Effect of crude protein content on growth performance and carcass characteristics of late-fattening Hanwoo steers

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Abstract

Hanwoo is a unique breed of cattle in Korea, and is mainly raised to produce beef. Various efforts are being made to reduce the cost of raising ruminants and to shorten the breeding period in various ways to reduce methane and manure produced by ruminants. Among them, a method of improving beef productivity and shortening the breeding period by adjusting the protein content of cocentrate feed is being reviewed.This study investigated the effects of crude protein (CP) content of concentrate feed (CF) on rumen fermentation, growth performance, and carcass characteristics of late-fattening Hanwoo steers. In vitro and in situ experiments were conducted using three Hanwoo cows (423.0 ± 44.8 kg) equipped with rumen fistulas. The experimental diets were classified into three categories based on CP content: CF -I, CF-II, and CF-III containing 15.5, 16.5, and 17% CP, respectively. A total of 24 late-fattening Hanwoo steers (619.3 ± 65.7 kg) were randomly assigned to either a control group fed CF-I or a treatment group fed CF-II. The disappearance rates of ruminal dry matter were higher in CF-II and CF-III than in CF-I after 48 h of incubation ($p<0.01$). CP disappearance rates were higher in CF-I compared to CF-II and CF-III throughout the incubation period ($p<0.05$). Propionate concentrations after 48 h of incubation were higher in CF-II and CF-III than in CF-I ($p<0.01$). Concentrations of total volatile fatty acids (VFAs) at 24 h of incubation were higher in CF-II and CF-III than in CF-I ($p<0.05$), with a trend of increasing VFAs concentration at 48h correlating with higher CP content in CF. Although average daily gain was higher in the treatment group than in the control group, the difference was not significant. Roughage intake was higher in the treatment group than in the control group ($p<0.05$). Plasma glucose concentration was higher in the control group ($p<0.05$), whereas total protein concentration was higher in the treatment group ($p<0.05$). Carcass weight and rib eye area were slightly, but not significantly, higher in the treatment group than in the control group. Marbling score did not differ significantly between groups. While the CP content in CF influenced CP disappearance rates, VFAs concentrations, and plasma total protein concentrations, increasing CP content alone, without considering the energy content of CF, appears unwarranted given the environmental implications and productivity outcomes for late-fattening Hanwoo steers.

Keywords: Hanwoo steers; concentrate feed; crude protein; rumen fermentation; growth performance; carcass characteristics

Introduction

Hanwoo is a native Korean cattle breed primarily raised for beef production. Of the 3.65 million head of cattle in Korea, Hanwoo accounts for approximately 3.5 million, reflecting its significant share in the industry. Due to the preference for highly marbled beef in Korean dietary culture, Hanwoo carcasses with higher intramuscular fat content receive premium prices. Consequently, to enhance marbling, the average slaughter age of Hanwoo steers was relatively long at 31.1 months as of 2023. However, given the rising costs of feed and management associated with prolonged rearing, a study by Lee & Lee (2024) reported that the most economical slaughter age, considering the balance between marbling-related carcass value and production costs, is 27.2 months.

Recently, the need to shorten the fattening period of Hanwoo steers has been highlighted as an increase in production costs due to an increase in feed costs, and environmental problems in livestock farming, such as greenhouse gases, excrement, and odor, have emerged as serious social problems. The practice of feeding additives like adsorbents and enzyme preparation has increased productivity of steers (Hamikoeva et al 2021; Kairov et al, 2021). If the raising period of Hanwoo steers is shortened from 30 to approximately 28 months, the annual greenhouse gas emissions per head can be reduced by approximately 456 kg CO2eq, manure emissions by 961.1 kg, and production costs can be reduced by KRW 116.1 billion annually (GIR, 2022). However, the average raising period of Hanwoo steer was 30.3 months in 2020, 30.4 months in 2021, 30.7 months in 2022, and 31.1 months in 2023, which was still around 30 months due to concerns over reduced productivity (i.e., carcass weight and intramuscular fat) (KIAPQE, 2023).

Recent studies have focused on developing concentrate feed (CF) with high energy and crude protein (CP) content to decrease beef production costs in Korea by shortening the raising period of Hanwoo steers (Chung et al., 2018; Kim et al., 2021). Feed protein is essential for the physiological and nutritional metabolism of beef cattle and has various effects on growth, reproduction, and fattening. Previous studies have mainly focused on increasing the CP content of feed to improve the productivity and economic returns of Hanwoo steers (Kamiya et al., 2020; Karr-Lilenthal et al., 2004). However, with a recent emphasis on carbon neutrality and environmentally friendly livestock farming, an increase in the CP content in feed has been identified as a cause of environmental pollution, such as manure generation, odor, and greenhouse gas emissions (methane, nitrous oxide, and carbon dioxide). Consequently, the government announced policies to limit the CP content of CF according to the feeding method and growth stage (GIR, 2022). Therefore, there is a need to qualitatively increase the amount of CP that can be metabolized within the body to enhance the utility and efficiency of feed protein within the legally permissible range.

