

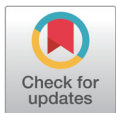
# High-level dietary crude protein decreased backfat thickness and increased carcass yield score in finishing Hanwoo beef cattle (*Bos taurus coreanae*)

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## Abstract

Recently, a high level of dietary crude protein (CP) has become of interest as a possible practice to improve the carcass quality of beef cattle, and its level has been increasing in the field. However, there is little scientific evidence that supports this. This study was conducted to test whether a high dietary CP level would improve growth performance, body metabolism, and carcass traits in Hanwoo beef cattle. A total of 32 Hanwoo finishing beef cattle (18 multiparous cows, six heifers, and eight steers) participated in a 12-weeks feeding trial. Two kinds of total mixed rations were prepared to contain two different CP; 156 g/kg for the control (CON) and 173 g/kg of CP for the treatment (HCP), while maintaining a similar level of metabolizable energy. The experiment was ended when more than half of the steers reached the target body weight (730 kg). Blood was collected at the end of the experiment. After harvesting, the carcass trait was evaluated at the slaughterhouse according to Korean standards. The carcass yield score and grade were also calculated based on revised criteria. Overall, dry matter intake, average daily gain, blood metabolites concentration, and the carcass traits, except for backfat thickness and the yield score, did not differ between the treatments. The HCP had lower backfat thickness than those of CON. There was no difference in the carcass yield grade, but the yield score was higher in the HCP treatment. According to the newly revised carcass grading criteria, both yield score and grade were higher in HCP than in CON. Increasing CP supply decreased the carcass's backfat thickness without altering growth performance and body metabolism, resulting in improved yield score and grade. Therefore, feeding a high CP diet may be beneficial in the farm income, although it may also increase feed cost and nitrogen excretion to the environment.

**Keywords:** Hanwoo, Finishing beef cattle, Crude protein, Growth performance, Blood metabolite, Carcass characteristics

**Competing interests**

No potential conflict of interest relevant to this article was reported.

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**Availability of data and material**

Upon reasonable request, the datasets of this study can be available from the corresponding author.

**Authors' contributions**

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**Ethics approval and consent to participate**

This study was conducted at the Center for Animal Science Research, Chungnam National University, Korea. Animal use and the protocols for this experiment were reviewed and approved by the Chungnam National University Animal Research Ethics Committee (CNU-00755).

## INTRODUCTION

The supply of a sufficient amount of protein is critical for beef cattle production. Dietary protein maintains normal physiological functions, such as musculoskeletal maintenance and immune cell and enzyme production [1]. A sufficient dietary protein supply helps to maintain the adequate level of digestive enzymes and rumen microbes required to increase feed utilization and nutrient absorption in beef cattle [2,3]. Nevertheless, in the past, the importance of dietary protein level during the fattening period has often been ignored since the rate of empty body protein gain decreases, while that of fat gain increases as an animal gets closed to mature body weight (BW) [4]. Recently, however, beef cattle's mature BW has increased due to continuous genetic improvement and there has been a growing demand to increase the dietary protein level in beef cattle [5,6].

Several studies have shown that feeding a high protein diet to beef cattle has positive effects on growth performance and carcass traits. Owens and Gardner [7] reported that a high crude protein (CP) diet (13% CP) for beef cattle could lead to a better carcass yield and marbling score than a low CP diet (11% CP). Lee *et al.* [8] reported that average daily gain (ADG) tended to be higher in the Hanwoo beef cows fed a high protein diet (14.3% and 12.7% of CP at early fattening and finishing, respectively) compared with the control (12.4% of CP throughout the fattening stage). In Jeong *et al.* [6], a high CP diet group (14% CP) had a higher marbling score than the control diet (12% CP). Kim *et al.* [9] also showed an improvement in the marbling score in Hanwoo steers with a high CP feeding program (16, 15, and 14% CP during growing, fattening, and finishing periods, respectively) compared with the control (14, 13, and 11% CP during growing, fattening, and finishing periods, respectively). Subsequently, a high level of dietary protein has become of interest to improve the carcass quality of beef cattle, and its level has been increasing in the field.

