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Effect of starch and crude protein in supplemental feed on rumen fermentation, growth performance, and carcass characteristics in early- and late-fattening Hanwoo steers

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Abstract

Recently, there has been an increased focus on developing high-energy and high-protein feeds for Hanwoo steers in Korea to reduce the fattening period and decrease production costs. The aim of this study was to investigate the effects of different starch and crude protein contents in supplemental feed on rumen fermentation, growth performance, plasma metabolites, and carcass characteristics in early- and late-fattening Hanwoo steers. Sixty steers were randomly assigned to the following two groups based on the fattening stages: moderate-protein and high-starch (MPHS) and high-protein and moderate-starch (HPMS). The *in vitro* ruminal pH and ammonia concentrations in steers fed the MPHS supplemental feed were lower than those on HPMS supplemental feeds. The propionate concentration was higher in the MPHS supplemental feed in both the early- and late-fattening stages. During late fattening, average daily gain was 0.93 and 0.82 in the MPHS and HPMS groups, respectively, with a higher value in the MPHS group. In early-fattening Hanwoo steers, plasma creatinine, calcium, phosphorus, and magnesium concentrations in the MPHS group were higher than in the HPMS group. The rib-eye area in the MPHS group was 98.60 and 93.60 in the HPMS group. The marbling score in the MPHS group tended to be higher than in the HPMS group. Increasing the starch energy content rather than the crude protein content in the supplemental feed exerts a positive effect on rumen fermentation, growth performance, carcass weight, and rib-eye area in Hanwoo steers during the early and late fattening periods.

Keywords: Hanwoo steers; starch; crude protein; ruminal characteristics; average daily gain; rib-eye area
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Introduction

The growth performance and carcass characteristics of beef cattle are dependent on the feeding system (León-Llanos *et al*., 2022). In particular, meat quality is dependent on the breed, feed type, and fattening period (Chung *et al*., 2006). Recently, there has been an increased focus on developing high-energy and high-protein feeds for Hanwoo steers in Korea to reduce the fattening period and decrease production costs (Chung *et al*., 2015).

The energy and crude protein requirements for Hanwoo steers vary depending on the fattening stage (Choi, 2018). Increasing the crude protein contents in feeds during the early- and late-fattening periods was reported to exert a positive effect on average daily gain (ADG), feed conversion ratio (FCR), carcass weight, rib eye area, back-fat thickness, and intramuscular fat (Martin *et al*., 1979; Rossi *et al*., 2000). In contrast, Kang *et al*. (2020) reported that ADG was highly correlated with energy level rather than the crude protein content of feed in growing and fattening cattle. It is known that ADG is markedly affected by the energy content rather than the protein content in supplemental feed during the early- and late-fattening periods (MAFRA, 2007).

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The energy level of feed is a major factor influencing the growth performance, carcass characteristics, and fat deposition in beef cattle (Chung *et al*., 2015). Various studies have reported that increasing the energy level in supplemental feeds maintains the marbling score and shortens the fattening period (Chung *et al*., 2018). Increasing the energy levels in late-fattening supplemental feed improves the energy availability and ADG and promotes the accumulation of intramuscular fat (Chang *et al*., 2007; Ki *et al*., 2009; Chung *et al*., 2015; Ryu, 2017).

Previous studies examining the energy and protein content in beef cattle have focused on energy (mainly TDN) and protein supply levels based on the growth stages. However, most studies are limited to studies on the energy or protein levels of supplemental feeds. Limited studies have focused on the effects of starch and crude protein contents in supplemental feed during the early- and late fattening periods. Therefore, this study aimed to investigate the effect of starch and crude protein levels in supplemental feed on rumen fermentation characteristics, growth performance, plasma metabolites, and carcass characteristics of Hanwoo steers during the early- and late fattening periods.