The rumen degradable protein (RDP) ratio is a nutritional concept used in ruminants and refers to the portion of feed protein excluding rumen undegradable protein (RUP), which is broken down and used by rumen microorganisms. Rumen microorganisms break down proteins into urea and ammonia, resulting in a decrease in the absorption of amino acids needed for livestock growth and meat production in the small intestine (NRC, 2016). Soybean meal, cottonseed, and lupin are representative ingredients with high rumen undegradable protein content. They are known for their superior nutritional balance, palatability, and digestibility, which enhance the growth potential and body characteristics of Hanwoo steers (Kang et al., 2012). However, the relatively high cost of these ingredients requires their use at a minimum level that positively affecting productivity.

We hypothesized that improving the protein quality by increasing the CP content and high quality ingredients ratio in the CF could prevent a decrease in productivity caused by the shortened raising period of Hanwoo steers. Among various ingredients, soybean meal, cottonseed, and lupin flake were evaluated as potential ingredients that could enhance productivity at low supplementing levels. Therefore, this study investigated the effects of CP content including the ratio of three protein ingredients in the CF on rumen fermentation, growth performance, and carcass characteristics of late-fattening Hanwoo steers.

Materials and methods

This study was approved by the Animal Experimental Ethics Committee of Kangwon National University (approval number: KW-220927-1).

Location of experiment

This experiment was performed in Hanwoo Farm, Yanggu-gun, Gangwon-do, Korea (38°2'37"N, 127°59'6"E) and laboratory, Chuncheon-si, Gangwon-do, Korea(37°52'9", 127°44'48").

Animals, treatments, and management

This study was conducted for approximately 8 months from April to December 2023, and the feeding experiment was conducted for 6 months (168 days) from April. In vitro and in situ experiments were conducted using three Hanwoo cows (423.0 ± 44.8 kg) equipped with rumen fistulas. The experimental diets were classified into three categories based on CP content of CF: CF-I containing 15.5% CP (4.0% soybean meal, 3.0% cottonseed, and 0% lupin flake), CF-II containing 16.5% CP (5.2% soybean meal, 4.4% cottonseed, and 1.6% lupin flake), and CF-III containing 17.0% CP (6.1% soybean meal, 5.4% cottonseed, and 2.9% lupin flake). The addition ratio of each ingredient was determined at the minimum level required to adjust CP content for each CF. In addition, regardless of the CP content of the CFs, the TDN of all CFs was set to approximately the same level of 81% based on dry matter (DM). The feeding experiment was conducted by selecting CF-I and II according to the results of in vitro and in situ experiments. For the feeding experiments, 24 Hanwoo steers (619.3 ± 65.7 kg, 24.1 ± 0.1 months of age) were randomly assigned to one of two groups (i.e., the control and treatment groups): control group fed CF-I, and treatment group fed CF-II. CF and roughage were fed twice daily (8:00 AM and 5:00 PM), and water was provided ad libitum. Table 1 shows the ingredients and chemical compositions of the experimental diets.

In situ ruminal DM and CP disappearance rates

For the in situ DM and CP disappearance experiment, samples (5 g each) were measured in three groups and placed in a nylon bag (5 × 10 cm). Three nylon bags were produced for each treatment per incubation time, and the nylon bags were placed in a mesh and then inserted into the rumen fistula. In situ experiments were performed for 48 h, and meshes were collected through a fistula at 3, 6, 9, 12, 24, and 48 h. The nylon bag was washed until clear water was obtained and then dried in a 70 ℃ forced-air drying oven for 72 h. A total of three repetition experiments were performed using the same method.

In vitro ruminal fermentation characteristics

Rumen fluid was collected from the rumen fistula, filtered through four layers of cheese cloth, and stored in a thermos flask at 39 ℃. Thereafter, O2-free CO2 gas was injected for 1 min to eliminate air in the flask and incubated in an incubator at 39 ℃ for 30 min to be used as inoculum for in vitro. In vitro culture solutions were prepared by mixing 4,788 mL of the buffer solution with 1,200 mL of rumen inoculum. The prepared in vitro culture solutions (45 ml) were divided into 100 ml bottles. Samples (5 g) were added according to the treatment groups, and then CO2 gas without O2 was injected. Incubation was carried out for 3, 6, 9, 12, 24, and 48 h in a 39 ℃ incubator, and three 10 mL culture solutions were collected for each sampling point and used to analyze rumen fermentation characteristics. A total of three repetition experiments were performed using the same method.

