12.8	11.3	0.6	12.7	9.4
RUP, % of CP	50.1	37.2	46.2	83.2	37.0	47.0
RUP, %	2.3	7.6	9.7	3.1	7.5	8.4
TDN, %	58.2	72.0	72.0	60.7	74.0	74.0

SBM, low RUP ratio diet (37% CP); FSBM, high RUP ratio diet (47% CP); OM. organic matter; CP, crude protein; NDF, neutral detergent fiber; ADF, acid detergent fiber; RDP, rumen degradable protein; RUP, rumen undegradable protein; TDN, total digestible nutrients. [1]Growing stage = from 8 to 17 months of age. [2]Fattening stage = from 18 to 26 months of age. [3]FSBM = basal concentrate for Exp.2.

2. 2. Growth Performance and feed intake

Body weight was measure once a month during entire experiment period. Refusal amount of concentrate and forage were recorded three consecutive days per 10 days interval and dry matter intake (DMI) was calculated on a pen basis throughout the experiment. Feed conversion ratio (FCR) was calculated as total feed intake divided by total BW gain.

2. 3. Rumen fluid and Blood collection and analysis

Rumen fluid and blood sampling of all steers were taken at 15 months of age. Approximate 200ml of rumen fluid from each Hanwoo steers was collected by using a stomach tube equipped with an Erlenmeyer flask and a vacuum pump at 2 hours after morning feeding. The first 50ml was thrown away to prevent saliva contamination. Immediately after filtering the rumen fluid through four layer of cheesecloth , rumen pH was measured by using pH meter (model AG8603; Seven Compact™ pH/Ion S220, Mettler-Toledo, Schwerzenbach, Switzerland). The rumen fluid samples were stored at -20 ºC for further analysis of ammonia-nitrogen (NH_3-N) and volatile fatty acid (VFA) concentration. The concentration of NH_3-N was measured by a spectrophotometer (Spectra Max® iD3 Multi-Mode Micro-Plate Reader, Molecular Devices, United states of America), using a colorimetric method (Chaney and Marbach, 1962) based on the reaction of NH_3-N with hypochlorite and phenol. A 5.0mL aliquot of rumen fluid was mixed with 1.0mL 25% HPO_3 and 0.2mL 2% pivalic acid to measure VFAs concentration. using an Agilent 7890B gas chromatography (GC) system (Agilent Technologies, Santa Clara, CA, USA) with flame ionization detector

(FID) detector. Aliquot (1μl) were injected with a split ratio of 10:1 into a 39m × 0.25mm × 0.25μm Nukol™ fused-silica capillary column (Catalog No. 24107, Supelco, Sigma-Aldrich, St. Louis, Mo, United State of America) with helium carrier gas set to a flow rate of 1mL/ min and initial over temperature of 80ºC. The over temperature was held constant at the initial temperature for 1 min, and thereafter increased at 20ºC/min to a temperature of 180ºC and held for 1 min, and increased at 10ºC/min to final temperature of 200ºC, and a final run time of 14 min.

Blood sampling from each Hanwoo steers was performed 3 hours after morning feeding. using jugular vein using a venipuncture syringe (18G) and transferred to anticoagulant-free 8.5ml yellow-capped BD vacutainer® SST™II advance tubes. The blood collected in vacutainer was centrifuged at 3000 × g for 5 min. (1580R, Labogene, Seoul, Korea) and serum was transferred to 2ml micro tubes for storage at -80 ºC until further analysis. Serum biochemical parameter, including total protein (TP), albumin, total cholesterol, triglyceride (TG), glucose, blood urea nitrogen (BUN), creatinine, aspartate aminotransferase (AST), gamma-glutamyl transferase (GGT), alkaline-phosphatase (ALP), non-esterified fatty acid (NEFA), β-hydroxybutyrate were analyzed, using an automatic analyzer (BS-400, Mindray, China).

2. 4. Tissue Biopsy

Tissue biopsy (2g per head) were obtained at 17 months of age, from the left-side rear of the third lumbar vertebra of eight steers in each treatment group (2 steers selected randomly per pen, Exp.1; n=8, Exp.2; n=8), using a spring-

loaded biopsy instrument (Biotech, Republic of Slovakia) as detailed by Cheah, K.S.; Cheah, A.M.; Just (1997). The collected *longissimus lumborum* muscle samples were immediately frozen in liquid nitrogen and were transferred to deep freezer for storage at -80 ºC until analysis.

2. 5. Carcass evaluation and grading standards

All steers were transported to the local slaughterhouse at 26 months of age and then fed with water as *ad-libitum* without feed for approximately 12 hours prior to slaughter. On the following day the animals were slaughtered in accordance with Korean rules and regulations for animal care and standard procedures and ethical guidelines for animal welfare (APQA, 2013). After 24 hours chilling, carcasses were assessed Yield Grade and Quality Grade by official grader according to the Korean Carcass Grading Procedure (KAPE, 2019). The yield grade was determined by reflecting the rib eye area (cm^2), back fat thickness (mm) and carcass weight (kg) and the quality grade was determined by considering the degree of marbling, meat color, fat color, firmness of rib eye and maturity. In addition, the *longissimus lumborum* muscle of non-biopsy Hanwoo steers (Exp.1; n=16, Exp.2; n=16) was taken from between 13^{th} *thoracis* vertebrae and 1^{st} *lumbar* vertebrae of cold carcasses. The *longissimus lumborum* muscle samples were stored in ice box with dry ice, and then, transported to laboratory stored at -80 ºC for gene extract and long chain fatty acid analysis.

2. 6. Image analysis for marbling fleck characteristics

After chilling carcasses for 24 hours, digital images of *longissimus dorsi* sections of the cold carcasses were taken by mirror-type camera (HK-333, HayasakaRikoh co. Ltd., Sapporo, Japan; Kuchida et al., 2001) to analyze marbling fleck characteristics (Konarska et al., 2016). The image analysis was carried out by using the Beef Analyzer II software (Hayasaka Ricoh Co. Ltd., Sapporo, Japan). First, a boundary line (1pixel line width) was drawn semi-automatically to designate the muscle region and corrected manually using an image analysis program (Kuchida, 1997). Further procedures of Kuchida et al. (2006) were executed to take rib eye area (cm^2), marbling percentage, number of marbling particles, coarseness index: 15 round thinning, number of coarse marbling particle: 15 round thinning, coarseness index 1 to 5: 15 round thinning, marbling area (cm^2), number of fine marbling particle and fineness of marbling. the marbling area percentage was derived by dividing the muscle surface area from the total marbling area. The total marbling area engrossed marbling particles above 0.01cm^2 was carried out. Fineness index was calculated as the number of small particles from 0.01 to 0.05cm^2 per loin-eye area (cm^2).

2. 7. Chemical and Fatty acid compositions analysis of samples

Feed samples were dried in forced-air oven at 65ºC for 72h to calculate the dry matter (DM) content and ground samples passed through a 1mm screen (Thomas Scientific Model 4, New Jersey, USA) were obtained for analyzing chemical composition; crude protein by combustion (Method 990.03, AOAC. 2007) using an Elementar rapid N-cube protein/nitrogen apparatus (Elementar Americas, Mt. Laurel, NK, USA), ash (Method 942.05, AOAC. 2007), and ether extract (EE; Method 960.39, AOAC. 2007). Neutral detergent fiber (NDF) and acid detergent fiber (ADF) content were measured by using the method (Van Soest et al., 1991).

The direct method of O'Fallon et al., (2007) was used to measure the fatty acid quantitative compositions in intramuscular of biopsy and slaughter samples. *Longissimus lumborum* muscle samples collected from biopsy and slaughter, respectively were analyzed using an Agilent 7890B GC system (Agilent Technologies, Santa Clara, CA, United State of America) with a FID detector. The inlet and detector temperature were maintained at 250 ºC and 260 ºC, respectively. Aliquots (1µl) were injected with a split ratio of 30:1 into a 100m × 0.25mm × 0.20µm SP-2560 biscyanopropyl polysiloxane capillary column (catalog. No: 24056, Supelco, Sigma-Aldrich, St. Louis, Mo, United State of America) with helium carrier gas set to a flow rate of 1.18 ml/min and initial oven temperature of 100 ºC. The oven temperature was held constant at

the initial temperature for 5 min, and thereafter increased at 4 ºC /min to a final temperature of 240 ºC and held for 14 min. 37-Component FAME mix (CRM47885, Supelco, Sigma-Aldrich, St. Louis, Mo, United State of America) was used for identification fatty acid contents and tridecanoic acid (C13:0) was used for internal standard (Supelco, Sigma-Aldrich, St. Louis, Mo, United State of America). The standard ampule of 1mL includes 37 types of FAME from C4 to C24, containing major mono-unsaturated fatty acid (MUFA) and poly unsaturated fatty acid (PUFA). Identified fatty acid contents were quantified after normalization to an internal standard using the FAME standard as described in AOCS Official Method Ce 1j-07 of AOCS. (2018) and were calculated as mg/ 100g FAME or Meat.

2. 8. RNA extraction and real-time quantitative PCR

The RNA was isolated from biopsy tissues and *longissimus* muscle using RNeasy Lipid Tissue Mini Kits (Qiagen, Hilden, Germany) according to the manufacturer's instructions. The both quality and quantity for total RNA were analyzed using a NanoDrop™ 2000/2000c spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA), and the integrity of total RNA was checked based on the 28S and 18S bands using eco dye stained (Biofact, Daejeon, Republic of Korea) agarose gel electrophoresis. Total RNA of 1μg was reverse-transcribed into cDNA using PrimeScript™ 1st strand cDNA Synthesis Kit (TAKARA) according to the manufacturer's instructions. Real-time PCR was carried out using SYBR Green real-time-PCR Master Mix (Bioneer, Daejeon,

South Korea) and the CFX96 Touch™ Real-Time PCR Detection System (Bio-Rad Laboratories, INC., Hercules, CA, USA). Briefly, the PCR was performed in 20μL total reaction volumes containing 30ng cDNA, 10μL SYBR Green RT-PCR Master Mix, and 1.0μL of each 10μM primer. The thermal cycling parameters were as follows by 2 steps: 95ºC for 5 min, followed by 42 cycles at 94ºC for 15s, 60ºC for 30s. All primers were designed using the Primer BLAST (Ye et al., 2012) tool, based on National Center for Biotechnology Information (NCBI) published sequences (www.ncbi.nlm.nih.gov; Table 3). The $2^{-\Delta\Delta CT}$ method was used to determine the relative fold changes (Livak and Schmittgen, 2001). all data were normalized based on the housekeeping β-actin gene.

2. 9. Statistical analysis

Data analysis for rumen fluid characteristics, blood metabolism, fatty acid composition, gene expression, carcass characteristics and animal performance was performed using the PROC MIXED of SAS 9.4 (SAS Institute, Cary, NC, USA). The model contained the fixed effect of diet such as RUP rate (Exp.1), feed intake level (Exp.2). Random effect was animal nested within treatment. LSMEANS was used for calculation means. Experiment units was considered as pen basis. FSBM was used in both Exp.1 and Exp.2. Therefore, a total of 32 Hanwoo steers were used in Exp.1 and Exp.2, respectively. Treatment differences were considered significant at less than $P < 0.05$. A trend was considered to exist at significance between $0.05 < P < 0.1$.

Table 3. List of primers used for real-time quantitative PCR for Exp.1 and Exp.2

	NCBI Acc. No.	Primer name	Primer sequence (5' – 3')	Product size (bp)
Transcription factors:				
Peroxisome proliferator activated receptors (PPARα)	NM_001034036.1	PPARα FP	CAATGGAGATGGTGGACACA	95
		PPAR αRP	TTGTAGGAAGTCTGCCGAGAG	
Peroxisome proliferator activated receptors (PPARγ)	NM_181024	PPARγFP	TGGCCATTGAATGCCGGGTC	207
		PPAR γRP	ACATCCCCACAGCAAGGCAC	
Sterol regulatory element-binding proteins	NM_001113302.1	SREBP FP	GAGCCACCCTTCAACGAA	88
		SREBP RP	TGTCTTCTATGTCGGTCAGCA	
Zinc finger protein 423	NM_001101893	ZFP423 FP	GGATTCCTCCGTGACAGCA	120
		ZFP423 RP	TCGTCCTCATTCCTCTCCTCT	
Lipogenesis:				
Acetyl-CoA carboxylase	NM_174224.2	ACACA FP	CGCTCGGTGATTGAAGAGAA	117
		ACACA RP	CGTCATGTGGACGATGGAAT	
Fatty acid synthase	NM_001012669.1	FASN FP	ATCGAGTGCATCAGGCAAGT	92
		FASN RP	TGTGAGCACATCTCGAAAGCCA	
Stearoyl-CoA desaturase	NM_173959.4	SCD FP	TTATTCCGTTATGCCCTTGG	83
		SCD RP	TTGTCATAAGGGCGGTATCC	

Fatty acid uptake:				
Lipoprotein lipase	NM_001075120.1	LPL FP LPL RP	CTCAGGACTCCCGAAGACAC GTTTTGCTGCTGTGGTTGAA	98
Fatty acid binding protein 4	NM_174314.2	FABP4 FP FABP4 RP	GGATGATAAGATGGTGCTGGA ATCCCTTGGCTTATGCTCTCT	80
Fatty acid translocase (CD36)	NM_174010.3	CD36 FP CD36 RP	GGTCCTTACACATACAGAGTTCG ATAGCGAGGGTTCAAAGATGG	115
Fatty acid esterification:				
Glycerol-3-phosphate acyltransferase-1	NM_001012282.1	GPAT1 FP GPAT1 RP	TGTGCTATCTGCTCTCCAATG CTCCGCCACTATAAGAATG	116
Diacylglycerol acyltransferase-2	NM_205793.2	DGAT2 FP DGAT2 RP	CACCGATTGCTGGCTCATTG GACCTCCTGCCACCTTTCTT	84
Lipolysis:				
Adipose triglyceride lipase	NM_001046005.2	ATGL FP ATGL RP	TGACCACACTCTCCAACA AAGCGGATGGTGAAGGA	100
Very long chain acyl-CoA dehydrogenase	U30817.1	VLCAD FP VLCAD RP	TCTTCGAGGGGACAAATGAC AGCATTCCCAAAAGGGTTCT	116
Adipocyte size:				
Synaptosome-associated protein 23	BT030678.1	SNAP23 FP SNAP23 RP	GGAGGGGAGGCAAGAGATAA AAACCAAGCACTGGCCTAAA	148

Berardinelli-Seip congenital lipodystrophy2-seipin	BC105396.1	BSCL2 FP BSCL2 RP	CGAAAGGTCTCTGCCCATC GTTTTCTCCTCCTCGGACAG	140
Housekeeping:				
Beta-actin	BC142413.1	β-Actin FP β -Actin RP	GTCCACCTTCCAGCAGATGT CAGTCCGCCTAGAAGCATTT	90

3. Result

3.1 Experiment1: Effect of RUP ratio on productivity of Hanwoo steers for shortening feeding period

3.1.1 Animals performance

There were no significant differences in DM, CP and TDN intakes between SBM and FSBM diets throughout the whole experimental period. RUP intake was significantly higher in FSBM in all stages (Figure 5 and 6). Diet with different RUP:RDP ratio did not affect body weight during the whole experiment period (Figure 7 and Table 4). However, average daily gain (ADG) was significantly ($p<0.01$) higher in FSBM than SBM by 0.07 kg/d and 0.06 kg/d during the fattening and whole feeding period, respectively. Steers fed FSBM diet had a significantly lower FCR ($p<0.05$) than SBM during the fattening stage and whole feeding period (Table 5).