Materials and Methods

This study followed all animal experimental procedures indicated in the Kangwon National University Animal Experimental Ethics Committee (Institutional Animal Care and Use Committee, IACUC). Three cows equipped with rumen fistula was used for the *in vitro* experiment (427.0 ± 41.5 kg; aged 42 months). The supplemental feed and rice straw were used as the basal feed. The formulated feed was given twice a day (09:00 and 18:00) at 1.7% of their body weight. The field trial was performed using 30 (434.5 ± 67.0 kg; aged 13 months) early-fattening and 30 (668.1 ± 30.3 kg; aged 24 months) late-fattening Hanwoo steers. The Hanwoo steers (n = 60) were randomly assigned to the following two groups: moderate-protein and high-starch (MPHS) and high-protein and moderate-starch (HPMS). The steers were housed in 12 pens (5 m × 10 m) where the floor was covered with 20 cm of sawdust. Supplemental feed was provided twice daily (07:30 and 17:00) at 1.7% of their body weight using an automatic feeding system (SEOCHANG 65M/M, Seochang Co. Ltd., Cheonan, Korea). The cows and steers had free access to rice straw, water, and mineral blocks. Other feeding management procedures were conducted according to the practices of the experimental farm. The ingredients and chemical composition of the experimental feeds are listed in Tables 1 and 2.

The experimental feeds were dried at 65 °C for 72 h, ground to a particle size of 1 mm, and used for the analysis. Dry matter, crude protein, acid detergent fibre (ADF), and crude ash of experimental feeds were analysed according to the methods of the AOAC (2005). Ether extract was analysed according to the method of the AOAC (2006). The crude protein content was calculated as: 6.25 × total nitrogen content. The total nitrogen content of the feed was analysed using a Leco FP-528 nitrogen combustion analyser (Leco, MI, USA).

To evaluate the fibre content, neutral detergent fibre (NDF) and acid detergent lignin (ADL) were analysed following the methods of Van Soest *et al*. (1991). Heat-stable α-amylase was used for NDF analysis. Among the carbohydrate fractions, ethanol-soluble carbohydrate (ESC) and starch were analysed following the methods of Hall (2009). Soluble protein (SOLP) was analysed following the method of Mohamed and Chaudhry (2008).

Neutral detergent insoluble crude protein (NDICP) and acid detergent insoluble crude protein (ADICP) were analysed following the methods of Åkerlind *et al*. (2011). TDN content in experimental feeds was evaluated using the above analysis results. NRC (2001) was used to evaluate the energy value of feed as TDN.

Based on the measured carbohydrate and protein contents, the carbohydrate and protein fractions of the Cornell net carbohydrate and protein system (CNCPS) were evaluated according to the methods of Fox *et al*. (2004) with modifications (Jeon *et al*., 2016). Sugar and organic acids were estimated as fraction A (carbohydrate A fraction, CA), which was measured as the content of ESC in the feed component analysis.

Table 1 Ingredient composition of experimental diets

Items	Early-fattening		Late fattening	
	MPHS[1]	HPMS[2]	MPHS	HPMS
Corn (%)	33.05	22.47	33.39	27.06
Wheat (%)	9.00	11.00	9.00	12.00
Rice (%)	5.00	3.00	6.00	3.00
Lupin (%)	3.00	3.00	2.00	3.00
Cottonseed (%)	-	-	2.00	-
Wheat flour (%)	-	-	2.00	2.00
Wheat bran (%)	5.00	10.00	3.00	9.32
Soybean meal (%)	2.00	5.00	-	3.00
Corn gluten feed (%)	20.57	20.00	22.00	22.00
Sunflower seed meal (%)	-	4.00	-	1.30
Palm kernel meal (%)	12.00	8.00	9.66	4.00
Corn-DDGS[3] (%)	2.00	6.00	2.60	6.00
Cane molasses (%)	4.60	3.00	4.60	3.00
Salt dehydrated (%)	0.60	0.60	0.70	0.60
Limestone (%)	2.10	2.85	2.09	2.72
Vitamin premix[4] (%)	0.10	0.10	0.10	0.10
Mineral premix[5] (%)	0.30	0.30	0.20	0.30
Sodium bicarbonate (%)	0.50	0.50	0.50	0.50
Feed additives (%)	0.18	0.18	0.16	0.10

[1]MPHS: moderate-protein and high-starch; [2]HPMS: high-protein and moderate-starch; [3]Corn-DDGS: corn dried distiller's grains with solubles; [4]Vitamin premix: providing the following quantities of vitamins per kilogram of diet: vitamin A 10,000 IU, vitamin D3 1,500 IU, vitamin E 25 IU, [5]Mineral premix: providing the following quantities of minerals per kilogram of diet: Fe 50 mg, Cu 7mg, Zn 30 mg, Mn 24 mg, I 0.6 mg, Co 0.15 mg, Se 0.15 mg