The in vitro ruminal pH was measured using a pH meter (Corning 445, Corning, NY, USA) in a 100 mL bottle for each incubation time. Ammonia concentration was measured using automatic analyzer (Quik Chem 8500 series 2, Lachat, CO, USA) after collecting 5 mL of culture solution in a 100 mL bottle for each incubation time.

To analyze volatile fatty acids (VFAs) concentration, 10 mL of the culture solution was collected in a 100 mL bottle for each incubation time, followed by addition of 1 mL of 20% HPO3 and 0.5 mL of saturated HgCl2 and centrifugation at 4 °C at 1,250 × g for 15 min. The supernatant was discarded and the VFAs concentration was measured via gas chromatography (Shimadzu-17A, Shimadzu, Kyoto, Japan).

Plasma metabolites

To analyze blood metabolites, blood samples were collected end of the examination. This was done via the jugular vein using a 10 ml syringe (18-gauge needle) and blood collection tube coated with heparin (Vacutainer, Becton-Dickinson, Franklin Lakes, NJ, USA). The samples collected in tubes were centrifuged at 1,250 × g for 10 minutes to separate the plasma, and then albumin (ALB), alanine aminotransferase (ALT), aspartate aminotransferase (AST), blood urea nitrogen (BUN), calcium (Ca), cholesterol (CHO), gamma glutamyl transferase (GGT), glucose (GLU), magnesium (Mg), non-esterified fatty acid (NEFA), phosphorus (P), triglyceride (TG), and total protein (TP) were analyzed using an automatic blood analyzer (Hitachi Ltd., Tokyo, Japan).

Growth performance

The weights of the steers were measured at the beginning and end of the experiment using a cattle weight bridge. The average daily gain (ADG) was calculated by dividing the weight gain by the number of feeding days. Dry matter intake (DMI) was measured for three consecutive days each month during the experiment, and the DMI was determined by measuring the remaining feed before the morning feeding. The feed conversion ratio (FCR) was calculated by dividing DMI by the ADG.

Table 1. Ingredient and chemical compositions of experimental diets

Items	CF-I[1]	CF-II[2]	CF-III[3]
	---------- Ingredient composition ----		
Corn grain (%)	37.25	36.11	35.26
Wheat grain (%)	6.00	6.33	6.58
Corn gluten feed (%)	13.00	12.04	11.32
Soybean meal (%)	4.00	5.21	6.12
Lupin flakes (%)	-	1.64	2.87
Corn-DDGS[4] (%)	5.00	5.00	5.00
Cottonseed whole (%)	3.00	4.36	5.38
Palm kernel meal (%)	14.00	12.88	12.04
Coconut meal (%)	4.20	3.86	3.61
Wheat bran (%)	2.00	1.84	1.72
Cassava residue (%)	2.00	1.84	1.72
Almond meal (%)	1.76	1.62	1.51
Cane molasses (%)	2.00	1.84	1.72
MSG-CMS[5] (%)	0.50	0.46	0.43
Salt dehydrated (%)	0.60	0.55	0.52
Limestone (%)	1.54	1.50	1.46
Probiotics (%)	0.15	0.14	0.13
Sodium bicarbonate (%)	0.40	0.38	0.36
Vitamin/mineral premix[6] (%)	0.10	0.10	0.10
Feed additives (%)	2.50	2.30	2.15
	---------- Chemical composition (DM basis)		
Dry matter (%)	91.52±0.27	91.53±0.26	91.54±0.25
Crude protein (%)	15.52±0.20	16.46±0.09	17.02±0.21
Ether extract (%)	3.85±0.83	3.91±0.71	3.96±0.63
Crude fiber (%)	6.43±0.19	6.33±0.16	6.26±0.13
Crude ash (%)	11.53±1.81	11.98±1.82	12.32±1.83
NDF[7] (%)	34.36±1.61	34.08±1.59	33.88±1.59
ADF[8] (%)	15.05±1.14	15.27±1.18	15.44±1.20
TDN[9] (%)	80.78	80.79	80.80