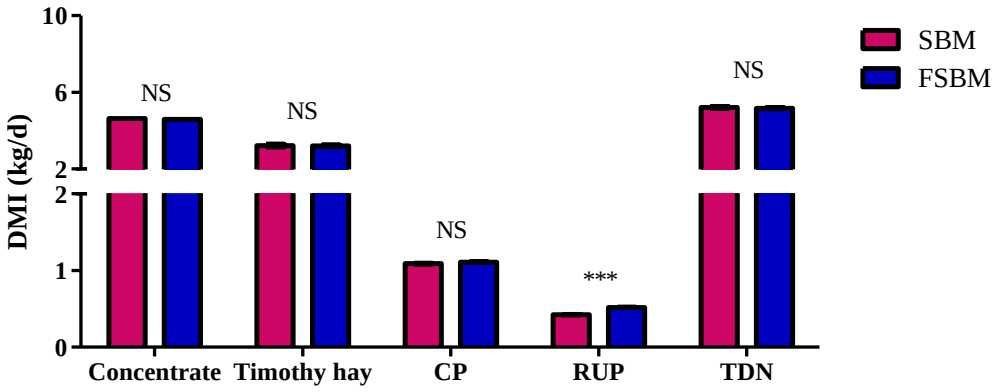


Figure 5. Intake amounts of concentrate, timothy hay, crude protein, RUP and TDN for steers fed SBM (RUP 37% of CP) and FSBM (RUP 47% of CP) during growing stage of Exp.1. $^{+}p < 0.1$, $^{*}p < 0.05$,** $p < 0.01$, $^{***}p < 0.001$. Values are the least-squares means with the standard error.

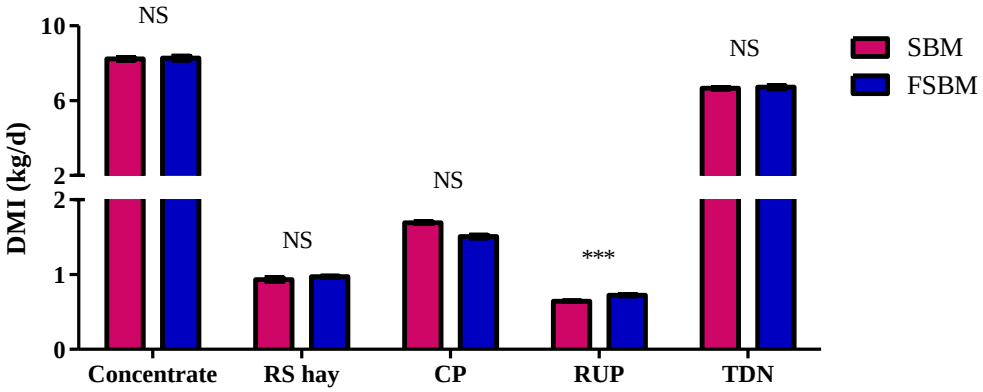


Figure 6. Intake amounts of concentrate, rice straw hay, crude protein, RUP and TDN for steers fed SBM (RUP 37% of CP) and FSBM (RUP 47% of CP) during fattening stage of Exp.1.$^{+}p < 0.1$, $^{*}p < 0.05$,** $p < 0.01$, $^{***}p < 0.001$. Values are the least-squares means with the standard error.

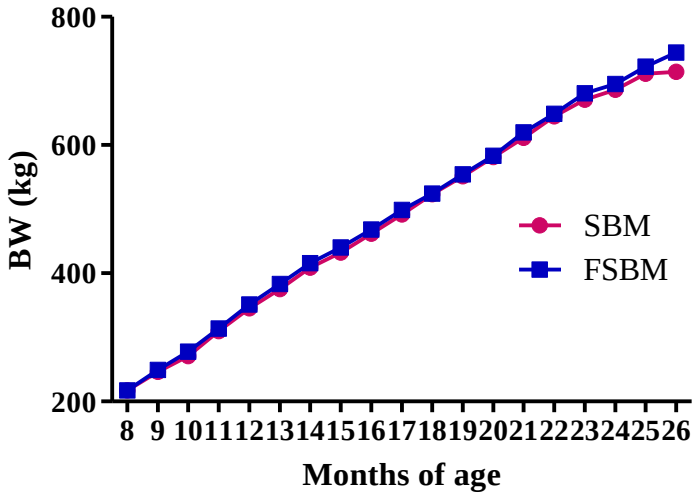


Figure 7. Changes in live body weight of SBM (RUP 37% of CP) and FSBM (RUP 47% of CP) during whole feeding period in Exp.1.

Table 4. Changes in body weight of steers fed diets with different RUP ratio during whole feeding period in Exp.1

Item	SBM	FSBM	SEM	*P*-value
8 months of age	217.1	217.1	12.84	0.996
9 months of age	246.1	249.2	11.56	0.796
10 months of age	270.9	277.6	11.76	0.587
11 months of age	309.9	313.6	11.79	0.761
12 months of age	345.1	351.1	13.53	0.673
13 months of age	375.3	383.0	12.47	0.560
14 months of age	408.9	415.8	14.21	0.643
15 months of age	432.3	440.3	14.26	0.592
16 months of age	461.8	468.0	15.22	0.698
17 months of age	491.6	498.7	14.74	0.649
18 months of age	523.1	524.4	15.05	0.933
19 months of age	551.1	554.0	17.11	0.872
20 months of age	581.4	583.4	17.90	0.912
21 months of age	611.1	619.8	18.74	0.662
22 months of age	644.5	648.6	18.13	0.830
23 months of age	670.9	680.8	19.64	0.633
24 months of age	686.4	695.4	18.93	0.651
25 months of age	711.4	722.4	16.88	0.539
26 months of age	714.4	744.0	17.40	0.140

SBM**,** low RUP ratio diet (37% CP)**;** FSBM, high RUP ratio diet (47% CP)**.**

Table 5. Growth performance of steers fed experimental diets with different RUP ratio in Exp.1

Item	SBM	FSBM	SEM	*P*-value
Average daily gain, ADG, kg/d				
Growing stage[1]	1.00	1.03	0.03	0.289
Fattening stage[2]	0.81	0.88	0.02	0.004
Whole stage[3]	0.90	0.96	0.01	0.006
Average body weight, BW, kg				
Initial	217.1	217.1	12.84	0.996
Growing stage	491.6	498.7	14.74	0.649
Fattening stage	714.4	744.0	17.40	0.140
Feed conversion ratio, FCR[4]				
Growing stage	7.9	7.6	0.19	0.173
Fattening stage	11.4	10.5	0.25	0.013
Whole stage	9.8	9.3	0.14	0.008

SBM**,** low RUP ratio diet (37% CP)**;** FSBM, high RUP ratio diet (47% CP). [1]Growing stage = from 8 to 17 months of age. [2]Fattening stage = from 18 to 26 months of age. [3]Whole stage = from 8 to 26 months of age. [4]Feed conversion ratio = average daily DM intake / average daily gain.

3.1.2 Rumen fluid fermentation and Blood metabolic characteristics at growing stage

Rumen fluid fermentation characteristics

Table 6 shows the rumen fermentation properties of Hanwoo fed diets with different RUP:RDP ratio at 15 months of age in the growing stage. There was no significant difference in pH and total volatile fatty acid (TVFA) concentration between SBM and FSBM. However, FSBM showed a lower rumen NH_3-N concentration ($p=0.062$), acetate ($p<0.05$) and A: P ratio ($p<0.001$) than SBM. In addition, valerate ($p<0.05$) was significantly lower in FSBM than SBM, however, iso-valerate concentrate ($p<0.1$) tended to be higher in FSBM than SBM.

Blood metabolic characteristics

Table 7 indicates blood characteristics of Hanwoo steers fed diets with RUP:RDP ratio. There was no significant differences in serum metabolites associated with dietary protein, fat and carbohydrates between SBM and FSBM.

Table 6. Changes in rumen fermentation characteristics of steers fed diets with different RUP ratio at 15 months of age of the growing stage in Exp.1

Item	SBM	FSBM	SEM	*P*-value
Rumen fluid pH	6.3	6.0	0.16	0.126
Rumen NH_3-N, mg/dl	22.0	17.0	2.18	0.062
Total VFA, mM	102.2	95.2	3.80	0.116
Individual VFA, mM				
Acetate	64.6	58.1	2.48	0.040
Propionate	19.4	19.6	0.79	0.767
Iso-butyrate	0.93	0.87	0.03	0.135
Butyrate	14.0	13.5	0.58	0.438
Iso-valerate	1.24	1.43	0.97	0.097
Valerate	2.11	1.72	0.13	0.027
A:P ratio	3.34	2.96	0.05	0.0002

SBM**,** low RUP ratio diet (37% CP)**;** FSBM, high RUP ratio diet (47% CP); VFA, volatile fatty acid; A:P ratio, acetate: propionate ratio.

Table 7. Blood metabolic characteristics of steers fed diets with different RUP ratio at 15 months of age of growing stage in Exp.1

Item	SBM	FSBM	SEM	*P*-value
Total Protein (g/dl)	7.3	7.3	0.11	0.955
Albumin (g/dl)	3.8	3.9	0.07	0.558
Creatinine (mg/dl)	1.3	1.4	0.06	0.242
Blood urea nitrogen (mg/dl)	20.3	20.5	0.78	0.770
AST (U/l)	66.7	72.7	4.09	0.193
ALP (U/l)	142.4	159.6	25.62	0.526
GGT (U/l)	16.4	24.0	4.43	0.141
Glucose (mg/dl)	72.2	74.7	2.15	0.290
NEFA (mmol/l)	0.15	0.17	0.02	0.561
β-hydroxybutyrate (mmol/l)	0.56	0.57	0.06	0.918
Total-Cholesterol (mg/dl)	168.6	172.4	15.36	0.812
Triglycerides (mg/dl)	18.5	18.8	1.29	0.788

SBM**,** low RUP ratio diet (37% CP)**;** FSBM, high RUP ratio diet (47% CP); AST, aspartate aminotransferase; ALP, alkaline-phosphatase; GGT, gamma-glutamyl transferase; NEFA, non-esterified fatty acid.

3.1.3 Carcass characteristics and marbling fleck characteristics on *longissimus lumborum* of Hanwoo

The differences in dietary RUP: RDP ratio in the current experiment did not influence on carcass auction price, shrunk weight and carcass weight (Table 8). However, the dressing percentage, which is the ratio of carcass weight to shrunk weight, tended to be lower in FSBM than SBM ($p<0.1$). On the other hand, the rib-eye area in FSBM tended to be higher than SBM, which is one of the factors determining the yield grade. However, other factors related to the yield traits did not show significant differences. The appearance rates of high grade (B and A) were 93.8% in SBM and 87.5% in FSBM, respectively, and A grade was appeared 62.5% which is 25%p higher than that in FSBM (37.5%). The appearance rates of 1++ grade were 25% in SBM and 6.3% in FSBM, respectively. However, all factors related to quality grade were not affected by dietary RUP: RDP ratio.

Table 8. Carcass and marbling fleck characteristics of steers fed diets with different RUP ratio in Exp.1

	SBM	FSBM	SEM	*P*-value
Animals productivity				
Price, Korean won	8,854,130	9,220,578	280,300	0.239
Shrunk weight, kg	703.3	731.6	15.62	0.119
Carcass weight, kg	418.1	426.3	8.79	0.387
Yield grade traits				
Dressing percentage[1], %	59.4	58.3	0.57	0.085
Yield grade (A : B : C, head)	10 : 5 : 1	6 : 8 : 2		
Yield grade score[2]	2.5	2.3	0.27	0.390
Rib eye area ,cm^2	81.5	83.9	1.14	0.081
Back fat thickness, mm	12.1	11.0	1.90	0.596
Yield index	62.0	62.2	0.63	0.712
Quality grade traits				
Quality grade (1^{++} : 1^{+} : 1 : 2, head)	4 : 1 : 7 : 4	1 : 3 : 10 : 2		
Marbling score	5.0	4.8	0.58	0.758
Image analysis				
Marbling percentage	0.19	0.19	0.02	0.829
Number of marbling particles	3741.2	2665.5	733.77	0.193
Coarseness index: 15 round thinning	0.16	0.17	0.03	0.690
Number of coarse marbling particle: 15 round thinning	61.8	63.4	7.15	0.828
Marbling area, cm^2	16.0	16.3	1.57	0.838
Number of fine marbling particle	186.8	190.4	13.70	0.800
Fineness of marbling	2.30	2.25	0.15	0.735

SBM, low RUP ratio diet (37% CP)**;** FSBM, high RUP ratio diet (47% CP)**.** [1]Dressing percentage = carcass weight / shrunk weight, [2] A=3, B=2 and C=1.