Carbohydrate fraction B1 (CB1) was estimated as starch in the feed component analysis. Carbohydrate fraction B2 (CB2) was calculated as:

$$NFC - CA - CB1 \quad (1)$$

Carbohydrate B3 fraction (CB3) was calculated as:

$$(NDF - NDICP) - (2.4 \times ADL) \quad (2)$$

Carbohydrate fraction C (CC) was calculated as:

$$2.4 \times ADL \text{ content} \quad (3)$$

The CNCPS protein fraction was determined as follows. Non-protein nitrogen and soluble true protein were expressed as protein A + B1 fraction (PA + PB1), which was the same as the content of SOLP in feed analysis. PA + PB1 represents the protein fraction that is immediately lysed and degraded in the rumen. Protein B2 fraction (PB2) was calculated as:

$$\text{Protein B2 fraction (PB2)} = 100 - NDICP - SOLP \quad (4)$$

PB2 represents a protein that is not immediately soluble but rapidly digested in the rumen (intermediate degradable CP). Protein B3 fraction (PB3), which refers to a slowly degradable fibre-bound CP that is gradually digested in the rumen, was calculated as:

$$PB3 = [NDICP - ADICP] \quad (5)$$

Protein C fraction (CC) is an indigestible protein in the rumen and was determined as the ADICP content of the feed.

Ruminal fluid was collected from the rumen fistula of a cow before morning feeding. The collected rumen fluid was immediately stored at 39 °C in a thermos flask, transferred to the laboratory, filtered through eight layers of cheesecloth, and diluted in an *in vitro* buffer (Goering & Van Soest, 1970) at a ratio of 1:3. Diluted rumen fluid was maintained under O_2-free, CO_2-bubbling conditions until inoculation into serum bottle to maintain complete anaerobic conditions. Under completely anaerobic conditions, each 60 mL of the rumen fluid was dispensed into 125 mL serum bottles containing 1 g of the experimental diet, and completely sealed using a butyl rubber stopper and aluminium cap. The sealed serum bottles were incubated in an incubator at 39 °C for 48 h.

The *in vitro* ruminal pH was measured using a pH meter (FP20, Mettler Toledo). Gas production was measured using a pressure transducer (EA-6, SunBee Instrument, Seoul, Korea), following the methods of Theodorou *et al*. (1994). To analyse the ammonia concentration, 10 mL of culture solution was centrifuged at 3,000 × *g* and 4 °C for 15 min. Next, 5 mL of the supernatant was mixed with 0.05 mL of $HgCl_2$ and the mixture was centrifuged at 3,000 × *g* and 4 °C for 15 min. The ammonia concentration in 1 mL of the supernatant was analysed following the methods of Chaney & Marbach (1962).

To analyse volatile fatty acid (VFA) concentration, 10 mL of the culture solution was mixed with 1 mL of 20% HPO_3 and 0.5 mL of saturated $HgCl_2$. The mixture was centrifuged at 1,250 × *g* and 4 °C for 15 min. The VFA concentration in the supernatant was measured using gas chromatography (Agilent 7890A, Agilent Technology, CA, USA).

The body weight (BW) was measured before morning feeding at 2-month intervals using a cattle scale. ADG was calculated based on the BW difference and the number of days of feeding. Feed intake was calculated by measuring the quantity of residual feed before feeding in the morning. The FCR was calculated based on the dry matter intake (DMI) and ADG values.

The blood samples (3 mL) were collected at 2-month intervals from the jugular vein of the experimental animals using an 18-gauge needle and were transferred to a heparin-coated blood collection tube (Vacutainer, Becton-Dickinson, Franklin Lakes, NJ, USA) to analyse the metabolites. Additionally, 3 mL of blood was collected in another blood collection tube containing ethylenediaminetetraacetate to analyse complete blood count (CBC). The blood samples were stored in an icebox and transferred to the laboratory within 6 h of collection. Blood samples were centrifuged at 1,250 × *g* for 10 min to separate the plasma and analysed using an automatic blood analyser (Hitachi 7020, Hitachi Ltd, Tokyo, Japan). The following parameters were analysed; glucose, non-esterified fatty acid, creatinine, cholesterol, triglyceride, blood urea nitrogen, total protein, albumin, bilirubin, calcium, phosphorus, and magnesium.