[1]CF-1: concentrate feed containing 15.5% crude protein; [2]CF-2: concentrate feed containing 16.5% crude protein; [3]CF-3: concentrate feed containing 17.0% crude protein; [4]Corn-DDGS: corn-dried distillers grains; [5]MSG-CMS: mono sodium glutamate condensed molasses solubles; [6]Vitamin-mineral premix provided the following quantities of vitamins and minerals per kilogram of diet: vitamin A, 10,000 IU; vitamin D3, 1,500 IU; vitamin E, 25 IU; Fe, 50 mg; Cu, 7 mg; Zn, 30 mg; Mn, 24 mg; I, 0.6 mg; Co, 0.15 mg; Se, 0.15mg; [7]NDF: neutral detergent fiber; [8]ADF: acid detergent fiber; [9]TDN: total digestible nutrient.

Carcass characteristics

All steers were slaughtered after the feeding experimental period. The carcass yield grades (carcass weight, back fat thickness, rib eye area, and yield index) and quality grades (marbling score, meat color, fat color, texture, and maturity) were examined according to the criteria of the Korean carcass grading system (MAFRA, 2017). The carcass was chilled for 24 h, and its weight was measured. Next, the left side of each carcass was cut between the thirteenth rib and the first lumbar vertebra and used to determine yield and quality traits. The rib eye area was measured from the longissimus muscle of the thirteenth rib. The back fat thickness was measured on the thirteenth rib. The yield index was calculated as follows: yield index = [(11.06398 − 1.25149 × back fat thickness (mm)] + [0.28293 × rib eye area (cm2)] + [0.56781 × carcass weight (kg)] / [carcass weight (kg) × 100]. Yield grades were classified as grade A (best, yield index > 67.20), grade B (yield index 63.30 – 67.20), and grade C (worst, yield index

< 63.30) as determined by the yield index. The yield grade scores were calculated as follows: grade A = 3, grade B = 2, and grade C = 1. The quality grade was determined by assessing the degree of marbling on the cut surface of the rib eye based on the maturity, texture, meat color, and fat color of the carcass. Marbling scores were graded on a scale of 1 – 9, with higher numbers indicating better quality (1 = devoid and 9 = abundant). Additional scores included meat color (1 = bright red, 7 = dark red), fat color (1 = creamy white, 7 = yellowish), maturity (1 = youthful and 9 = old), and texture (1 = soft and 5 = firm). The quality grades were evaluated as follows: 1++ (excellent quality), 1+, 1, 2, and 3 (low quality). Quality grade scores were calculated as follows: 1++ = 5, 1+ = 4, 1 = 3, 2 = 2, 3 = 1. The auction price was calculated as the final successful bid price at the auction, based on quality grade and carcass yield.

Statistical Analysis

Data of in situ and in vitro experiments were analyzed using the generalized linear model procedure in the SAS package 9.1 software program (SAS Institute Inc.; Carey, NC, USA). Significant differences between the treatments were analyzed using Tukey's test at the 95% significance level. Results of feeding experiment were analyzed by t-tests using the least significant difference procedure of the SAS package program (release. 9.1.3 version, 2005). Significant differences were accepted if $p < 0.05$.

Results and discussion

Ruminal DM or CP disappearance rates

Table 2 shows the effect CP content including the ratio of protein ingredients in CF on DM or CP disappearance rates in the rumen. Ruminal DM disappearance rates were higher in the CF-II and CF-III groups than in the CF-I group after 12 h of rumen incubation, especially after 48 h of incubation ($p<0.01$). The CP disappearance rates were higher in the CF-I group than in the CF-II and CF-III group throughout the incubation period ($p<0.05$). The DM disappearance rate varies depending on the levels of DM or CP available for microbial utilization (Karr-Lilienthal et al., 2004). Soybean, cottonseed, and lupin flake used in this study were the preferred ingredients with excellent palatability and digestibility. Consistent with the results of this study, Pedrosa et al. (2012) indicated that the DM disappearance rate of soybean meal, cottonseed, and lupin was higher than that of other ingredients. In this study, the decrease in the CP disappearance rate with an increase in CP content was attributed to the inclusion of soybean, cottonseed, and lupin flake, which have lower RDP ratios, leading to a decrease in overall RDP. In general, a higher DM disappearance rate is associated with a higher CP disappearance rate, but CP disappearance rates can vary depending on protein structure and fraction, RDP ratio, etc. In addition, previous study (Sahroni and Permana, 2021) showed that CP disappearance rate decreased with decreasing RDP ratio in the diets with the same TDN content, but DM disappearance rate increased, which supports the present study. Therefore, an increase in the protein ingredient ratio can reduce the level of RDP while increasing the proportion of protein that can be digested in the small intestine, thus efficiently improving amino acid utilization. It is also expected to positively affect DM or CP metabolism and energy supply by increasing the amount of DM or CP available for utilization in the rumen.