3.1.4 Fatty acid composition and fat contents of intramuscular samples obtained from biopsy and carcass

Table 9 and Table 10 shows the fatty acid compositions of *longissimus lumborum* tissues collected by biopsies performed during the growing stage and carcasses obtained after slaughter, respectively. Not only biopsy but also carcass *longissimus lumborum* samples showed no significant differences in palmitic acid (C16:0), stearic acid (C18:0), and oleic acid (C18:1n9c), which accounted for the majority component in beef. In addition, total saturated fatty acid (SFA), monounsaturated fatty acid (MUFA), polyunsaturated fatty acid (PUFA), and total fatty acids (TFAs) contents were also not affected by dietary RUP: RDP ratio. The carcass *longissimus lumborum* intramuscular fat contents (%) in both SBM and FSBM showed no significant difference (Figure 8)

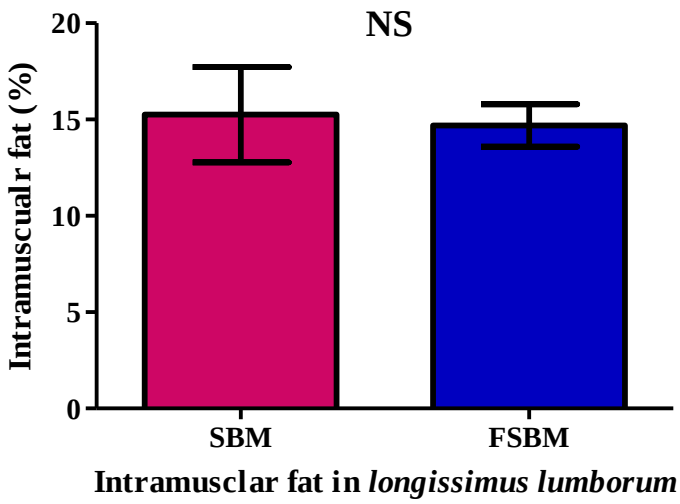


Figure 8. Percentage of intramuscular fat in *longissimus lumborum* of carcass samples in SBM (RUP 37% of CP) and FSBM (RUP 47% of CP) of Exp.1..

Table 9. Fatty acid composition of biopsy *longissimus lumborum* samples in growing stage (mg/100g FAME) in Exp.1

Fatty acid	SBM	FSBM	SEM	*P*-value
Myristic acid, C14:0	2727	3195	336.1	0.213
Palmitic acid, C16:0	23986	24684	926.1	0.479
Palmitoleic acid, C16:1	4173	4208	562.8	0.952
Stearic acid, C18:0	9915	9728	961.2	0.852
Elaidic acid, C18:1n9t	1372	1301	103.7	0.521
Oleic acid, C18:1n9c	36340	34726	1499.4	0.323
Linoleic acid(LA), C18:2n6c	3041	2845	383.5	0.628
α-linolenic acid(ALA), C18:3n3	169	133	35.8	0.031
Others[1]	4130	4071	217.5	0.797
SFA[2]	38748	39638	1706.2	0.621
MUFA[3]	43384	41786	2029.1	0.461
PUFA[4]	3720	3469	444.6	0.593
Omega-6	3514	3307	431.9	0.649
Omega-3	206	162	16.8	0.038
Total fatty acids (mg/100g FAME)	85852	84893	2530.1	0.718

SBM, low RUP ratio diet (37% CP); FSBM, high RUP ratio diet (47% CP); SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; [1]Others= C4:0+C6:0+C8:0+C10:0+C11:0+C12:0+C13:0+C14:1+C15:0+C15:1+C17:0+C17:1+C18:2n6t +C20:0+C18:3n6+C20:1n9+C21:0+C20:2+C22:0+C20:3n6+C22:1n9+C20:3n3+C23:0+C20:4 n6+C22:2+C24:0+C20:5n3+C24:1n9+C22:6n3. [2]SFA = C10:0 + C11:0 + C12:0 + C14:0 + C15:0 + C16:0 + C17:0 + C18:0 + C20:0 + C21:0 +C22:0 + C24:0. [3] MUFA = C14:1n5 + C16:1n7 + C17:1n7 + C18:1n7 + C18:1n9 + C20:1n9 + C22:1n9 + C24:1n9. [4] PUFA = C18:2n6 + C18:2c9,t11 + C18:3n3 + C18:3n6 + C20:2n6 + C20:3n3 + C20:3n6 + C20:4n6 + C20:5n3 + C22:2n6 + C22:4n6 + C22:5n3 + C22:6n3

Table 10. Fatty acid compositions of *longissimus lumborum* muscle after slaughter (mg/100g Meat) in Exp.1

Fatty acid	SBM	FSBM	SEM	*P*-value
Myristic acid, C14:0	469	456	122.3	0.916
Myristoleic acid, C14:1	144	116	26.9	0.337
Palmitic acid, C16:0	3351	3294	679.7	0.936
Palmitoleic acid, C16:1	668	593	127.4	0.576
Stearic acid, C18:0	1168	1199	222.5	0.895
Elaidic acid, C18:1n9t	164	203	49.5	0.456
Oleic acid, C18:1n9c	5140	5351	945.4	0.831
Linoleic acid(LA), C18:2n6c	286	292	37.0	0.880
α-linolenic acid(ALA), C18:3n3	15	8	3.1	0.068
Others[1]	327	357	54.7	0.598
SFA[2]	5182	5160	1046.8	0.984
MUFA[3]	6216	6375	1124.6	0.892
PUFA[4]	336	335	43.6	0.983
Omega-6	321	326	41.2	0.890
Omega-3	15	8	3.1	0.068
Crude fat, %	15	15	2.7	0.842
Total fatty acids (mg/100g Meat)	11734	11870	2172.6	0.952

SBM, low RUP ratio diet (37% CP)**;** FSBM, high RUP ratio diet (47% CP); SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; [1]Others= C4:0+C6:0+C8:0+C10:0+C11:0+C12:0+C13:0+C15:0+C15:1+C17:0+C17:1+C18:2n6t+C20:0 +C18:3n6+C20:1n9+C21:0+C20:2+C22:0+C20:3n6+C22:1n9+C20:3n3+C23:0+C20:4n6+C22 :2+C24:0+C20:5n3+C24:1n9+C22:6n3. SFA = C10:0 + C11:0 + C12:0 + C14:0 + C15:0 + C16:0 + C17:0 + C18:0 + C20:0 + C21:0 +C22:0 + C24:0. [3] MUFA = C14:1n5 + C16:1n7 + C17:1n7 + C18:1n7 + C18:1n9 + C20:1n9 + C22:1n9 + C24:1n9. [4] PUFA = C18:2n6 + C18:2c9,t11 + C18:3n3 + C18:3n6 + C20:2n6 + C20:3n3 + C20:3n6 + C20:4n6 + C20:5n3 + C22:2n6 + C22:4n6 + C22:5n3 + C22:6n3

3.1.5 Lipid metabolic gene expression of intramuscular samples obtained from biopsy and carcass

Gene expressions of biopsy longissimus lumborum

The relative expressions of the peroxisome proliferator activated receptors-gamma (PPARγ) and sterol regulatory element-binding protein (SREBP) genes involved in transcription in FSBM were significantly lower ($p<0.01$) than SBM (Figure 9). In addition, there was significantly less expression in acetyl-CoA carboxylase (ACACA) related to lipid biosynthesis ($p<0.01$) than SBM. Conversely, adipose triglyceride lipase (ATGL) associated with lipolysis tended to be up-regulated ($p<0.1$) by the high ratio of RUP, and berardinelli-seip congenital lipodystrophy 2-seipin (BSCL2) was also up-regulated significantly ($p<0.05$).

Gene expression in carcass longissimus lumborum

The expression of stearoyl-CoA desaturase (SCD) and ACACA, which affects lipid biosynthesis, tended to be up-regulated ($p<0.1$) by the high ratio of RUP in the diet (Figure 10). In addition, the high dietary RUP up-regulated the expression of synaptosome-associated protein 23 (SNAP23) ($p<0.1$), ATGL ($p<0.05$) and BSCL2 ($p<0.1$). However, fatty acid translocase (CD36) and zinc finger protein 423 (Zfp423), which plays a role in fatty acid transport and regulates transcription factors, respectively, were significantly down-regulated by increasing dietary RUP ratio.

Comparison of gene expression related to lipid metabolism between 15 and 26 months of age

The lipid metabolism-related gene expression of Hanwoo steers at 26 months of age showed significant high expression of transcription factors PPARγ ($p<0.05$), lipogenesis-related genes (ACACA, FASN and SCD) ($p<0.001$), fatty acid uptake genes (FABP4 and CD36) ($p<0.001$) and fatty acid esterification genes (GPAT1 and DGAT2) ($p<0.001$) in *longissimus lumborum* samples. On the other hand, ATGL ($p<0.001$) and very long chain acyl-CoA dehydrogenase (VLCAD) ($p<0.1$) involved in lipolysis were down-regulated at 26 months of age. According to the overall gene expression, the intramuscular fat synthesis was significantly more active at the age of 26 months than at the age of 17 months (Figure 11)

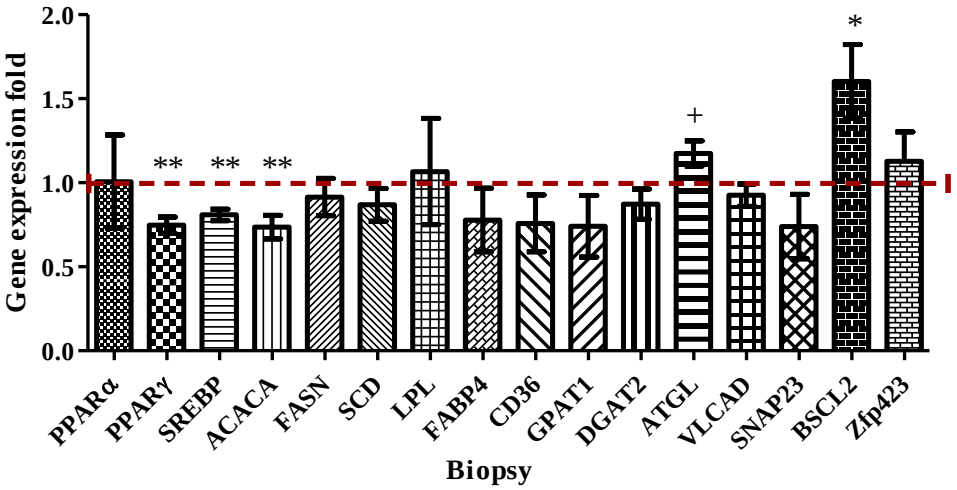


Figure 9. Effect of RUP ratio of diet on relative expression of genes related to lipid metabolism in intramuscular tissue at growing stage of SBM (RUP 37% of CP) and FSBM (RUP 47% of CP) in Exp. 1.$^{+}p < 0.1$, $^{*}p < 0.05$,** $p < 0.01$. Values are the least-squares means with the standard error.

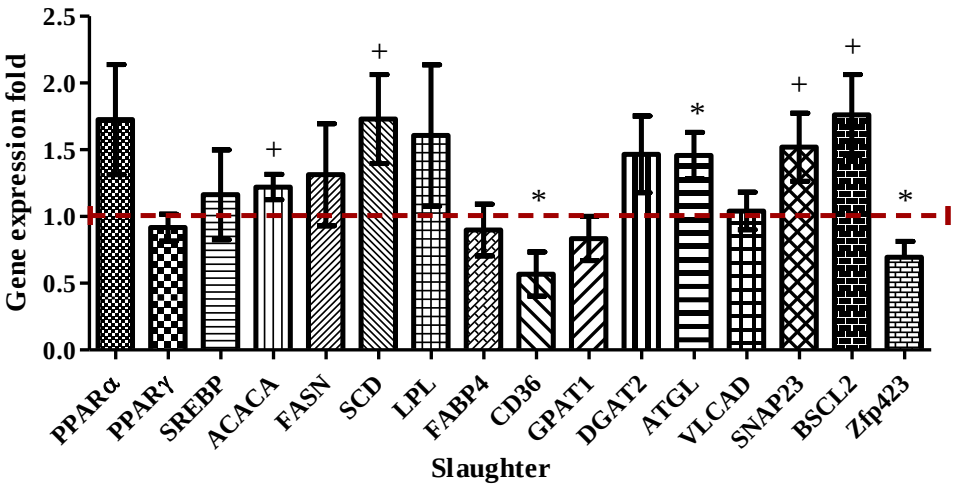


Figure 10. Effect of RUP ratio of diet on relative expression of genes related to lipid metabolism on intramuscular after slaughter of SBM (RUP 37% of CP) and FSBM (RUP 47% of CP) in Exp. 1. $^{+}p < 0.1$, $^{*}p < 0.05$,** $p < 0.01$. Values are the least-squares means with the standard error.

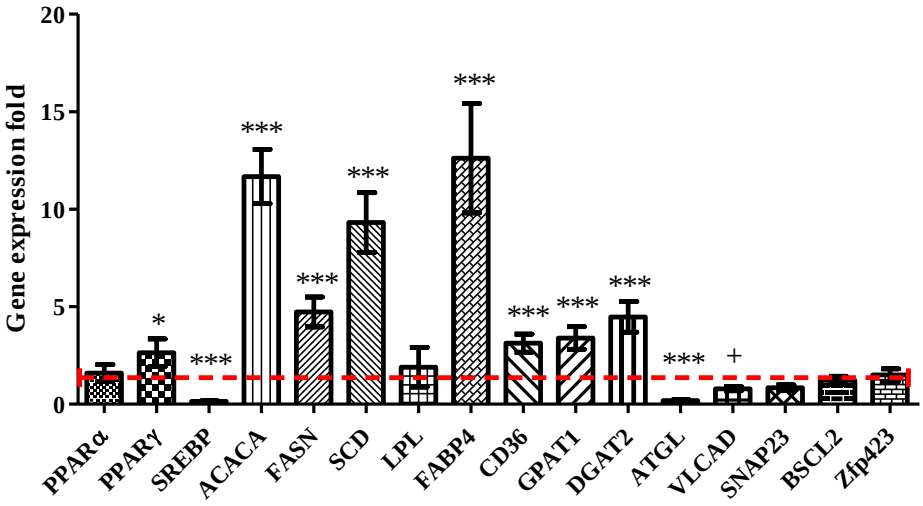


Figure 11. Relative gene expression related to lipid metabolism of Hanwoo steers at 26 months of age to 17 months of age in Exp. 1. $^{+}p < 0.1$, $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$. Values are the least-squares means with the standard error.