CBC was analysed using an automatic blood cell counter (IDEXX Procyte Dx, USA) while agitating vacuum tubes with a roller mixer. The following parameters were analysed: red blood cell count, haematocrit, haemoglobin, mean corpuscular volume, mean corpuscular haemoglobin volume, mean corpuscular haemoglobin concentration, red cell distribution width, reticulocyte count, white blood cell, neutrophil, lymphocytes, monocytes, eosinophils, basophils, and platelets.

All steers were slaughtered at 30 months of age to assess the meat yield grades (based on carcass weight, backfat thickness, and rib eye area) and meat quality grades (based on marbling score, meat colour, fat colour, texture, and maturity) of the carcasses at the local slaughterhouse. Carcass traits were determined from the sirloins of each carcass. Meat graders evaluated the carcass grade according to the criteria of the Korean Carcass Grading System (MAFRA, 2018).

Carcass characteristics, such as meat yield and quality grades were assessed 24 h post-mortem by a carcass grader from the Animal Products Grading Service (APGS), Korea. After 24 h of chilling, the weight of cold carcass was measured. The left side of each carcass was cut between the last rib and the first lumbar vertebra to assign the quality grade. The quality grade was determined by assessing the marbling score and texture in the cut surface of the rib eye area relative to the maturity, meat colour, and fat colour of the carcass.

Table 2 Chemical composition, carbohydrate, and protein fractions of experimental diets fed to early- and late-fattening Hanwoo steers

Items	Early-fattening		Late fattening	
	MPHS	HPMS	MPHS	HPMS
Chemical composition				
DM[1] (%)	87.90	88.10	87.90	88.20
CP[2] (% DM)	14.00	19.00	14.80	17.10
SolP[3] (% DM)	4.30	5.60	4.70	5.40
NDICP[4] (% DM)	1.97	1.73	1.64	1.41
ADICP[5] (% DM)	1.26	1.16	1.01	1.07
NDF[6] (% DM)	27.10	27.60	24.70	25.80
ADF[7] (% DM)	11.70	13.00	12.50	10.90
Lignin (% DM)	3.31	3.39	3.10	2.73
Sugar (% DM)	5.00	5.20	5.00	5.10
Starch (% DM)	40.90	31.40	40.70	34.90
EE[8] (% DM)	3.65	4.52	4.04	3.65
Ca (% DM)	1.46	1.39	1.05	1.31
P (% DM)	0.49	0.59	0.52	0.54
NFC[9] (% DM)	50.20	43.30	51.70	47.90
TDN[10] (%)	74.40	75.00	76.40	75.50
Carbohydrate fraction				
CA[11] (% CHO[12])	6.63	7.52	6.69	7.06
CB1[13] (% CHO)	54.27	45.38	54.47	48.33
CB2[14] (% CHO)	5.71	9.68	8.03	10.94
CB3[15] (% CHO)	22.80	25.63	20.90	24.70
CC[16] (% CHO)	10.54	11.76	9.96	9.07
Protein fraction				
PA[17]+PB1[18] (% CP)	30.30	29.70	31.70	31.40
PB2[19] (% CP)	55.21	61.42	57.16	60.18
PB3[20] (% CP)	5.10	3.00	4.30	2.00
PC[21] (% CP)	9.00	6.10	6.80	6.30

[1]DM: dry matter; [2]CP: crude protein; [3]SOLP: soluble protein; [4]NDICP: neutral detergent insoluble protein; [5]ADICP, acid detergent insoluble protein; [6]NDF: neutral detergent fibre; [7]ADF: acid detergent fibre; [8]EE: ether extract; [9]NFC, non-fibre carbohydrate; [10]TDN: total digestible nutrient; [11]CA: rapidly fermented; [12]CHO: carbohydrate; [13]CB1: moderate fermented; [14]CB2: intermediately fermented; [15]CB3: slowly fermented; [16]CC: unfermentable; [17]PA: non-protein nitrogen; [18]PB1: rapidly degradable protein; [19]PB2: intermediately degradable protein; [20]PB3: intermediately degradable protein; [21]PC: unavailable protein