In vitro ruminal fermentation characteristics

Table 3 shows the effect CP content of CF on ruminal fermentation characteristics. In vitro rumen pH of all treatment groups were decreased in progress of incubation time, with small differences in pH between treatment groups at each incubation time. Ammonia concentrations were similar between treatment groups up to 6 h of incubation, but were lower in CF-III group compared to CF-I and CF-II groups at 9 and 24 h of incubation ($p<0.05$).

The acetate concentrations in in vitro rumen fluid increased proportionally with CP content including the ratio of protein ingredients in the CF throughout the incubation period. However, the difference was not statistically significant. The propionate concentrations tended to increase proportionally with CP content including the ratio of protein ingredients in the CF, and they were significantly higher in the CF-II and CF-III groups than in the CF-I group at 48 h of incubation ($p<0.01$). Butyrate concentrations were similar between the treatment groups after 9 h of incubation. However, from 12 to 48 h of incubation, the butyrate concentrations tended to be higher in the CF-II and CF-III groups than in the CF-I group, although the difference was not statistically significant. The total VFAs concentrations at 24 h of incubation were higher in the CF-II and CF-III groups than in the CF-I group ($p<0.05$). At 48 h of incubation, the total VFAs concentrations tended to increase proportionally with the CP content including the ratio of protein ingredients in the CF, with significantly higher levels observed ($p<0.01$).

The rumen pH is known to be influenced by the non-fiber carbohydrate (NFC) and acid detergent **Table 2**. Effect of crude protein content in concentrate feed on dry matter and crude protein disappearance rates during ruminal fermentation

Table 2. Effect of crude protein content in concentrate feed on dry matter and crude protein disappearance rates during ruminal fermentation

Items	Time (hr)	CF-I[1]	CF-II[2]	CF-III[3]	SEM[4]	*P*-value
DM[5] (%)	0	28.41	28.47	29.76	0.412	0.318
	3	31.20	33.94	34.35	0.738	0.156
	6	37.41	39.64	39.82	1.215	0.670
	9	39.85	40.33	43.97	0.859	0.087
	12	46.77	48.55	49.68	1.423	0.711
	24	49.29	53.01	58.69	1.727	0.067
	48	67.07[c]	73.04[b]	78.18[a]	1.327	0.001
CP[6] (%)	0	35.90[a]	29.76[b]	27.63[b]	0.901	0.001
	3	36.10[a]	34.35[a]	28.54[b]	0.928	0.001
	6	39.64[a]	36.57[ab]	32.01[b]	1.324	0.046
	9	43.97[a]	39.74[b]	32.73[c]	1.241	0.001
	12	49.68[a]	41.62[b]	38.87[b]	1.449	0.002
	24	58.69[a]	42.72[b]	39.82[b]	2.031	0.001
	48	78.18[a]	63.96[b]	55.85[c]	2.266	0.001

[a,b,c]Means in the same row with different superscripts differ significantly (p<0.05).[1]CF-1: concentrate feed containing 15.5% crude protein; [2]CF-2: concentrate feed containing 16.5% crude protein; [3]CF-3: concentrate feed containing 17.0% crude protein; [4]SEM: standard error of mean; [5]DM: dry matter; [6]CP: crude protein.

fiber (ADF) contents of the feed (Rustomo et al., 2006), and the rumen pH is reduced by the VFAs, lactic acid, and organic acids produced during fermentation (NRC, 2016). The small differences in pH between the treatment groups at different incubation times in this study may be related to the similar levels of ADF content between the treatment groups (Table 1). RDP contents are known to be associated with rumen ammonia concentrations (Colmenero and Broderick, 2006; Wiese et al., 2003), which may be due to the high inclusion ratios of soybean meal, cottonseed meal, and lupin, which are known to be high in RUP, in the CF-III in this study. Addition of heat-treated soybean meal to diets has been shown to increase nitrogen utilization and decrease ammonia concentrations (Rafiee-Yarandi et al., 2016), and feeding heat-treated cottonseed meal has been shown to decrease rumen CP disappearance rate and increase RUP content (Hahm et al., 2013).