3.2 Experiment2: Effect of feed intake levels of FSBM diets on productivity of Hanwoo steers for shortening feeding period

3.2.1 Animals performance

Figure 12 and 13 shows daily feed and nutrient intakes of low intake level of FSBM (L-FSBM) and high intake level of FSBM (H-FSBM). Average concentrate intake of steers fed H-FSBM diet was greater ($p<0.001$) than L-FSBM but hay intake was lower ($p<0.1$) in H-FSBM treatment compared to L-FSBM in growing stage. However, there were no differences in intake amount of concentrate as well as hay in fattening stage between treatments. With respect to nutrient intakes, there were significant differences ($p<0.001$) in CP, RUP and RDP during the growing stage, and TDN in H-FSBM also showed higher intake amount ($p<0.1$) than L-FSBM. However, CP, RUP, RDP and TDN intakes in H-FSBM treatment did not differ significantly to those in L-FSBM during the fattening stage. In terms of weight change, there was no effect of feed intake in entire feeding period (Table 11 and Figure 14), and ADG and FCR were not also influenced by high concentrate intake during growing (Table 12).

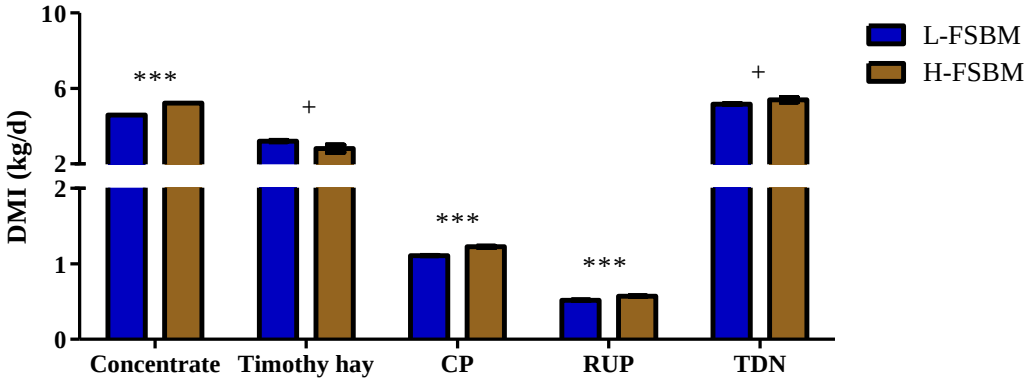


Figure 12. Intake amounts of concentrate, timothy hay, crude protein, RUP and TDN in low intake level of FSBM (L-FSBM) and high intake level of FSBM (H-FSBM) during growing stage of Exp. 2. $^{+}p < 0.1$, $^{*}p < 0.05$,** $p < 0.01$, $^{***}p < 0.001$. Values are the least-squares means with the standard error.

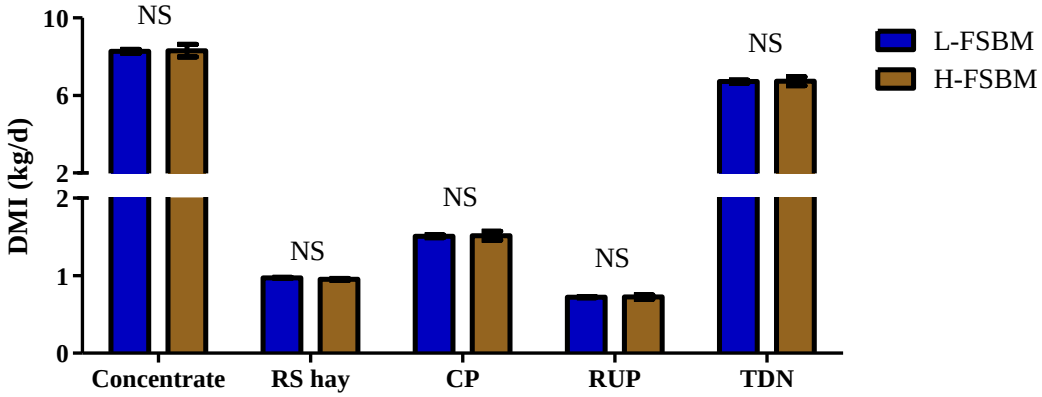


Figure 13. Intake amounts of concentrate, rice straw hay, crude protein, RUP and TDN in L-FSBM low intake level of FSBM (L-FSBM) and high intake level of FSBM (H-FSBM) during fattening stage of Exp. 2. $^{+}p < 0.1$, $^{*}p < 0.05$,** $p < 0.01$, $^{***}p < 0.001$. Values are the least-squares means with the standard error.

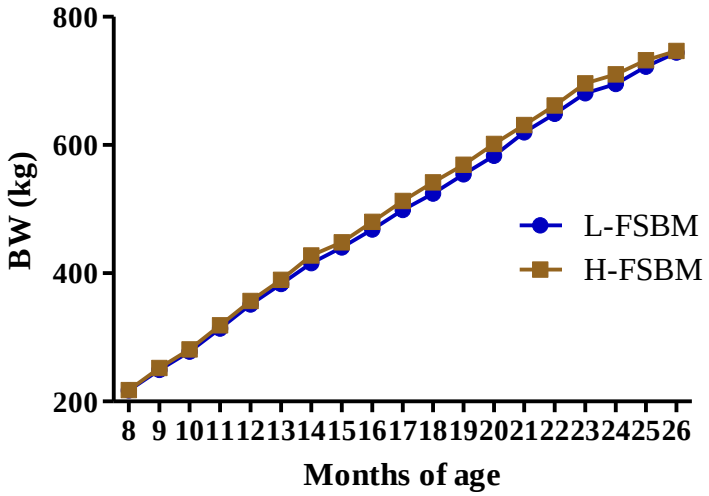


Figure 14. Changes in live body weight of steers fed low intake level of FSBM (L-FSBM) and high intake level of FSBM (H-FSBM) during whole feeding period in Exp. 2

Table 11. Changes in body weight of steers fed diets with different feed intake ratio during whole feeding period in Exp.2

Item	L-FSBM	H-FSBM	SEM	*P*-value
8 months of age	217.1	217.9	13.58	0.954
9 months of age	249.2	252.4	12.41	0.806
10 months of age	277.6	281.2	12.47	0.785
11 months of age	313.6	318.5	12.69	0.714
12 months of age	351.1	356.6	12.86	0.687
13 months of age	383.0	389.5	12.34	0.617
14 months of age	415.8	727.6	13.95	0.432
15 months of age	440.3	448.4	12.22	0.531
16 months of age	468.0	480.3	13.90	0.410
17 months of age	498.7	512.6	15.73	0.410
18 months of age	524.4	541.8	16.76	0.339
19 months of age	554.0	569.1	18.21	0.438
20 months of age	583.4	601.9	18.50	0.357
21 months of age	619.8	631.1	21.68	0.619
22 months of age	648.6	661.6	22.79	0.587
23 months of age	680.8	696.4	23.71	0.534
24 months of age	695.4	710.0	24.29	0.569
25 months of age	722.4	732.0	26.87	0.732
26 months of age	744.0	746.9	26.62	0.918

L-FSBM, low intake level of FSBM; H-FSBM, high intake level of FSBM.

Table 12. Growth performance of steers fed experimental diets with different feed intake in Exp.2

Item	L-FSBM	H-FSBM	SEM	*P*-value
Average daily gain, ADG, kg/d				
Growing stage[1]	1.03	1.08	0.03	0.211
Fattening stage[2]	0.88	0.84	0.05	0.406
Whole stage[3]	0.96	0.96	0.04	0.979
Average body weight, BW, kg				
Initial	217.1	217.9	13.58	0.954
Growing stage	498.7	512.6	15.73	0.410
Fattening stage	744.0	746.9	26.62	0.918
Feed conversion ratio, FCR[4]				
Growing stage	7.6	7.5	0.21	0.722
Fattening stage	10.5	11.1	0.42	0.209
Whole stage	9.3	9.4	0.23	0.534

L-FSBM, low intake level of FSBM; H-FSBM, high intake level of FSBM. [1]Growing stage = from 8 to 17 months of age. [2]Fattening stage= from 18 to 26 months of age. [3]Whole stage= from 8 to 26 months of age. [4]Feed conversion ratio= average daily DM intake/average daily gain.

3.2.2 Rumen fluid fermentation & Blood metabolic characteristics at growing stage

Table 13 shows the rumen fermentation characteristics at 15 months of age during the growing stage. There were no significant differences in pH and rumen NH_3-N concentration between L-FSBM and H-FSBM. However, the total VFA concentration was significantly higher ($p=0.002$) in H-FSBM with increasing feed intake level. In addition, acetate, propionate and butyrate, which constitute most of the rumen VFA, showed significantly higher concentrations in H-FSBM ($p<0.01$). Both iso-valerate ($p=0.06$), one of the branched chain volatile fatty acid (BCVFA) and valerate ($p=0.001$) increased with increasing feed intake level. There was no significant differences in blood metabolic characteristics between L-FSBM and H-FSBM (Table 14).

Table 13. Changes in rumen fermentation characteristics of steers fed diets with different feed intake at 15 months of age of the growing stage in Exp.2

Item	L-FSBM	H-FSBM	SEM	*P*-value
Rumen fluid pH	6.0	6.0	0.07	0.523
Rumen NH3-N, mg/dl	17.0	17.9	3.00	0.763
Total VFA, mM	95.2	114.5	3.49	0.002
Individual VFA, mM				
Acetate	58.1	68.1	2.59	0.008
Propionate	19.6	25.2	1.06	0.002
Iso-butyrate	0.87	0.99	0.08	0.176
Butyrate	13.5	16.1	0.64	0.006
Iso-valerate	1.43	1.80	0.16	0.064
Valerate	1.72	2.20	0.09	0.001
A:P ratio	2.96	2.71	0.14	0.131

L-FSBM, low intake level of FSBM; H-FSBM, high intake level of FSBM; VFA, volatile fatty acid; A: P ratio, acetate: propionate ratio.

Table 14. Blood metabolic characteristics of steers fed diets with different feed intake at 15 months of age of growing stage in Exp.2

Item	L-FSBM	H-FSBM	SEM	*P*-value
Total Protein (g/dl)	7.3	7.3	0.13	0.816
Albumin (g/dl)	3.9	3.9	0.45	0.894
Creatinine (mg/dl)	1.4	1.3	0.06	0.205
Blood urea nitrogen (mg/dl)	20.5	21.4	1.00	0.414
AST (U/l)	72.7	65.3	4.08	0.118
ALP (U/l)	159.6	123.9	25.55	0.211
GGT (U/l)	23.9	18.7	4.45	0.283
Glucose (mg/dl)	74.7	71.5	1.73	0.116
NEFA (mmol/l)	0.17	0.14	0.02	0.187
β-hydroxybutyrate (mmol/l)	0.57	0.56	0.05	0.863
Total-Cholesterol (mg/dl)	172.4	197.4	20.36	0.266
Triglycerides (mg/dl)	18.8	19.7	0.97	0.403

L-FSBM, low intake level of FSBM; H-FSBM, high intake level of FSBM; AST, aspartate aminotransferase; ALP, alkaline-phosphatase; GGT, gamma-glutamyl transferase; NEFA, non-esterified fatty acid.

3.2.3 Grade characteristics and marbling fleck characteristics of *longissimus lumborum* Hanwoo

There was no significant differences in whole auction price of carcass, carcass weight and shrunk body weight between L-FSBM and H-FSBM. Appearance rate of yield grade A in H-FSBM (18.8%) was about 18.7%p lower than L-FSBM (37.5%) (Table 15). On the other hand, H-FSBM showed a lower appearance rate of grade C compared to L-FSBM. However, the rib-eye area in H-FSBM tended to be higher ($p<0.1$) than L-FSBM. In the quality grade, H-FSBM (87.5%) showed 62.5%p higher rate of 1+ grade appearance than L-FSBM (25%). Steers fed H-FSBM (37.5%) had a higher appearance rate of 1++ grade, the highest grade in Hanwoo quality grade, than L-FSBM (6.3%). In the characteristics related to quality grade, the H-FSBM of marbling score was significantly higher ($p=0.052$) than L-FSBM, and the marbling percentage ($p=0.055$) and marbling area ($p=0.031$) also showed higher in H-FSBM than L-FSBM. In addition, not only the area of marbling but also the number of coarseness and fineness marbling of H-FSBM showed a significant greater ($p<0.05$) than L-FSBM. Overall, the quality grade traits were improved by increasing feed intake level.

Table 15. Carcass and marbling fleck characteristics of steers fed diets with different feed intake in Exp.2

	L-FSBM	H-FSBM	SEM	*P*-value
Animals productivity				
Price, Korean won	9,220,578	9,878,089	427,851	0.175
Shrunk weight, kg	731.6	736.5	25.59	0.855
Carcass weight, kg	426.3	435.9	18.02	0.615
Yield grade traits				
Dressing percentage[1], %	58.3	59.1	0.74	0.311
Yield grade (A : B : C, head)	6 : 8 : 2	3 : 12 : 1		
Yield grade score[2]	2.3	2.1	0.24	0.620
Rib eye area ,cm^2	83.9	89.5	2.75	0.088
Back fat thickness, mm	11.0	14.3	1.93	0.136
Yield index	62.2	61.7	0.57	0.395
Quality grade traits				
Quality grade (1^{++} : 1^{+} : 1 : 2, head)	1 : 3 : 10 : 2	6 : 8 : 1 : 1		
Marbling score	4.8	6.3	0.62	0.052
Image analysis				
Marbling percentage	0.19	0.23	0.02	0.055
Number of marbling particles	2665.5	2021.8	453.06	0.205
Coarseness index: 15 round thinning	0.17	0.19	0.25	0.441
Number of coarse marbling particle: 15 round thinning	63.4	80.4	5.41	0.020
Marbling area, cm^2	16.3	20.4	1.47	0.031
Number of fine marbling particle	190.4	229.2	13.70	0.030
Fineness of marbling	2.25	2.58	0.17	0.104

L-FSBM, low intake level of FSBM; H-FSBM, high intake level of FSBM**.** [1]Dressing percentage = carcass weight / shrunk weight. [2]A=3, B=2 and C=1.