Quality grades were classified as 1^{++} (best), 1^{+}, 1, 2, and 3 (worst) according to the Korean Beef Quality Grading System (MAFRA, 2018). The degree of marbling was evaluated using the Korean Beef Marbling Standard. Meat colour and fat colour scores were determined using a colour standard. The scores for texture and maturity were calculated using the APGS reference index. The grading for marbling score ranged from 1 to 9 with higher numbers indicating better quality (1 = devoid, 9 = abundant), meat colour (1 = bright red, 7 = dark red), fat colour (1 = creamy white, 7 = yellowish), texture (1 = soft, 3 = firm), and maturity (1 = youthful, 9 = old). Meanwhile, the quality grades ranged from 3 (low quality) to 1^{++} (very high quality). The rib eye area and back-fat thickness were measured at the thirteenth rib. The yield index was calculated as follows:

$$\text{yield index} = \{68.184 - [0.625 \times \text{back-fat thickness (mm)}] + [0.130 \times \text{rib eye area (cm}^2\text{)}] - [0.024 \times \text{carcass weight (kg)}]\} \times 3.23 \quad (6)$$

Yield grades were classified as A (best), B, and C (worst) according to the Korean beef yield grading system. The yield index indicated grade A > 67.20; grade B > 63.30, but < 67.20; and grade C, < 63.30.

All statistical analyses were performed using SAS 9.2 (SAS Institute, Inc., Cary, NC, US). The means between different groups were determined using independent *t*-tests. Differences were considered significant at $P < 0.05$.

Results and Discussion

Table 3 shows the effects of the crude protein and starch contents in the supplemental feed on the parameters of the *in vitro* rumen culture. The *in vitro* ruminal pH and ammonia concentrations of supplemental feeds in the MPHS were lower than those of the supplemental feed (HPMS) group ($P < 0.05$). Gas production was similar between the two groups in the same fattening stage. The propionate concentration was higher in the MPHS group than in the HPMS group in both the early- and late-fattening supplemental feed ($P < 0.05$). The acetate, butyrate, and total VFA concentrations were similar between the two groups in the same fattening stage.

The optimal pH for the growth and fermentation activity of rumen microorganisms is reported to be 5.8–7.2 (Ryu *et al*., 2022). The rumen pH is lowered due to the effects of VFAs and organic acids produced during the microbial fermentation of feed (Cooper *et al*., 2001). In the current study, the *in vitro* ruminal pH of the HPMS group was higher than that of the MPHS group. This was presumed to be due to the buffering effect (reduction of the proton concentration) according to the high NH_3-N concentration in the HPMS group.

Ammonia is produced by the degradation of the feed crude protein by microorganisms in the rumen. Rumen microorganisms synthesize microbial proteins using ammonia (May *et al*., 2009). Additionally, NH_3-N, which is generated as a final product through the decomposition of feed protein by rumen microorganisms, is closely related to the microbial protein availability and the microbial conversion efficiency of ammonia (Firkins *et al*., 2007). If the ruminant microorganisms do not use ammonia efficiently, the concentration of ammonia in the rumen fluid may increase due to enhanced proteolysis. However, considering the total VFA concentration measured in this study, the fermentation characteristics of the rumen microorganisms were assumed to be physiological. Therefore, the low NH_3-N concentration in the MPHS groups in this study can be attributed to the low crude protein contents of the supplemental feeds (Table 2).

Table 3 Effects of crude protein and starch content of the supplemental feed of early- and late-fattening Hanwoo steers on ruminal parameters after 48 h *in vitro* incubation

Item	Early-fattening		SEM[3]	Late fattening		SEM
	MPHS[1]	HPMS[2]		MPHS	HPMS	
pH	5.70[b]	5.86[a]	0.012	5.69[b]	5.78[a]	0.019
NH_3-N (mg/dL)	37.2[b]	42.0[a]	0.765	37.7[b]	42.1[a]	1.000
Gas (mL/g DM)	279.9	275.7	2.913	281.8	281.9	3.301
Volatile fatty acid						
Acetate (mmol/mol)	606.8	610.0	6.119	608.6	613.6	5.112
Propionate (mmol/mol)	196.2[a]	184.7[b]	2.005	198.1[a]	184.5[b]	1.458
Butyrate (mmol/mol)	105.7	113.7	2.300	105.4	111.4	2.214
Total-VFA (mM)	105.4	105.7	7.563	103.8	102.7	7.413

[a,b]Means followed by different letters in the same row are significantly different (P <0.05)

[1]MPHS: moderate-protein and high-starch; [2]HPMS: high-protein and moderate-starch; [3]SEM: standard error mean

Seo *et al*. (2009) reported that gas production in the rumen is directly affected by the soluble carbohydrate content in the digestive tract. In the current study, gas production was similar between the two groups. This can be due to the small difference in the CA + CB1 + CB2 fractions representing soluble carbohydrates and the CC fraction representing the indigestible fibre content in the rumen among the CNCPS carbohydrate fractions between the two groups (Table 2).