VFAs produced in the rumen are used as the final metabolic products of microbial fermentation as an energy source for ruminants. The production of VFAs is influenced by the levels of feed DM or CP that can be degraded in the rumen (NRC, 2016). In this study, the increase in the ratio of protein ingredients in the CF was considered the cause of the increased production of VFAs, as it increased the level of DM or CP that rumen microorganisms could metabolize. In particular, the increase in propionate concentration in the in vitro rumen fluid is thought to be related to an increase in the degradation rate of energy (starch) in the CF. Rumen concentrations of acetate, propionate, and butyrate increase in proportion to the DM degradability of feed in the rumen (Kim et al., 2014), and Miyaji et al. (2012) reported that the addition of lupin increased the concentration of VFAs in the rumen. Therefore, an increase in the CP content including the ratio of protein ingredients in the CF may decrease ruminal ammonia production by reducing the proportion of RDP. However, this was not expected to have a negative impact on microbial growth. Furthermore, increasing the rumen availability of nutrients (e.g., starch) other than CP may positively affect energy metabolism by increasing the production of VFAs.

Growth performance

Table 4 shows the effect CP content of CF on growth performance of late-fattening Hanwoo steers. ADG was higher in the treatment group than in the control group, although the difference was not significant. Roughage intake was higher in the treatment group than in the control group (p<0.05). There were no significant differences in DMI or FCR between the control and treatment groups.

The late-fattening stage is a period of substantial intramuscular fat deposition during which general body growth typically decreases and fat synthesis increases (KFSKC). There was no significant difference in ADG between the treatment groups based on the CP content including the ratio of protein ingredients in the CF. This can be attributed to the physiological characteristics to the late-fattening stage, where the influence of protein is less pronounced than that of the growing stage. Kim et al. (2012) reported similar trends, indicating that increasing dietary CP content did not significantly affect the ADG of late-fattening Hanwoo steers, which is consistent with the results of this study. DMI is closely related to feed digestibility (Um and Park, 2022), and the increased DMI in the treatment groups was partially associated with improved feed digestibility due to the use of soybean meal, cottonseed, and lupin flake.

Plasma metabolites

Table 5 shows the effect CP content of CF on plasma metabolites concentrations of late-fattening Hanwoo steers. Plasma glucose concentration was higher in the control group than in the

Table 3. Effect of crude protein content in concentrate feed on ruminal fermentation characteristics.

Items	Time (hr)	CF-I[1]		CF-III[3]	SEM[4]	*P*-value
pH	0	6.61	6.64	6.65	0.012	0.326
	3	6.63	6.62	6.58	0.025	0.208
	6	5.77	5.99	5.75	0.061	0.201
	9	4.76	4.93	4.92	0.045	0.118
	12	4.51	4.59	4.59	0.025	0.118
	24	4.87	4.88	4.84	0.044	0.720
	48	4.75	4.74	4.77	0.007	0.865
NH_3-N (mg/dl)	0	3.34	3.37	3.54	0.067	0.426
	3	4.06	3.82	3.82	0.085	0.410
	6	4.09	4.35	4.07	0.075	0.204
	9	4.19^{ab}	4.49^{a}	3.61^{b}	0.169	0.038
	12	4.26	4.58	4.13	0.101	0.167
	24	4.34^{a}	4.57^{a}	3.52^{b}	0.206	0.032
	48	4.15	4.59	4.03	0.131	0.154
Acetate (mg/dl)	0	99.1	112.9	119.5	5.999	0.368
	3	114.4	149.1	161.5	9.775	0.072
	6	121.5	163.6	166.2	11.055	0.137
	9	161.3	170.1	176.3	10.843	0.870
	12	172.0	177.9	186.7	10.037	0.854
	24	188.2	187.8	208.3	13.582	0.806
	48	199.4	224.6	279.7	16.036	0.057
Propionate (mg/dl)	0	79.0	81.6	95.6	4.749	0.295
	3	97.3	104.4	112.1	6.640	0.690
	6	111.1	119.3	133.6	9.023	0.616
	9	123.4	133.9	148.9	9.690	0.585
	12	153.9	162.1	224.1	19.396	0.253
	24	197.9	245.6	269.0	15.527	0.117
	48	275.1^{b}	356.1^{a}	388.9^{a}	20.257	0.010
Butyrate (mg/dl)	0	33.1	29.9	25.8	2.425	0.493
	3	33.2	32.3	30.2	2.133	0.859
	6	34.2	35.6	38.9	2.369	0.735
	9	36.5	38.6	39.5	3.311	0.941
	12	41.1	83.2	88.8	13.541	0.277
	24	109.9	168.1	133.9	13.199	0.156
	48	168.9	203.3	223.1	14.277	0.279
Total-VFAs (mg/dl)	0	211.1	224.4	241.0	6.954	0.181
	3	244.9	285.8	303.7	13.219	0.134
	6	266.8	318.5	338.7	17.574	0.197
	9	321.2	342.6	364.7	17.809	0.637
	12	367.0	423.3	499.7	31.453	0.194
	24	496.0^{b}	601.5^{a}	611.2^{a}	23.818	0.034
	48	643.4^{c}	784.0^{b}	891.7^{a}	41.489	0.004