On the other hands, some other studies were against increasing CP beyond a necessary level. They suggested an excessive increase in the dietary protein level does not increase animal performance or efficiency, but rather incurs an energy cost for detoxifying excess ammonia [10]. Gleghorn *et al.* [11] reported quadratic effect on ADG and dry matter intake (DMI) and no difference for the gain:feed ratio with increased dietary CP contents. McBride *et al.* [12] indicated that as dietary CP concentration increased, the N retention efficiency decreased. Protein rich ingredients are usually expensive, so it may be an inefficient feeding strategy if there is no performance enhancement. In addition to the efficiency aspect, unused protein in the body is excreted as manure, which may increase environmental pollution [13–15]. In this regard, there is a need to know the upper level of dietary protein that positively affects growth performance and carcass traits. However, to the best of our knowledge, there is no study on how much to increase the level of dietary protein in Hanwoo beef cattle.

Therefore, this study aimed to test whether a dietary CP level higher than a commercial diet would improve growth performance, body metabolism, and carcass traits in Hanwoo beef cattle. We prepared two kinds of total mixed ration (TMR), which provided similar dietary energy but had different dietary protein levels. Since there is a possibility that the high dietary CP level may differ by gender and age due to differences in body composition, the experiment was performed on beef cows, heifers, and steers, and their growth performance, blood metabolites, and carcass characteristics were analyzed.

## MATERIALS AND METHODS

This study was conducted at the Center for Animal Science Research, Chungnam National University, Korea. Animal use and the protocols for this experiment were reviewed and approved by

the Chungnam National University Animal Research Ethics Committee (CNU-00755).

### Experimental design, animals, and diets

A total of 32 Hanwoo beef cattle participated in this three-month (12 weeks) feeding trial. There were 18 multiparous cows ( $663 \pm 58.6$  kg,  $49 \pm 0.6$  months old), six heifers ( $555 \pm 45.8$  kg,  $26 \pm 0.5$  months old), and eight steers ( $649 \pm 34.7$  kg,  $26 \pm 0.3$  months old). The cattle were distributed in a completely randomized block design according to gender and BW. Two cattle of the same gender and similar BW were grouped and housed in pen. Each pen (5 m  $\times$  5 m) was equipped with one feed bin, which automatically measured individual feed intake by recognizing each animal using the radio-frequency identification tag attached to them (Dawoon, Incheon, Korea).

Two different TMR were prepared for this experiment. The forage sources and amounts were the same in the two TMR. The control TMR (CON) was mixed with a commercial concentrate mix containing 168 g CP/kg dry matter (DM). The high CP TMR (HCP) was prepared with a concentrate mix formulated to contain 183 g CP/kg DM and the same level of metabolizable energy (ME) content as the control. The feed composition and the analyzed chemical composition of the experimental TMR are given in Tables 1 and 2, respectively. The final CP level of CON (156 g/kg DM) was similar to that of a general high protein diet [16], and the final CP content of HCP (173 g/kg DM) was 10% higher than that of CON. Within each block, cattle were randomly allocated to one of the two TMR treatments. The cattle were fed twice daily at 09:00 h and 18:00 h. The TMR, drinking water, and mineral block were freely accessible to the animals throughout the experiment.

**Table 1.** Diet formulation (g/kg DM or as stated) of the experimental diets

Item	Treatments <sup>1)</sup>	
	Control	HCP
Ingredients		
Tall fescue	62	62
Ryegrass straw	62	62
Corn (flaked)	225	222
Corn (ground)	58	26
Wheat (ground)	195	202
Corn gluten feed	192	170
DDGS	41	94
Soy hulls	84	83
Whole cottonseed	28	27
Extruded linseed	5	5
Palm oil	2	3
Molasses	22	22
CMS	10	0
Urea	0	8
NH <sub>4</sub> Cl	2	2
Limestone	8	10
Salt	2	2
Vitamin and mineral mix <sup>2)</sup>	2	1

<sup>1)</sup>Control, containing 156 g/kg DM of CP; HCP, high level of crude protein, containing 173 g/kg DM of CP.

<sup>2)</sup>33,330,000 IU/kg vitamin A, 40,000,000 IU/kg vitamin D, 20.86 IU/kg vitamin E, 20 mg/kg Cu, 90 mg/kg Mn, 100 mg/kg Zn, 250 mg/kg Fe, 0.4 mg/kg I, and 0.4 mg/kg Se.

DDGS, dried distillers grain with solubles; CMS, condensed molasses fermentation solubles.