It is known that VFAs, which are important energy sources for ruminants (Schwandt, 2015), are affected by feed type, nutrient composition, feed processing, pH, and microbial communities (Manríquez *et al*., 2016). In particular, non-structural carbohydrates, such as starch, are converted to lactic acid and propionate by rumen microbes, which affects rumen pH (Keles & Demirci, 2011). In addition, Ahn *et al*., (2019) reported that corn flakes have high starch digestibility, resulting in high VFAs and the lowest pH during ruminal incubation. Likewise, in the current study, the propionate concentrations in the MPHS groups were higher, which can be attributed to the high starch, NFC, and CB1 contents in the feed (Table 2).

Table 4 shows the effects of crude protein and starch contents in the supplemental feed on the growth performance of early- and late-fattening Hanwoo steers. During early-fattening, crude protein and starch contents in supplemental feed did not markedly affect ADG, supplemental feed intake, rice straw intake, and FCR. In contrast, during late-fattening, the ADG in the MPHS group was higher than that in the HPMS group (P <0.05). However, supplemental feed intake, rice straw intake, and FCR were similar between the two groups during late-fattening.

The results of the current study were consistent with those of MAFRA (2007), who reported that the protein content in supplemental feed did not markedly affect ADG of early- and late-fattening Hanwoo steers. Prior (1977) reported that the ADG for the fattening period was highly correlated with the energy level rather than the crude protein level in the supplemental feed. The energy level of the supplemental feed during the late-fattening period affects ADG and FCR (Ahn *et al*., 2019). This is because ADG during late-fattening is closely related to energy demand (Kim *et al*., 2013). Additionally, the late fattening period is the stage where the meat quality is determined. Thus, increasing the energy level of formulated feed during the late fattening period is more effective than during the growing period (Kim, 2015). Moreover, increasing the TDN level in supplemental feed promotes dry matter degradability and energy availability (Ki, 2009), increases the DMI (Chung *et al*., 2015), and improves ADG (Jin *et al*., 2012).

Table 4 Effects of crude protein and starch contents in the supplemental feed on growth performance of Hanwoo steers

Items	Early-fattening		SEM[3]	Late fattening		SEM
	MPHS[1]	HPLS[2]		MPHS	HPLS	
Initial body weight (kg)	434.5	434.5	10.857	667.9	668.2	5.221
Final body weight (kg)	541.9	538.9	11.271	792.0	776.6	6.180
Average daily gain (kg/d)	0.88	0.86	0.063	0.93[a]	0.82[b]	0.031
Supplemental feed intake (kg DM)	6.61	6.59	0.150	8.13	8.13	0.030
Rice straw intake (kg DM)	2.56	2.56	0.081	0.94	0.94	0.029
Feed conversion ratio	10.93	11.60	0.592	10.17	11.36	0.396

[a,b]Means followed by different letters in the same row are significantly different (P <0.05)

[1]MPHS: moderate-protein and high-starch; [2]HPMS: high-protein and moderate-starch; [3]SEM: standard error mean

Previous studies (Jin *et al*., 2012; Chung *et al*., 2015) have reported that increasing the feed energy level affects the ADG and that the energy level is directly proportional to the weight gain in the late-fattening Hanwoo steers. Consistently, ADG was high in the MPHS groups. These results suggest that increasing the content of starch rather than the crude protein content in the supplemental feed increases ADG of late-fattening Hanwoo steers.

Table 5 shows the effects of crude protein and starch contents in the supplemental feed on plasma metabolites and CBC profiles of early- and late-fattening Hanwoo steers. In early-fattening Hanwoo steers, the plasma creatinine, calcium, phosphorus, and magnesium concentrations in the MPHS group were higher than those in the HPMS group (P <0.05). The concentrations of other blood metabolites were similar between the two groups. The crude protein and starch contents in the supplemental feed did not markedly affect the plasma metabolite concentration in late-fattening Hanwoo steers. Crude protein and starch contents in the supplemental feed did not affect CBC profiles in the early- and late-fattening Hanwoo steers.