3.2.4 Fatty acid composition (mg/ 100g) and fat contents (% DM) on intramuscular tissue and meat of biopsy and carcass

Table 16 and Table 17 shows the fatty acid compositions of *longissimus lumborum* collected from biopsy performed during the growing stage and carcass after slaughter, respectively. Both biopsy and carcass *longissimus lumborum* showed similar amounts of palmitic acid (C16:0), stearic acid (C18:0) and oleic acid (C18:1n9c). In addition, total SFA and TFA also did not show significant differences between treatments. However, the proportion of MUFA in biopsies was positively influenced by raising feed intake level, and H-FSBM showed higher omega-3, omega-6, and total PUFA amounts ($p<0.05$) than L-FSBM in carcass samples. The fat content of *longissimus lumborum* for H-FSBM treatment was significantly higher ($p<0.05$) than L-FSBM (Figure 15).

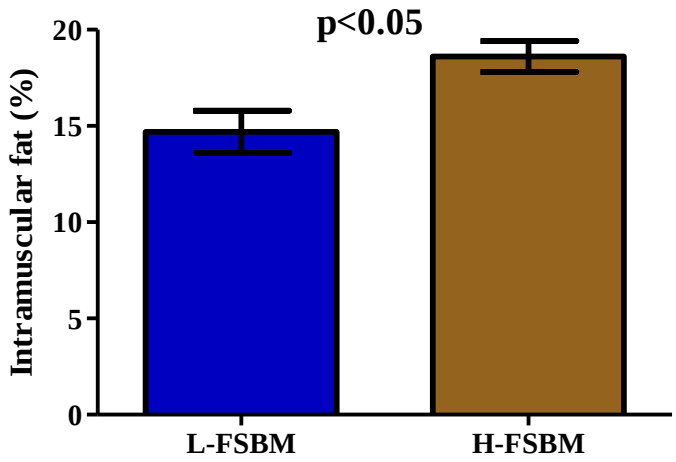


Figure 15. Percentage of intramuscular fat in *longissimus lumborum* of carcass samples in low intake level of FSBM (L-FSBM) and high intake level of FSBM (H-FSBM) of Exp. 2.

Table 16. Fatty acid composition of biopsy *longissimus lumborum* samples in growing stage (mg/100g FAME) in Exp.2

Fatty acids	L-FSBM	H-FSBM	SEM	*P*-value
Myristic acid, C14:0	3195	3485	368.2	0.462
Palmitic acid, C16:0	24684	26002	1166.3	0.301
Palmitoleic acid, C16:1	4208	4473	341.7	0.469
Stearic acid, C18:0	9728	10284	528.5	0.333
Elaidic acid, C18:1n9t	1301	1544	115.7	0.080
Oleic acid, C18:1n9c	34726	37057	1308.8	0.125
Linoleic acid(LA), C18:2n6c	2845	2843	398.4	0.996
α-linolenic acid(ALA), C18:3n3	133	127	8.3	0.474
Others[1]	4071	4193	272.4	0.669
SFA[2]	39638	41813	1796.2	0.272
MUFA[3]	41786	44789	1464.0	0.086
PUFA[4]	3469	3407	461.1	0.897
Omega-6	3307	3261	449.3	0.922
Omega-3	162	146	14.6	0.312
Total fatty acids (mg/100g FAME)	84893	90009	3102.4	0.150

L-FSBM, low intake level of FSBM; H-FSBM, high intake level of FSBM; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; [1]Others=C4:0+C6:0+C8:0+C10:0+C11:0+C12:0+C13:0+C14:1+C15:0+C15:1+C17:0+C17:1+ C18:2n6t+C20:0+C18:3n6+C20:1n9+C21:0+C20:2+C22:0+C20:3n6+C22:1n9+C20:3n3+C23: 0+C20:4n6+C22:2+C24:0+C20:5n3+C24:1n9+C22:6n3. SFA = C10:0 + C11:0 + C12:0 + C14:0 + C15:0 + C16:0 + C17:0 + C18:0 + C20:0 + C21:0 +C22:0 + C24:0. [3] MUFA = C14:1n5 + C16:1n7 + C17:1n7 + C18:1n7 + C18:1n9 + C20:1n9 + C22:1n9 + C24:1n9. [4] PUFA = C18:2n6 + C18:2c9,t11 + C18:3n3 + C18:3n6 + C20:2n6 + C20:3n3 + C20:3n6 + C20:4n6 + C20:5n3 + C22:2n6 + C22:4n6 + C22:5n3 + C22:6n3.

Table 17. Fatty acid compositions of *longissimus lumborum* muscle after slaughter (mg/100g Meat) in Exp.2

Fatty acids	L-FSBM	H-FSBM	SEM	*P*-value
Myristic acid, C14:0	456	563	86.9	0.264
Myristoleic acid, C14:1	116	158	28.2	0.188
Palmitic acid, C16:0	3294	3855	417.4	0.228
Palmitoleic acid, C16:1	593	687	99.0	0.379
Stearic acid, C18:0	1199	1372	142.8	0.271
Elaidic acid, C18:1n9t	203	239	45.4	0.458
Oleic acid, C18:1n9c	5351	5863	675.4	0.477
Linoleic acid(LA), C18:2n6c	292	357	25.8	0.046
α-linolenic acid(ALA), C18:3n3	8	12	1.5	0.050
Others[1]	357	405	37.3	0.260
SFA[2]	5160	6037	636.3	0.217
MUFA[3]	6375	7063	783.9	0.414
PUFA[4]	334.93	412.08	27.6	0.032
Omega-6	326	400	27.3	0.036
Omega-3	8	12	1.5	0.050
Crude fat, %	15	19	1.4	0.028
Total fatty acids (mg/100g Meat)	11870	13513	1380.0	0.279

L-FSBM, low intake level of FSBM; H-FSBM, high intake level of FSBM; SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; [1]Others=C4:0+C6:0+C8:0+C10:0+C11:0+C12:0+C13:0+C15:0+C15:1+C17:0+C17:1+C18:2n6t+C20:0+C18:3n6+C20:1n9+C21:0+C20:2+C22:0+C20:3n6+C22:1n9+C20:3n3+C23:0+C20:4n6+C22:2+C24:0+C20:5n3+C24:1n9+C22:6n3. SFA = C10:0 + C11:0 + C12:0 + C14:0 + C15:0 + C16:0 + C17:0 + C18:0 + C20:0 + C21:0 +C22:0 + C24:0. [3] MUFA = C14:1n5 + C16:1n7 + C17:1n7 + C18:1n7 + C18:1n9 + C20:1n9 + C22:1n9 + C24:1n9. [4] PUFA = C18:2n6 + C18:2c9,t11 + C18:3n3 + C18:3n6 + C20:2n6 + C20:3n3 + C20:3n6 + C20:4n6 + C20:5n3 + C22:2n6 + C22:4n6 + C22:5n3 + C22:6n3.

3.2.5 Lipid metabolic gene expression of intramuscular samples obtained from biopsy and carcass

Gene expressions of biopsy longissimus lumborum

Peroxisome proliferator activated receptors-alpha (PPARα), a transcription factor, was significantly ($p<0.05$) down-regulated in H-FSBM compared to L-FSBM (Figure 16). However, ACACA involved in lipid biosynthesis and diacylglycerol acyltransferase-2 (DGAT2) involved in fatty acid esterification tended to show higher expression in H-FSBM ($p<0.1$) than L-FSBM, and lipoprotein lipase (LPL) involved in fatty acid transport also showed higher expression ($p<0.01$) in H-FSBM than L-FSBM (Figure 16).

Gene expressions of carcass longissimus lumborum

Steers fed H-FSBM showed highly suppressed expression of CD36 in H-FSBM compared to L-FSBM. Contrary to the biopsy samples, DGAT2 was downregulated in H-FSBM compared to L-FSBM. ($p<0.01$) (Figure 17).

Comparison of gene expression related to lipid metabolism between 15 and 26 months of age

Lipid metabolism-related gene expression between biopsy and carcass *longissimus lumborum* samples showed that PPARγ ($p<0.05$), lipogenesis-related genes (ACACA, FASN and SCD) ($p<0.001$), fatty acid uptake gene (FABP4 and CD36) ($p<0.01$) and fatty acid esterification gene (GPAT1 and DGAT2) ($p<0.001$) were all significantly increased in carcass samples compared to biopsy samples. On the other hand, ATGL and VLCAD involved

in lipolysis decreased ($p<0.001$) in carcass samples compared to biopsy samples. In the aspects of overall gene expressions at biopsy and carcass *longissimus lumborum* samples, it is obvious that the intramuscular fat synthesis was significantly more active at the age of 26 months than at the age of 17 months (Figure 18).

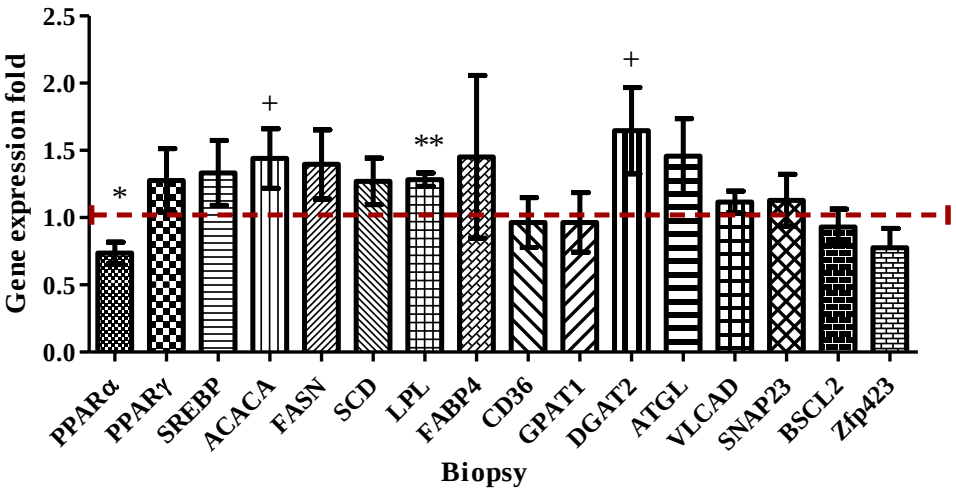


Figure 16. Relative gene expression related to lipid metabolism on intramuscular at growing stage of low intake level of FSBM (L-FSBM) and high intake level of FSBM (H-FSBM) in Exp.2. $^{+}p < 0.1$, $^{*}p < 0.05$,** $p < 0.01$. Values are the least-squares means with the standard error.

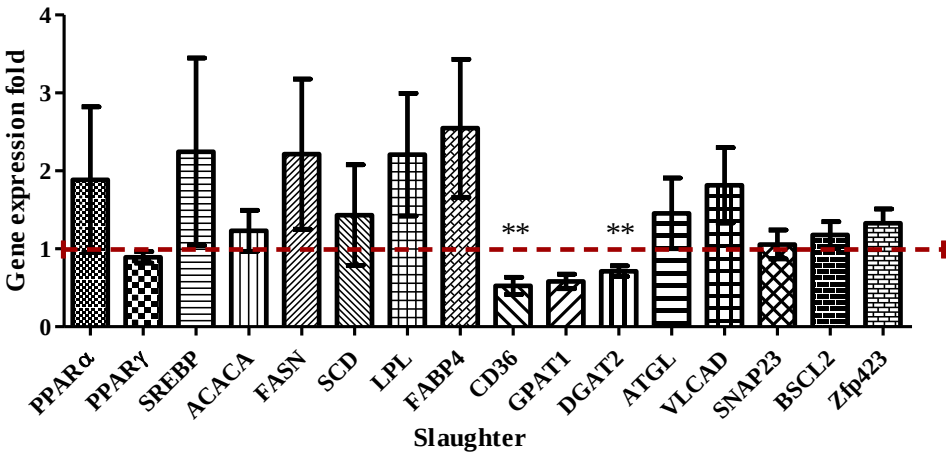


Figure 17. Relative gene expression related to lipid metabolism on intramuscular after slaughter of low intake level of FSBM (L-FSBM) and high intake level of FSBM (H-FSBM) in Exp.2. $^{+}p < 0.1$, $^{*}p < 0.05$, $^{**}p < 0.01$. Values are the least-squares means with the standard error.

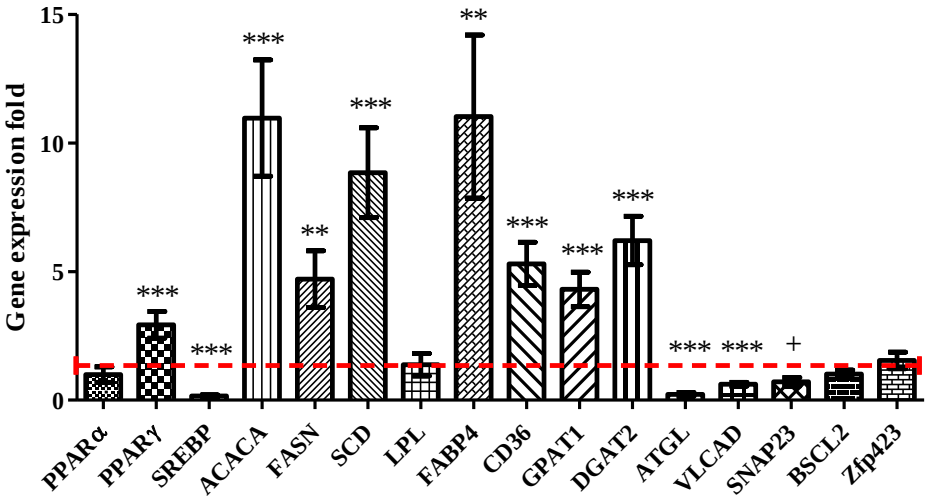


Figure 18. Relative gene expression related to lipid metabolism of Hanwoo steers at 26 months of age to 17 months of age in Exp. 2. $^{+}p < 0.1$, $^{*}p < 0.05$,** $p < 0.01$, $^{***}p < 0.001$. Values are the least-squares means with the standard error.