[a.b.c]Means in the same row with different superscripts differ significantly ($p<0.05$).
[1]CF-1: concentrate feed containing 15.5% crude protein; [2]CF-2: concentrate feed containing 16.5% crude protein; [3]CF-3: concentrate feed containing 17.0% crude protein; [4]SEM: standard error of mean.

Table 4. Effect of crude protein content in concentrate feed on growth performance of late-fattening Hanwoo steers

Items	Control[1]	Treatment[2]	SEM[3]	P-value
Body weight at 22 month of age (kg)	619.5	619.1	11.8	0.988
Body weight at 28 month of age (kg)	745.6	760.9	13.2	0.564
Average daily gain (kg/d)	0.75	0.81	0.034	0.296
Intakes (DM kg/steer/d)				
Concentrate feed	9.44	9.42	0.092	0.936
Roughage	1.52[b]	1.92[a]	0.092	0.023
DMI[4]	10.96	11.35	0.149	0.189
Feed conversion ratio	14.56	14.01	0.293	0.523

[a,b]Means in the same row with different superscripts differ significantly (p<0.05).
[1]Control: concentrate feed containing 15.5% crude protein; [2]Treatment: concentrate feed with 16.5% crude protein; [3]SEM: standard error of mean; [4]DMI: dry matter intake.

Table 5. Effect of crude protein content in concentrate feed on plasma metabolite concentrations of late-fattening Hanwoo steers

Items	Control[1]	Treatment[2]	SEM[3]	*P*-value
Glucose (mg/dl)	100.4[a]	82.1[b]	3.511	0.003
NEFA[4] (uEq/L)	251.3	197.7	24.00	0.167
BUN[5] (mg/L)	18.6	19.7	0.708	0.472
Albumin (g/dl)	4.1	4.3	0.060	0.108
Total bilirubin (mg/dl)	0.2	0.1	0.019	0.481
Total protein (g/dl)	8.1[b]	8.8[a]	0.164	0.023
Creatinine (mg/dl)	1.5	1.5	0.073	0.556
Cholesterol (mg/dl)	208.3	199.4	11.90	0.717
Triglycerides (mg/dl)	23.3	25.6	2.501	0.658
AST[6] (U/L)	82.9	89.9	7.970	0.362
ALT[7] (U/L)	27.6	31.2	1.240	0.136
GGT[8] (U/L)	31.8	33.6	5.569	0.603
Inorganic phosphorus (mg/dl)	7.1	7.7	0.171	0.098
Calcium (mg/dl)	9.7	10.0	0.073	0.055
Magnesium (mg/dl)	2.5	2.6	0.055	0.383

[a,b]Means in the same row with different superscripts differ significantly (p<0.05).
[1]Control: concentrate feed containing 15.5% crude protein; [2]Treatment: concentrate feed with 16.5% crude protein; [3]SEM: standard error of mean; [4]NEFA: non esterified fatty acid; [5]BUN: blood urea nitrogen; [6]AST: aspartate-amino-transferase; [7]ALT: alanine-amino-transaminase; [8]GGT: γ-glutamyl-transferase.

treatment group (p<0.05), and NEFA concentrations numerically higher in the control group compared to the treatment group, although the difference was not statistically significant. Plasma BUN, albumin, total bilirubin, and creatinine concentrations did not differ between treatment groups, but total protein concentration was higher in the treatment group than in the control group (p<0.05). There was no effect of CP contents in CF on plasma cholesterol and triglyceride concentrations. Plasma AST, ALT, and GGT concentrations were numerically higher in the treatment group compared to the control group, although there was no statistical significance between treatments. Inorganic phosphorus, calcium, and magnesium concentrations did not differ between treatment groups.