The plasma creatinine concentration tends to increase proportionally to the BW (muscle mass) and age (Mohri *et al*., 2007). Therefore, the creatinine concentration in the early-fattening MPHS group, which exhibited a high BW, was considered to be upregulated. Plasma phosphorus is involved in energy metabolism (Lippy *et al*., 2022), while plasma magnesium plays an important role in energy-consuming metabolic processes, such as protein synthesis and neurotransmission. The plasma magnesium concentration varies according to feed intake (Laires *et al*., 2004). In the current study, the plasma calcium, phosphorus, and magnesium concentrations were high in the early-fattening MPHS group, which was considered to be related to the increase in energy (Table 2) and supplemental feed intake (Table 4).

Table 5 Effects of protein and starch contents in supplemental feeds on plasma metabolites and complete blood count of Hanwoo steers

Items	Early-fattening		SEM[3]	Late fattening		SEM
	MPHS[1]	HPMS[2]		MPHS	HPMS	
Plasma metabolites[4]						
Glucose (mg/dL)	104.80	101.01	2.520	93.17	92.87	1.099
NEFA (uEq/L)	115.63	138.20	8.782	178.61	201.94	11.937
Creatinine (mg/dL)	1.76[a]	1.55[b]	0.040	1.69	1.73	0.033
Cholesterol (mg/dL)	124.12	115.60	5.132	178.11	190.53	7.444
Triglyceride (mg/dL)	20.37	20.46	0.884	26.10	23.03	1.034
BUN (mg/dL)	18.78	19.53	0.669	19.21	19.35	0.476
Total protein (g/dL)	9.66	8.81	0.150	9.13	9.02	0.084
Albumin (g/dL)	5.31	5.02	0.081	4.71	4.80	0.041
Bilirubin (mg/dL)	0.11	0.11	0.004	0.11	0.12	0.005
Calcium (mg/dL)	11.30[a]	10.59[b]	0.176	10.34	10.45	0.086
Phosphorus (mg/dL)	9.95[a]	9.26[b]	0.179	8.65	8.54	0.117
Magnesium (mg/dL)	3.00[a]	2.73[b]	0.074	2.87	2.81	0.038
Complete blood count[5]						
RBC (M/uL)	9.58	8.87	0.182	8.31	8.60	0.132
HCT (%)	41.80	39.33	0.900	42.67	42.78	0.720
HGB (g/dL)	13.85	13.09	0.261	13.87	13.94	0.213
MCV (fL)	43.67	44.52	0.656	51.42	49.73	0.516
MCH (pg)	14.48	14.81	0.176	16.74	16.39	0.153
MCHC (g/dL)	33.19	33.36	0.216	32.56	32.98	0.178
RDW (%)	36.66	35.46	0.516	29.87	30.26	0.307
RETIC (K/uL)	3.16	3.51	0.336	6.33	5.28	0.362
WBC (K/uL)	10.49	11.06	0.398	9.69	9.56	0.252
NEU (K/uL)	4.57	6.12	0.779	3.11	3.38	0.100
LYM (K/uL)	4.44	5.00	0.199	5.06	4.60	0.182
MONO (K/uL)	0.58	0.61	0.026	0.53	0.52	0.021
EOS (K/uL)	1.00	0.98	0.159	0.98	1.06	0.059
BASO (K/uL)	0.01	0.01	0.001	0.00	0.00	0.001
PLT (K/uL)	432.4	422.3	21.831	300.5	326.9	16.345

[1]MPHS: moderate-protein and high-starch; [2]HPMS: high-protein and moderate-starch; [3]SEM: standard error mean; [4]NEFA: non-esterified fatty acid (NEFA), BUN: blood urea nitrogen; [5]RBC: red blood cell, HCT: haematocrit, HGB: haemoglobin, MCV: mean corpuscular volume, MCH: mean corpuscular haemoglobin, MCHC: mean corpuscular haemoglobin concentration, RDW: red cell distribution width, RETIC: reticulocyte count, WBC: white blood cell, NEU: neutrophil, LYM: lymphocytes, MONO: monocytes, EOS: eosinophil, BASO: basophil, and PLT: platelets

Table 6 shows the effects of crude protein and starch contents in the supplemental feed on carcass characteristics of Hanwoo steers. The carcass weight in the MPHS group was non-significantly higher than that in the HPMS group. The rib eye area in the MPHS group was higher than that in the HPMS group (P <0.05). The marbling score in the MPHS group was non-significantly higher than that in the HPMS group. Meat colour, fat colour, texture, and maturity were similar between the two groups. The incidence of grade 1^{+} meat or higher in the MPHS group was higher than that in the HPLS group. Carcass weight is highly correlated with the live weight. Consistently, the weight of the MPHS group tended to increase during the late-fattening period in this study.