4. Discussion

4.1. Experiement1 1

In dairy science, a number of studies related to dietary RUP: RDP ratio were carried out to determine protein requirements for high yield dairy cows. However, some studies stated that milk yield was improved by increasing RUP ratio (Bateman et al., 2005; Ipharraguerre et al., 2005). Conversely, there is a study that increasing the RUP ratio did not affect milk yield (Wang et al., 2008). This discrepancy can be occurred by deficiency of MCP synthesis due to low RDP provision by increasing RUP content (Santos et al., 1998). However, it is obvious that the RUP ratio affects positively animal growth and FCR in dairy cow (Swartz et al., 1991; Tomlinson et al., 1997). In addition, relatively high RUP ratio improved nitrogen efficiency as well as body weight gain (Corea et al., 2020). In the current study, steers fed FSBM diet with 47% RUP of CP showed positive effects on animal performance and feed efficiency. Rib-eye area also improved by increasing RUP ratio, which implies that the more RUP in the diet may cause the adequate supply of essential amino acid compositions to small intestine and high protein digestibility of total digestive tract (Carbone and Pasiakos, 2019). In turn, it induced positive protein balance, the more protein synthesis rather than degradation in the skeletal muscles.

The NH_3-N concentration of steers fed FSBM diet during growing stage was 23% lower than SBM, which indicates that protein degradation rate in FSBM was less than SBM in the rumen and high amount of by-pass protein was supplied to small intestine. However, NH_3-N concentration in FSBM was

17mg/dl is higher than minimum NH_3-N concentration (6.0mg/dl) (Henning et al., 1993) for adequate MCP synthesis in the rumen. Even though the crude protein content in feed was declined from 18% for growing stage to 16%, as fed basis, for fattening stage, RUP: RDP ratio was not different between both stages. Therefore, the RUP amounts provided to small intestine would be remained higher in FSBM not only in growing but also in fattening stage compared to SBM. The high amount of RUP might have a positive effect on improvement of rib eye area in FSBM compared to SBM.

Feizi et al. (2020) reported that acetate was reduced by providing high RUP, and Griswold et al. (1996) pointed out that not only acetate but also BCVFAs and valerate decreased with increasing RUP ratio. BCVFAs such as iso-butyrate and iso-valerate are produced from protein metabolism by rumen microbes (Ipharraguerre et al., 2005; Syamsi et al., 2019) and are an important substrate to synthesize microbial protein (MCP) in the rumen (Kim et al., 1999a; b; 2005b). In current study, acetate and valerate was declined by increasing RUP ratio, while, iso-valerate was higher in FSBM than SBM. The difference in iso-valerate concentration between both diets might be due to the different protein sources of concentrate. Apajalahti et al. (2019) reported that a conversion rate from leucine to iso-valerate was depending on protein source, even though there were no significant difference in protein source intake amount. In addition, the study stated that some of leucine can be utilized in MCP synthesis without the conversion to iso-valerate. Castro et al. (2007) also dedicated that degradation of branched-chain amino acid (BCAA) was variable depending on processing of soybean meal (SBM) to improve RUP ratio. In the present study, SBM and

fermented soybean meal (FSBM) were used to manipulate RUP: RDP ratio. Therefore, the source of protein, such as SBM and FSBM, might affect to the rate of degradation in the rumen, utilization in the small intestine and conversion of leucine to iso-valerate.

There are few studies demonstrating the effect of RUP on beef production. In crossbred beef steers (Faucitano et al., 2011) and Hanwoo (Lee et al., 2020) research, increasing RUP intake tended to affect marling sore. However, in the present study, higher RUP with same CP content in concentrate did not improve intramuscular fat and quality grade traits. In a study of beef cattle fed 14.5% CP (Wagner et al., 2010) with 42% RUP of CP, there was no any significant effect on meat quality traits. Therefore, the further investigation is needed to demonstrate the range of CP and RUP content in feed to expect intramuscular fat accumulation.

Beef cattle are slaughtered between 18 and 21 months of age in USA or Europe (Girard et al., 2012), whereas Hanwoo is slaughtered at an average 30 months of age (Lee et al., 2015, 2020; Mamuad et al., 2020) because the rate of intramuscular fat accumulate continues to increase more vigorously from 21 to 30 months of age. According to carcass comparisons experiment (Kim et al., 2007), accumulated energy per ADG (Mcal/kg) rapidly increased to 3.7, 3.9, 4.7, 5.8, and 8.0 as Hanwoo steers grew to 12, 16, 22, 26 and 30 months of age. Therefore, in this experiment, it is estimated that 17-month-old and 26-month-old, which were subjected to biopsy and slaughter, respectively, are the early and middle stages of conventional feeding program. Genes regulating lipid metabolism (PPARγ), fatty acid uptake (LPL, FABP4 and CD36), lipogenesis

(ACACA, FASN and SCD) and fatty acid esterification (GPAT1, DGAT2) in slaughter samples were higher than biopsy samples. Conversely, genes associated with lipolysis (ATGL and VLCAD) were less in slaughter sample than biopsy samples. One of the transcription factors, PPARγ, promotes upregulation of genes related to lipid synthesis and plays an important role in adipogenesis and lipogenesis (Kliewer et al., 1997a; Varga et al., 2011). Among the fatty acid uptake genes, LPL associated with transports fatty acid is evaluated as genetic marker of intramuscular fat (Jeong et al., 2012). In addition, LPL is related to pathway of free fatty acid (FFA) storage in tissue by degrading very low-density lipoprotein (VLDL) and chylomicron to produce FFA (Pillarisetti and Saxena, 2003). CD36 delivers FFA produced by LPL in intracellular (Love-Gregory and Abumrad, 2011; Jay and Hamilton, 2018) and is implicated as an important gene for the formation of intramuscular fat by inducing accumulation TGs in muscle (Bonen et al., 2004). Fatty acid binding protein 4 (FABP4) is upregulated during adipocyte differentiation (Furuhashi et al., 2014) and more expressed in obese than normal conditions (Garin-Shkolnik et al., 2014), which indicates a significant role for intramuscular fat accumulation in Wagyu and limousin crossbred cattle (Michal et al., 2006). There are genes that play a major role in lipogenesis. First of all, ACACA is a protein expressed in the first-step of de novo biosynthesis of long chain fatty acids (Piórkowska et al., 2020), and fatty acid synthase (FASN) has an important role in palmitic acid biosynthesis using malonyl-CoA produced by ACACA and acetyl-CoA (Grzes et al., 2016). Finally, SCD is an important protein for producing MUFAs by desaturation of stearic acid (C18:0) and

palmitic acid (C16:0) (Kim and Ntambi, 1999; Smith et al., 2009). Glycerol-3-phosphate acyltransferase-1 (GPAT1) and DGAT2 associated with fatty acids esterification have a role in terminal step for TG synthesis. This is why GPAT1 is evaluated genetic marker of accumulation intramuscular fat together with LPL. The final step in TG biosynthesis pathway is catalyzed by DGAT (Buchanan et al., 2013), which is known as a key protein for increasing intramuscular fat (Harris et al., 2011). Conversely, there are protein involved in lipolysis for lipid homeostasis. For example, VLCAD induces β-oxidation in mitochondria and inhibits intramuscular fat deposition (Jeong et al., 2012). ATGL is a protein that acts first for hydrolysis of TG in adipocyte and a negative effect on intramuscular fat (Smirnova et al., 2006).

However, in this experiment, there was no significant difference in gene expression related to lipid metabolism between SBM and FSBM in biopsy sample of 17 months of age. Decreasing transcription factors like PPARγ and SREBP by increasing RUP intake in growing stage did not affect genes involved in fatty acid uptake and lipogenesis. Therefore, the modulation of RUP ratio of diet might not have effect on genes expression of lipid metabolism in 17 months of age, which is considered as inactive period of fat accumulation It is presumed that there was no differences in genes expression of transcription factor in carcass samples as well as decrease in Zfp423 and CD36 in carcass samples. Zfp423 is a protein that controls transcription of pre-adipocyte differentiation, and promotes differentiation of pre-adipocyte by inducing genes expression related to pre-adipocyte as well as PPARγ (Gupta et al., 2010, 2012).

In addition, ATGL related to lipolysis was higher in FSBM than SBM in both biopsy and carcass samples. Although there was no difference in body weight between SBM and FSBM, the study that essential amino acid supplementation reduced body fat (Xiao and Guo, 2022) supports an increase in ATGL in carcass samples as well as biopsies. In addition, BSC2L, which was up-regulated in both biopsy and slaughter samples, affects adipogenesis and lipid droplet size in adipocyte (Kociucka et al., 2016a; b), while at the same time affecting lipolysis except adipocytes (Wee et al., 2014). However, there are still insufficient studies to support whether the increase in BSCL2 is due to the presence of more muscle cells in biopsy samples obtained at the early stage of intramuscular fat accumulation.

In carcass samples, SCD, SNAP23 and BSCL2 as well as ACACA, which was suppressed in biopsy samples, were up-regulated. However, GPAT1 and DGAT1, related to fatty acid esterification, were no difference between SBM and FSBM. In addition, there was no significant difference between FSBM and SBM in MUFA quantity, although SCD was upregulated in carcass samples. However, oleic acid or total MUFA quantity cannot be explained by SCD expression level alone (Archibeque et al., 2005). SNAP23 accumulates lipid in lipid droplet and participates in lipid synthesis as well as lipid droplet size (Kociucka et al., 2016a; Kociucka et al., 2016b). therefore, despite of ATGL expression kept higher in FSBM compared to SBM both biopsy and carcass samples, marbling might not be affected due to improvement in genes involved in lipid synthesis. In other word, it implies that lipolysis genes and lipid synthesis genes were counteracted to each other.

4.2. Experiment 2.

Steers fed more amount of nutrients such as CP and TDN in H-FSBM diet than L-FSBM during growing stage showed higher concentration of total VFA and individual VFAs. However, there was no difference in daily gain, FCR between L-FSBM and H-FSBM at both fattening stage and entire feeding period. Nevertheless, rib eye area, marbling score, marbling area and the number of fine marbling particle were improved for the steers fed H-FSBM. Therefore, it is assumed that the higher feed intake during growing stage might induce carryover effect on the more synthesis of skeletal muscle and lipogenesis in fattening stage. Jeong et al. (2010) reported that fat contents of *longissimus* and marbling score by increasing TDN and CP similar to this experiments. Reddy et al. (2018) also demonstrated that carcass trait and body weight gain were improved by increasing TDN and CP in the feeding program for 28 months .

The results in Experiment 2 also revealed that transcription factor for lipid metabolism (PPARγ), fatty acid uptake (LPL, FABP4 and CD36), lipogenesis (ACACA, FAS, SCD) and fatty acid esterification (GPTA1, DGAT2) were significantly greater in carcass samples, which slaughtered 26 months of age, than biopsy samples collected in 17 months of age. Conversely, ATGL and VLCAD involved in lipolysis were suppressed in carcass samples compared to biopsy samples. The results support that characteristics of changes in the fat accumulation rate per body weight of Hanwoo from growing to late fattening stage (Kim et al., 2007).

Increased expression of ACACA, LPL and DGAT2 in biopsy samples might reflect the higher intramuscular fat and improved of beef quality of carcass. On the other hand, down-regulation of PPARα, which one of transcription factor related to β-oxidation (Kliewer et al., 1997b; Varga et al., 2011), induces low energy expenditure with PPARγ (Grygiel-Górniak, 2014) during the growing stage. However, even though the expression of lipid synthesis genes such as CD36 and DGAT2 was suppressed in carcass sample, the intramuscular fat contents and quality grade were improved by increasing CP and TDN intakes. It is similar to a previous study that that reported down-regulation of DGAT2 expression in cattle fed a high protein diet with fat (Segers et al., 2017). The adequate triglyceride biosynthesis could be sustained via multiple mechanism even under down-regulation of DGAT2

4.3 Perspective on Shortening feeding program

Since middle of 2000s, long terms fattening feeding program for around 30 months has been recommended for sufficient intramuscular fat accumulation (Kim et al., 2007; Kwon et al., 2009; Li et al., 2010) because fat deposition of Hanwoo is active from around 21 months of age to an average 30 months of age (Kim et al., 2007). Fat deposited between the collagen fibers is named marbling. The texture of the beef becomes soft by increasing marbling (Gotoh et al., 2018) and intramuscular fat has major role in juicy, flavor and tenderness of beef (Joo et al., 2013). It is well known that Hanwoo beef produced from the long terms fattening program has more fatty acid content in *longissimus lumborum* and higher palatability than imported (Hwang and Joo, 2017). The long terms fattening program has been an undeniable trend in the Hanwoo

industry for past 20 years, however, the need of a short-term fattening program less than 30 months has been raised continuously during that time. Because long-term fattening program was pointed out by production cost increases, prolonging cattle selling cycle and net profit decrease (Lee et al., 2013). Shortening the fattening period by 4 months (from 28 months to 24 months) in Japan reduced the total amount of feed supply by 1,000kg, although the contents of RUP, CP and TDN in the diet were increased (Abe et al., 2018). The current experiment is the first trial to evaluate the feeding program for rearing Hanwoo steers up to 26 months of age (Table 18). Final shrunk body weight at 26 months of age of Hanwoo steers fed H-FSBM diets was similar to the national average of body weight slaughtered at 30 months of age. The total amounts of feed consumed (4761kg) for H-FSBM treatment during the whole experimental period were around 1,000kg less than the average amounts of feed consumed for steers slaughter at 30 months of age.