Plasma glucose concentration was higher in the treatment group compared to the control group, which is similar to the previous studies (Foote et al., 2016) that showed an inverse correlation between DMI and plasma glucose concentration. In general, plasma total protein concentration tended to increase in proportion to CP intake or age (Law et al., 2009; Dhami et al., 2005). Similar to previous studies, in this study, the increase in plasma total protein concentration in the treatment group was likely due to the

Table 6. Effect of crude protein content in concentrate feed on carcass characteristics of late-fattening Hanwoo steers

Items	Control[1]	Treatment[2]	SEM[3]	*P*-value
Yield traits[4]				
Carcass weight (kg)	421.1	423.1	7.695	0.898
Rib eye area (cm^2)	87.2	89.4	2.477	0.653
Back fat thickness (mm)	10.8	9.3	0.621	0.225
Yield index (%)	62.3	63.0	0.219	0.154
Yield grade (A:B:C, steer)	3:9:0	5:7:0	-	-
Yield grade (A: B: C, %)	25:75:0	42:58:0	-	-
Quality traits[5]				
Marbling score	5.67	5.67	0.299	1.000
Meat color	4.92	5.08	0.106	0.436
Fat color	3.00	3.00	-	-
Texture	1.92	2.17	0.168	0.461
Maturity	2.00	2.08	0.043	0.328
Quality grade (1^{++}: 1^{+}: 1: 2: 3, steer)	2:4:6:0:0	2:4:6:0:0	-	-
Quality grade (1^{++}: 1^{+}: 1: 2: 3, %)	17:33:50:0:0	17:33:50:0:0	-	-
Carcass auction price (won/kg)	20,903	21,526	380.8	0.415

[1]Control: concentrate feed containing 15.5% crude protein; [2]Treatment: concentrate feed with 16.5% crude protein; [3]SEM: standard error of mean; [4]Area was measured from *longissmus* muscle taken at 13th rib and back fat thickness was also measured at 13th rib; Yield index was calculated using the following equation: 68.184 - (0.625 × back fat thickness (mm)) + (0.130 × rib eye area (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 color (1 = bright red, 7 = dark red); fat color (1 = creamy white, 7 = yellowish); texture (1 = soft, 3 = firm); quality grades from 3 (low quality) to 1^{++} (very high quality).

increase in CP content of CF. In particular, compared to the control group, the treatment group increased RUP (Table 2) (increasing CP content and high quality protein ingredients), which improved protein availability in the small intestine and affected plasma total protein. Plasma BUN concentration has been reported to increase with increased CP or NPN intake (Zhao, 2024). Similar to the previous study, BUN concentrations tended to be higher in the treatment group compared to the control group in the present study, which may be due to the higher CP content of CF in the treatment group compared to the control group.

Carcass characteristics

Table 6 shows the effect CP content of CF on carcass characteristics of late-fattening Hanwoo steers. The carcass weight and rib eye area tended to be higher in the treatment group than in the control group; however, the difference was not significant. The back fat thickness in the control group and treatment group was measured to be 10.8 and 9.3 mm, respectively, indicating a tendency for thinner back fat in the treatment group compared to the control group. However, the difference was not statistically significant. There were no significant differences in marbling score, meat color, fat color, texture, or maturity between the control and treatment groups. The treatment group had a high A-grade appearance and a high auction price. Jeon et al, (2021) reported that high level dietary crude protein decreased the backfat thickness of finishing Hanwoo cattle. As with previous research findings, the difference in dietary CP supply had a minimal impact on the increase in back fat thickness in this study. This can be attributed to the fact that there were no differences in energy (TDN) or DMI levels across the treatment groups. Ahn et al, (2019) reported that as the TDN content of CF increased in late-fattening steers, both back fat thickness and subcutaneous fat thickness increased. Chemical composition (e.g., fat content) determines beef quality (Muchenje et al., 2009). Consistent with the results of this study, previous studies (Vicenti et al., 2009; Jeon et al., 2021) have indicated that the CP content including the ratio of protein ingredients in CF does not alter the chemical composition of Hanwoo beef. Based on these findings, it can be inferred that improving the quality of CP through an increase in the CP content including the ratio of protein ingredients (e.g., soybean meal, cottonseed, and lupin flake) in CF did not contribute to improvements in beef quality of late-fattening Hanwoo steers.

Conclusion

In this study, the CP content in CF influenced ruminal CP disappearance rates, VFAs concentrations, and plasma metabolite (glucose and total protein) concentrations; however, the CP content in CF had little effect on growth performance and carcass grades in late-fattening Hanwoo steers. Therefore, we suggest increasing CP content alone, without considering the energy content of CF, appears unwarranted given the environmental implications and productivity outcomes for late-fattening Hanwoo steers.

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Conflicts of interest

The authors declare no conflicts of interest.

Data availability

All the data used in this manuscript is available on request to the corresponding author.

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