Table 6 Effects of protein and starch contents in the supplemental feed on carcass characteristics of Hanwoo steers

Item	MPHS[1] (n = 15)	HPMS[2] (n = 15)	SEM[3]
Yield traits[3]			
Carcass weight (kg)	459.20	448.5	4.571
Rib-eye area (cm^2)	98.60[a]	93.60[b]	1.443
Back-fat thickness (mm)	13.73	13.93	0.795
Yield index (%)	64.62	64.10	0.569
Yield grade (A:B:C, steer)	2:8:5	1:9:5	-
Quality traits[5]			
Marbling score	5.67	5.40	0.319
Meat colour	5.00	5.00	0.083
Fat colour	3.07	3.00	0.033
Texture	1.27	1.13	0.074
Maturity	2.27	2.40	0.088
Quality grade (1^{++}:1^{+}:1:2, steer)	2:5:4:4	0:5:8:2	-

[a,b]Means followed by different letters in the same row are significantly different (P <0.05)

[1]MPHS: moderate-protein and high-starch; [2]HPMS: high-protein and moderate-starch; [3]SEM: standard error mean; [4]Area was measured from rib eye area taken at 13th rib and back-fat thickness was also measured at 13th rib, Yield index was calculated using the following equation: 68.184 – [0.625 × back-fat thickness (mm)] + [0.130 × rib eye area (cm^2)] – [0.024 × dressed weight amount (kg)], Carcass yield grades from C (low yield) to A (high yield); [5]Grading ranges are 1 to 9 for marbling score with higher numbers for better quality (1 = devoid, 9 = abundant), meat colour (1 = bright red, 7 = dark red), fat colour (1 = creamy white, 7 = yellowish), texture (1 = soft, 3 = firm), maturity (1 = youthful, 9 = old), quality grades from 3 (low quality) to 1^{++} (very high quality)

The rib eye area in the MPHS group was higher than that in the HPMS group. Therefore, we suggest that energy sources such as starch, and not crude protein, are highly effective in increasing the rib eye area during the late-fattening period. Chung *et al*. (2015) reported that the back-fat thickness of Hanwoo steers slaughtered at 26 and 30 months of age in the high-energy feed-fed groups was higher than that in the control group. However, high energy (starch and TDN) levels did not affect the back-fat thickness in this study, which is presumably because there was no difference in DMI between treatment groups due to limited supplemental feed (Table 4). Similarly, Paek *et al*. (2005) examined energy levels in late-fattening concentrates and reported that the body fat ratio of the TDN (74%)-treated group was lower than that of the TDN (72%)-treated group. Compared with that in the HPMS group, the carcass yield index was higher in the MPHS group, which was attributed to the wide rib eye area and thin back-fat thickness (Lee *et al*., 2011).

Intramuscular fat is correlated with meat quality grade (Lee *et al*., 2004). Glucose derived from the breakdown of starch is involved in intramuscular fat synthesis and the regulation of fatty acid synthesis. Additionally, enhanced levels of energy derived from starch and NFC have been reported to enhance intramuscular fat accumulation (Chang *et al*., 2007). In the current study, however, the marbling score

was not significantly different between the two groups. This result is thought to be because the starch and NFC level control during the late-fattening stage on Hanwoo steers was not long enough to affect the marbling score. Therefore, additional studies on the carcass characteristics of Hanwoo steers fed from the early-fattening period are needed.

Conclusion

In this study, increasing the starch content rather than the protein content of supplemental feed during the fattening period in Hanwoo steers had a positive effect on rumen fermentation (NH_3-N and propionate concentrations), ADG, carcass weight, rib eye area, and meat quality grade. Therefore, it may be effective to increase the starch content rather than the CP content of the supplemental feed to shorten the fattening period or reduce production costs of Hanwoo steers.

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Authors’ Contributions

BKP conceived the research, collected and interpreted the data, and wrote the manuscript.

Conflict of Interest Declaration

The authors declare that they have no conflict of interest.

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