Table 18. Dry matter intake comparison between 26 months and 30 months fattening program

Item	SBM	FSBM	H-FSBM	Conventional 1[1]	Conventional 2[2]
Feed intake (kg DM/ head)					
Overall period[5]					
Concentrate	3545	3544	3730	4517	4049
Hay	1138	1145	1031	1263	2060
Total	4683	4689	4761	5780	6107
Index[6] (kg DM/ head)					
Feed intake					
Concentrate	-972	-973	-787		
Hay	-125	-118	-232		
Total	-1097	-1091	-1019		

SBM**,** low RUP ratio diet (37% CP)**;** FSBM, high RUP ratio diet (47% CP); H-FSBM, high intake level of FSBM; TDN, total digestible nutrients. [1]Conventional 1= Experiment using 30 months conventional fattening program of Hanwoo steer (Chung et al., 2017), [2]Conventional 2= Average feed provision of Hanwoo beef cattle (KOSIS, 2020), [3]Growing stage= from 8 to 17 months (SBM, FSBM and H-FSBM) and from 8 to12 months (Chung et al.,2017). [4]Fattening stage= from 18 to 26 months (SBM, FSBM and H-FSBM) and from 13 to 30 months (Chung et al., 2017). [5]Overall period = from 8 months to slaughter [6]Index= Experiment dry matter intake (SBM, FSBM and H-FSBM) – dry matter intake of Chung et al., 2017.

5. Conclusion

In Experiment 1, improvement RUP ratio had only effect on yield grade traits but not on quality grade traits. However, in Experiment 2 showed that increasing feed intake had effects on marbling score, marbling area, marbling percentage. In conclusion, maximizing dry matter intake of higher RUP ratio compared to the conventional feed composition one of strategy to attain similar daily gain and quality grade to the national average results in Hanwoo steers. This could shorten the feeding period by attaining the target BW earlier and reduce around 17% of total feed intake amount compared to the 30 months of conventional feeding program.

Bibliography

Abe, A., N. Naruso, M. Irie, A. Takano, Y. Arakawa, and T. Obitsu. 2018. Effect of bypass protein feeding in the early fattening of 24-month-old Black-haired Japanese breed steers on fattening performance. JAPANESE Soc. Anim. Sci. 89:329–337.

AOAC. 2007. Official Methods of Analysis of AOAC International. 18th ed. AOAC International, Gaithersburg Md.

AOCS. 2018. Determination of Cis-, Trans-, Saturated, Monounsaturated and Polyunsaturated Fatty Acids in Dairy and Ruminant Fats by Capillary GLC. Official Methods and Recommended Practices of the AOCS. 7th ed.

Apajalahti, J., K. Vienola, K. Raatikainen, V. Holder, and C.A. Moran. 2019. Conversion of Branched-Chain Amino Acids to Corresponding Isoacids - An in vitro Tool for Estimating Ruminal Protein Degradability. Front. Vet. Sci. 6:311. doi:10.3389/FVETS.2019.00311.

APQA. 2013. Animal Protection Act, Animal and Plant Quarantine Agency. Accessed September 27, 2021. https://www.qia.go.kr/bbs/lawAnn/viewLawWebAction.do?id=109281&type=0.

Bateman, H.G., J.H. Clark, and M.R. Murphy. 2005. Development of a System to Predict Feed Protein Flow to the Small Intestine of Cattle. J. Dairy Sci. 88:282–295. doi:10.3168/JDS.S0022-0302(05)72686-2.

Bonen, A., M.L. Parolin, G.R. Steinberg, J. Calles-Escandon, N.N. Tandon, J.F.C. Glatz, J.J.F.P. Luiken, G.J.F. Heigenhauser, and D.J. Dyck. 2004. Triacylglycerol accumulation in human obesity and type 2 diabetes is associated with increased rates of skeletal muscle fatty acid transport and increased sarcolemmal FAT/CD36. FASEB J. 18:1144–1146. doi:10.1096/FJ.03-1065FJE.

Bruns, K.W., R.H. Pritchard, and D.L. Boggs. 2004. The relationships among body weight, body composition, and intramuscular fat content in steers. J. Anim. Sci. 82:1315–1322. doi:10.2527/2004.8251315X.

Buchanan, J.W., A.J. Garmyn, G.G. Hilton, D.L. VanOverbeke, Q. Duan, D.C. Beitz, and R.G. Mateescu. 2013. Comparison of gene expression and fatty acid profiles in concentrate and forage finished beef. J. Anim. Sci. 91:1–9. doi:10.2527/JAS.2012-5154.

Carbone, J.W., and S.M. Pasiakos. 2019. Dietary protein and muscle mass: Translating science to application and health benefit. Nutrients 11:1–13. doi:10.3390/nu11051136.

Castro, S.I.B., L.E. Phillip, H. Lapierre, P.W. Jardon, and R. Berthiaume. 2007. Ruminal Degradability and Intestinal Digestibility of Protein and Amino Acids in Treated Soybean Meal Products. J. Dairy Sci. 90:810–822. doi:10.3168/JDS.S0022-0302(07)71565-5.

Chaney, A.L., and E.P. Marbach. 1962. Modified Reagents for Determination of Urea and Ammonia. Clin. Chem. 8:130–132. doi:10.1093/CLINCHEM/8.2.130.

Cheah, K.S., Cheah, A.M., Just, A. 1997. A Simple and Rapid Biopsy Technique for Obtaining Fat and Muscle Samples from Live Animals for Predicting Meat Quality. https://agris.fao.org/agris-search/search.do?recordID=DK1997022046.

Chung, C.S., W.K. Cho, I.S. Jang, S.S. Lee, and Y.H. Moon. 2017. Effects of feeding system on growth performance, plasma biochemical components and hormones, and carcass characteristics in Hanwoo steers. Asian-Australasian J. Anim. Sci. 30:1117–1123. doi:10.5713/ajas.17.0166.

Corea, E.E., J. Castro-Montoya, M. V. Mendoza, F.M. López, A. Martinez, M.E. Alvarado, C. Moreno, G.A. Broderick, and U. Dickhoefer. 2020. Effect of forage source and dietary rumen-undegradable protein on nutrient use and growth in dairy heifers. Anim. Feed Sci. Technol. 269:114658. doi:10.1016/J.ANIFEEDSCI.2020.114658.

Faucitano, L., R. Berthiaume, M. D'Amours, D. Pellerin, and D.R. Ouellet. 2011. Effects of corn grain particle size and treated soybean meal on carcass and meat quality characteristics of beef steers finished on a corn silage diet. Meat Sci. 88:750–754. doi:10.1016/J.MEATSCI.2011.03.008.

Feizi, L.K., S.S. Zad, S.A.H. Jalali, H. Rafiee, M.B. Jazi, K. Sadeghi, and R. Kowsar. 2020. Fermented soybean meal affects the ruminal fermentation and the abundance of selected bacterial species in Holstein calves: a multilevel analysis. Sci. Reports 2020 101 10:1–13. doi:10.1038/s41598-020-68778-6.

Furuhashi, M., S. Saitoh, K. Shimamoto, and T. Miura. 2014. Fatty Acid-Binding Protein 4 (FABP4): Pathophysiological Insights and Potent Clinical Biomarker of Metabolic and Cardiovascular Diseases. Clin. Med. Insights. Cardiol. 8:23. doi:10.4137/CMC.S17067.

Garin-Shkolnik, T., A. Rudich, G.S. Hotamisligil, and M. Rubinstein. 2014. FABP4 Attenuates PPARγ and Adipogenesis and Is Inversely Correlated With PPARγ in Adipose Tissues. Diabetes 63:900–911. doi:10.2337/DB13-0436.

Girard, I., J.L. Aalhus, J.A. Basarab, I.L. Larsen, and H.L. Bruce. 2012. Modification of beef quality through steer age at slaughter, breed cross and growth promotants. Can. J. Anim. Sci. 92:175–188. doi:10.4141/CJAS2012-001.

Gotoh, T., T. Nishimura, K. Kuchida, and H. Mannen. 2018. The Japanese Wagyu beef industry: current situation and future prospects — A review. Asian-Australasian J. Anim. Sci. 31:933. doi:10.5713/AJAS.18.0333.

Griswold, K.E., W.H. Hoover, T.K. Miller, and W. V. Thayne. 1996. Effect of form of nitrogen on growth of ruminal microbes in continuous culture. J. Anim. Sci. 74:483–491. doi:10.2527/1996.742483X.

Grygiel-Górniak, B. 2014. Peroxisome proliferator-activated receptors and their ligands: nutritional and clinical implications – a review. Nutr. J. 13:17. doi:10.1186/1475-2891-13-17.

Grzes, M., S. Sadkowski, K. Rzewuska, M. Szydlowski, and M. Switonski. 2016. Pig fatness in relation to FASN and INSIG2 genes polymorphism and their transcript level. Mol. Biol. Rep. 43:381. doi:10.1007/S11033-016-3969-Z.

Gupta, R.K., Z. Arany, P. Seale, R.J. Mepani, L. Ye, H.M. Conroe, Y.A. Roby, H. Kulaga, R.R. Reed, and B.M. Spiegelman. 2010. Transcriptional control of preadipocyte determination by Zfp423. Nat. 2010 4647288 464:619–623. doi:10.1038/nature08816.

Gupta, R.K., R.J. Mepani, S. Kleiner, J.C. Lo, M.J. Khandekar, P. Cohen, A. Frontini, D.C. Bhowmick, L. Ye, S. Cinti, and B.M. Spiegelman. 2012. Zfp423 Expression Identifies Committed Preadipocytes and Localizes to Adipose Endothelial and Perivascular Cells. Cell Metab. 15:230. doi:10.1016/J.CMET.2012.01.010.

Harris, C.A., J.T. Haas, R.S. Streeper, S.J. Stone, M. Kumari, K. Yang, X. Han, N. Brownell, R.W. Gross, R. Zechner, and R. V. Farese. 2011. DGAT enzymes are required for triacylglycerol synthesis and lipid droplets in adipocytes. J. Lipid Res. 52:657–667. doi:10.1194/JLR.M013003.

Henning, P.H., D.G. Steyn, and H.H. Meissner. 1993. Effect of synchronization of energy and nitrogen supply on ruminal characteristics

and microbial growth. J. Anim. Sci. 71:2516–2528. doi:10.2527/1993.7192516X.

Hwang, Y.H., and S.T. Joo. 2017. Fatty acid profiles, meat quality, and sensory palatability of grain-fed and grass-fed beef from Hanwoo, American, and Australian crossbred cattle. Korean J. Food Sci. Anim. Resour. 37:153–161. doi:10.5851/kosfa.2017.37.2.153.

Ipharraguerre, I.R., J.H. Clark, and D.E. Freeman. 2005. Rumen Fermentation and Intestinal Supply of Nutrients in Dairy Cows Fed Rumen-Protected Soy Products. J. Dairy Sci. 88:2879–2892. doi:10.3168/JDS.S0022-0302(05)72969-6.

Jay, A.G., and J.A. Hamilton. 2018. The enigmatic membrane fatty acid transporter CD36: New insights into fatty acid binding and their effects on uptake of oxidized LDL. Prostaglandins, Leukot. Essent. Fat. Acids 138:64–70. doi:10.1016/J.PLEFA.2016.05.005.

Jeong, J., E.G. Kwon, S.K. Im, K.S. Seo, and M. Baik. 2012. Expression of fat deposition and fat removal genes is associated with intramuscular fat content in longissimus dorsi muscle of Korean cattle steers. J. Anim. Sci. 90:2044–2053. doi:10.2527/JAS.2011-4753.

Jeong, J., N.-I. Seong, I.-K. Hwang, S.-B. Lee, M.-S. Yu, I.-S. Nam, and M.-I. Lee. 2010. Effects of Level of CP and TDN in the Concentrate Supplement on Growth Performances and Carcass Characteristics in Hanwoo Steers during Final Fattening Period. J. Anim. Sci. Technol. 52:305–312. doi:10.5187/jast.2010.52.4.305.

Joo, S.T., G.D. Kim, Y.H. Hwang, and Y.C. Ryu. 2013. Control of fresh meat quality through manipulation of muscle fiber characteristics. Meat Sci. 95:828–836. doi:10.1016/J.MEATSCI.2013.04.044.

KAPE. 2019. The Beef Carcass Grading, Korea Institute for Animal Products Quality Evaluation (KAPE). https://www.ekape.or.kr/contents/list.do.

Kim, K.H., J.J. Choung, and D.G. Chamberlain. 1999a. Effects of varying the degree of synchrony of energy and nitrogen release in the rumen on the synthesis of microbial protein in lactating dairy cows consuming a diet of grass silage and a cereal-based concentrate. J. Sci. Food Agric. 79:1441–1447. doi:10.1002/(SICI)1097-0010(199908)79:11.

Kim, K.H., J.H. Lee, Y.G. Oh, S.W. Kang, S.C. Lee, W.Y. Park, and Y.D. Ko. 2005a. The Optimal TDN Levels of Concentrates and Slaughter Age in Hanwoo Steers. J. Anim. Sci. Technol. 47:731–744.

Kim, K.H., S.S. Lee, and K.J. Kim. 2005b. Effect of intraruminal sucrose infusion on volatile fatty acid production and microbial protein synthesis in sheep. Asian-Australasian J. Anim. Sci. 18:350–353. doi:10.5713/ajas.2005.350.

Kim, K.H., Y.G. Oh, J.J. Choung, and C. DG. 1999b. Effects of varying degrees of synchrony of energy and nitrogen release in the rumen on the synthesis of microbial protein in cattle consuming grass silage.. J. Sci. Food Agric. 79:833–838. doi:10.1002/(SICI)1097-0010(19990501)79:6.

Kim, K.H., Y.G. Oh, S.C. Lee, K.J. Shin, W.T. Chung, S.W. Kang, S.K. Hong, J.C. Ju, and B.H. Baek. 2007a. Determination of Net Energy and Protein Requirements for Growth in Hanwoo Steers by Comparative Slaughter Experiment.. J. Anim. Sci. Technol. 49:41–50.

Kim, K.H., Y.G. Oh, S.C. Lee, K.J. Shin, W.T. Chung, S.W. Kang, S.K. Hong, J.C. Ju, and B.H. Baek. 2007b. Determination of Net Energy and Protein Requirements for Growth in Hanwoo Steers by Comparative Slaughter Experiment. J. Anim. Sci. Technol. 49:41–50. doi:10.5187/JAST.2007.49.1.041.

Kim, Y.C., and J.M. Ntambi. 1999. Regulation of Stearoyl-CoA Desaturase Genes: Role in Cellular Metabolism and Preadipocyte Differentiation. Biochem. Biophys. Res. Commun. 266:1–4. doi:10.1006/BBRC.1999.1704.

Kliewer, S.A., S.S. Sundseth, S.A. Jones, P.J. Brown, G.B. Wisely, C.S. Koble, P. Devchand, W. Wahli, T.M. Willson, J.M. Lenhard, and J.M. Lehmann. 1997a. Fatty acids and eicosanoids regulate gene expression through direct interactions with peroxisome proliferator-activated receptors α and γ. Proc. Natl. Acad. Sci. U. S. A. 94:4318. doi:10.1073/PNAS.94.9.4318.

Kociucka, B., T. Flisikowska, D. Mróz, and I. Szczerbal. 2016a. Expression of genes involved in lipid droplet formation (BSCL2, SNAP23 and COPA) during porcine in vitro adipogenesis. J. Appl. Genet. 2016 574 57:505–510. doi:10.1007/S13353-016-0350-9.

Kociucka, B., H. Jackowiak, M. Kamyczek, M. Szydlowski, and I. Szczerbal. 2016b. The relationship between adipocyte size and the transcript levels of SNAP23, BSCL2 and COPA genes in pigs. Meat Sci. 121:12–18. doi:10.1016/J.MEATSCI.2016.05.011.

Konarska, M., K. Kuchida, G. Tarr, and R.J. Polkinghorne. 2016. Relationships between marbling measures across principal muscles. Meat Sci. 123:67-68 doi:10.1016/j.meatsci.2016.09.005.

KOSIS. 2020. Livestock Production Cost Survey Report,2020. Accessed. https://kosis.kr/publication/publicationThema.do.

Kuchida, K.;, M.. Suzuki, and S. Miyoshi. 2001. Development of Photographing Equipment for the Cross Section of Carcass and Prediction of BMS Number by Using Obtained Image from That Equipment. Accessed September 27, 2021. https://agris.fao.org/agris-search/search.do?recordID=JP2001005049.

Kuchida, K. 1997. Development of accurate method for measuring fat percentage on rib-eye area by computer image analysis. Anim. Sci. Technol. 68:853–859.

Kuchida, K., T. Osawa, T. Hori, H. Kotaka, and S. Maruyama. 2006. Evaluation and genetics of carcass cross section of beef carcass by computer image analysis. J. Anim. Genet. 34:45–52. doi:10.5924/ABGRI2000.34.2_45.

Kwon, E.G., B.K. Park, H.C. Kim, Y.M. Cho, T. Il Kim, S.S. Chang, Y.K. Oh, N.K. Kim, J.H. Kim, Y.J. Kim, E.J. Kim, S.K. Im, and N.J. Choi. 2009. Effects of fattening period on growth performance, carcass characteristics and lipogenic gene expression in Hanwoo steers. Asian-Australasian J. Anim. Sci. 22:1654–1660. doi:10.5713/ajas.2009.90257.

Lee, S.-C., H.-H. Choi, and D.-W. Shin, Jeong-Seop;Kim, Kyoung-Hoon;Oh, Young-Kyoon;Cheon. 2013. Carcass Characteristics and Profitability Analysis Based on Slaughter Age of Hanwoo Steers. J. Anim. Sci. Technol. 55:315–323. doi:10.5187/JAST.2013.55.4.315.

Lee, S.J., D.H. Kim, L.L. Guan, S.K. Ahn, K.W. Cho, and S.S. Lee. 2015. Effect of medicinal plant by-products supplementation to total mixed ration on growth performance, carcass characteristics and economic efficacy in the late fattening period of hanwoo steers. Asian-Australasian J. Anim. Sci. 28:1729–1735. doi:10.5713/ajas.15.0290.

Lee, Y.H., F. Ahmadi, M. Lee, Y.-K. Oh, and W.S. Kwak. 2020. Effect of crude protein content and undegraded intake protein level on productivity, blood metabolites, carcass characteristics, and production economics of Hanwoo steers. Asian-Australasian J. Anim. Sci. 33:1599. doi:10.5713/AJAS.19.0822.

Li, S.G., Y.X. Yang, Y.J. Rhee, W.J. Jang, J.J. Ha, S.K. Lee, and Y.H. Song. 2010. Growth, behavior, and carcass traits of fattening hanwoo (Korean native cattle) steers managed in different group sizes. Asian-Australasian J. Anim. Sci. 23:952–959. doi:10.5713/ajas.2010.90276.

Livak, K.J., and T.D. Schmittgen. 2001. Analysis of Relative Gene Expression Data Using Real-Time Quantitative PCR and the 2−ΔΔCT Method. Methods 25:402–408. doi:10.1006/METH.2001.1262.

Love-Gregory, L., and N.A. Abumrad. 2011. CD36 genetics and the metabolic complications of obesity. Curr. Opin. Clin. Nutr. Metab. Care 14:527. doi:10.1097/MCO.0B013E32834BBAC9.

Mamuad, L.L., S.H. Kim, M.J. Ku, and S.S. Lee. 2020. Effect of γ-aminobutyric acid producing bacteria on in vitro rumen fermentation, growth performance, and meat quality of Hanwoo steers. Asian-Australasian J. Anim. Sci. 33:1087–1095. doi:10.5713/ajas.19.0785.

Michal, J.J., Z.W. Zhang, C.T. Gaskins, and Z. Jiang. 2006. The bovine fatty acid binding protein 4 gene is significantly associated with marbling and subcutaneous fat depth in Wagyu x Limousin F2 crosses. Anim. Genet. 37:400–402. doi:10.1111/J.1365-2052.2006.01464.X.

O'Fallon, J. V., J.R. Busboom, M.L. Nelson, and C.T. Gaskins. 2007. A direct method for fatty acid methyl ester synthesis: Application to wet meat tissues, oils, and feedstuffs. J. Anim. Sci. 85:1511–1521. doi:10.2527/JAS.2006-491.

Pillarisetti, S., and U. Saxena. 2003. Lipoprotein lipase as a therapeutic target for dyslipidemia. Front. Biosci. 8. doi:10.2741/958.

Piórkowska, K., M. Małopolska, K. Ropka-Molik, M.S. Nędza, A. Wiechniak, K. Żukowski, B. Lambert, and M. Tyra. 2020. Evaluation of SCD, ACACA and FASN Mutations: Effects on Pork Quality and Other Production Traits in Pigs Selected Based on RNA-Seq Results. Anim. 2020, Vol. 10, Page 123 10:123. doi:10.3390/ANI10010123.

Reddy, K.E., J.Y. Jeong, S.Y. Ji, Y.-C. Baek, S. Lee, M. Kim, Y.K. Oh, and H.-J. Lee. 2018. Effects of High Levels of Nutrients on Growth Performance and Carcass Characteristics of Hanwoo Cattle. J. Korean Soc. Grassl. Forage Sci. 38:180–189. doi:10.5333/kgfs.2018.38.3.180.

Santos, F.A.P., J.E.P. Santos, C.B. Theurer, and J.T. Huber. 1998. Effects of Rumen-Undegradable Protein on Dairy Cow Performance: A 12-Year Literature Review. J. Dairy Sci. 81:3182–3213. doi:10.3168/JDS.S0022-0302(98)75884-9.

Smirnova, E., E.B. Goldberg, K.S. Makarova, L. Lin, W.J. Brown, and C.L. Jackson. 2006. ATGL has a key role in lipid droplet/adiposome degradation in mammalian cells. EMBO Rep. 7:106–113. doi:10.1038/SJ.EMBOR.7400559.

Smith, S.B., C.A. Gill, D.K. Lunt, and M.A. Brooks. 2009. Regulation of fat and fatty acid composition in beef cattle. Asian-Australasian J. Anim. Sci. 22:1225–1233. doi:10.5713/AJAS.2009.R.10.

Van Soest, P.J., J.B. Robertson, and B.A. Lewis. 1991. Methods for Dietary Fiber, Neutral Detergent Fiber, and Nonstarch Polysaccharides in Relation to Animal Nutrition. J. Dairy Sci. 74:3583–3597. doi:10.3168/jds.S0022-0302(91)78551-2.

Swartz, L.A., A.J. Heinrichs, G.A. Varga, and L.D. Muller. 1991. Effects of Varying Dietary Undegradable Protein on Dry Matter Intake, Growth, and Carcass Composition of Holstein Calves. J. Dairy Sci. 74:3884–3890. doi:10.3168/JDS.S0022-0302(91)78581-0.

Syamsi, A.N., L. Waldi, H.S. Widodo, and H. Harwanto. 2019. Branched Chain Volatile Fatty Acids Profile of Rumen Fluids Suplemented by Different Meal Protein Sources and Protein-Energy Synchronization Index. IOP Conf. Ser. Earth Environ. Sci. 372. doi:10.1088/1755-1315/372/1/012060.

Tomlinson, D.L., R.E. James, G.L. Bethard, and M.L. McGilliard. 1997. Influence of Undegradability of Protein in the Diet on Intake, Daily Gain, Feed Efficiency, and Body Composition of Holstein Heifers. J. Dairy Sci. 80:943–948. doi:10.3168/JDS.S0022-0302(97)76018-1.

Toshiyuki, S., K. Kentaro, and H. NAGABUCHI, Masahiro OoHASHI. 2013. Effect of Feeding a Concentrate-Rich Diet on the Growing Period of Japanese-Black Steers. Bull. Miyazaki Livest. Exp. Stn. 45:79–84.

Varga, T., Z. Czimmerer, and L. Nagy. 2011. PPARs are a unique set of fatty acid regulated transcription factors controlling both lipid metabolism and inflammation. Biochim. Biophys. Acta 1812:1007. doi:10.1016/J.BBADIS.2011.02.014.

Wagner, J.J., T.E. Engle, and T.C. Bryant. 2010. The effect of rumen degradable and rumen undegradable intake protein on feedlot performance and carcass merit in heavy yearling steers. J. Anim. Sci. 88:1073–1081. doi:10.2527/JAS.2009-2111.

Wang, C., J.X. Liu, S.W. Zhai, J.L. Lai, and Y.M. Wu. 2008. Effects of rumen-degradable-protein to rumen-undegradable-protein ratio on nitrogen conversion of lactating dairy cows. Acta Agriculturae Scand Section A, 58:2, 100-103. http://dx.doi.org/10.1080/09064700802187210 58:100–103. doi:10.1080/09064700802187210.

Wee, K., W. Yang, S. Sugii, and W. Han. 2014. Towards a mechanistic understanding of lipodystrophy and seipin functions. Biosci. Rep. 34:583–591. doi:10.1042/BSR20140114.

Xiao, F., and F. Guo. 2022. Impacts of essential amino acids on energy balance. Mol. Metab. 57:101393. doi:10.1016/j.molmet.2021.101393.

Ye, J., G. Coulouris, I. Zaretskaya, I. Cutcutache, S. Rozen, and T.L. Madden. 2012. Primer-BLAST: A tool to design target-specific primers for polymerase chain reaction. BMC Bioinforma. 2012 131 13:1–11. doi:10.1186/1471-2105-13-134.

Yoshimi, N., N. Hiroyoshi, and T. Shin-ichi. 2014. The Effects of Feeding Dried Shochu By Product on the Fattening of Japanese Black Cattle. Bull. Miyazaki Livest. Exp. Stn. 26:17–21.

국문 초록

한우 거세우의 단기 비육은 생산비와 질소 및 메탄 등 환경 오염 물질 배출을 절감 할 수 있다. 따라서, 본 연구는 RUP 비율과 사료 섭취량 증가가 26 개월령 단기 비육에서 한우 거세우의 성장 및 도체 특성에 미치는 영향에 대해서 조사하기 위해서 실험 1 과 실험 2 를 수행하였다. 각 실험 당 평균 8 개월령의 한우 거세우 32 마리를 체중 자료에 기초해서 8 개의 우방에 배치하였다. 실험 1 에서는 CP 당 37% RUP 가 포함된 사료(SBM)와 CP 당 47% RUP 가 포함된 사료(FSBM)를 각 실험군에 급여하였으며, 실험 2 에서는 CP 당 47% RUP 의 기초 사료의 섭취량 차이인 저 사료 섭취량 실험군(L-FSBM)과 고 사료 섭취량 실험군(H-FSBM)를 조사하였다. 실험 1 에서는 SBM 과 비교하여, FSBM 의 평균 일당 증체량(ADG), 사료 전환 효율(FCR)이 향상되었고($p<0.01$), 등심 단면적도 높은 경향을 보였다($p<0.1$). 그러나, 육질 특성, 지방산 조성 및 지질 대사 관련 유전자 발현에서는 유의적인 차이가 존재하지 않았다. 실험 2 에서는 H-FSBM 이 L-FSBM 에 비하여 생검 등심 조직에서의 지질 대사 유전자 발현이 상대적으로 상승하였으며, 도체에서도 육량과 육질 특성이 모두 향상되었다($p<0.1$). 또한 47% RUP 가 포함된 사료를 섭취한 실험군 모두 전국 30 개월령 한우 거세우 평균 체중과도

유사하였다. 그러므로, RUP 비율과 사료 섭취량의 증가는 한우 거세우의 일당 증체를 향상 시키고, 적정 출하 체중을 조기에 달성함으로써, 비육 기간을 단축 할 수 있을 것이다.

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주요어: 사료 섭취량, 반추위 미분해 단백질, 단기 비육

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