**Table 2.** Analyzed chemical composition (g/kg DM or as stated) of the experimental diets

Item	Treatments <sup>1)</sup>	
	Control	HCP
DM (g/kg as fed)	898	911
OM	940	943
CP	156	173
SOLP	58	67
NDICP	17	18
ADICP	10	12
aNDF	338	353
ADF	162	188
ADL	30	35
Ether extract	44	55
Ash	60	57
Ca	8	9
P	5	5
K	11	11
Na	3	3
Cl	5	5
S	3	3
TDN	747	753
ME (MJ/kg DM)	11.2	10.8
NE <sub>m</sub> (MJ/kg DM)	7.4	7.5
NE <sub>g</sub> (MJ/kg DM)	4.7	4.8
Total carbohydrates	740	715
NFC	419	381
Carbohydrate fractions (g/kg carbohydrate)		
CA	63	63
CB1	430	393
CB2	73	76
CB3	336	350
CC	99	117
Protein fractions (g/kg CP)		
PA+B1	373	387
PB2	520	510
PB3	46	34
PC	61	70

<sup>1)</sup>Control, containing 156 g/kg DM of CP; HCP, high level of crude protein, containing 173 g/kg DM of CP.

DM, dry matter; OM, organic matter; CP, crude protein; SOLP, soluble CP; NDICP, neutral detergent insoluble CP; ADICP, acid detergent insoluble CP; aNDF, neutral detergent fiber analyzed using a heat-stable amylase and expressed inclusive of residual ash; ADF, acid detergent fiber; ADL, acid detergent lignin; TDN, total digestible nutrients; NE<sub>m</sub>, net energy for maintenance; NE<sub>g</sub>, net energy for growth; NFC, non-fiber carbohydrate; CA, carbohydrate A fraction, ethanol soluble carbohydrates; CB1, carbohydrate B1 fraction, starch; CB2, carbohydrate B2 fraction, soluble fiber; CB3, carbohydrate B3 fraction, available insoluble fiber; CC, carbohydrate C fraction, unavailable carbohydrate; PA+B1, protein A and B1 fractions, soluble CP; PB2, protein B2 fraction, intermediate degradable CP; PB3, protein B3 fraction, slowly degradable fiber-bound CP; PC, protein C fraction, unavailable CP.

### Measurements and chemical analysis

Daily DMI was measured throughout the 12-week feeding trial. Every four weeks, daily DMI with 2.5 times the SD above or below the mean were treated as outliers and omitted. Daily DMI were averaged over the 12 weeks. The BW was measured every four weeks before morning feeding.

The feed samples, collected every two or three weeks, were pooled and dried at 60°C for 96 h. They were then ground through a cyclone mill (Foss, Hillerød, Denmark) fitted with a 1 mm screen before chemical analysis. The nutrient composition of the samples was analyzed at Cumberland Valley Analytical Services (Waynesboro, PA, USA). The details of the methods used to analyze the samples' nutrient contents were the same as described in Jeon et al. [17]. The dietary total digestible nutrients (TDN) was calculated according to NRC [18]. The digestible energy (DE) and ME contents (Mcal/kg) were assumed to be 0.004409 times TDN (g/kg) and 0.82 times DE, respectively, based on NASEM [4].

### Carcass characteristics and blood metabolites

The experiment was ended when more than half of the steers reached the target body weight (730 kg). The beef cattle that reached 730 kg were transferred to a slaughterhouse (Nonghyup livestock joint market Eumseong center, Chungcheongbuk-do, Korea) in groups. Two months after the feeding trial was done, those that did not reach the target BW were transferred to the slaughterhouse regardless of their BW, including 17 cows, five heifers, and one steer. There was no difference in the mean experiment period (30 weeks) and the animal numbers of each gender between the treatment groups. At the slaughterhouse, the shrunk BW (SBW) of the cattle was also measured after 24 h fasting before slaughtering (i.e., the final BW in Table 5), which was different from the full BW on the last day of the feeding trial (i.e., the final BW in Table 3). Carcass characteristics were determined by an officer in the Korea Institute for Animal Products Quality Evaluation according to the detailed criteria for judging livestock product grades established by the Ministry of Agriculture, Food and Rural Affairs in Korea. The judging guidelines for livestock product grades changed after the judgment had been made [19]. Thus, the yield score and yield grade based on the current standards were also determined manually.

The blood of all cattle was collected a day before the first group was transferred to the slaughterhouse. Approximately 20 mL of blood was taken from each cattle's jugular vein and collected into a vacutainer serum tube containing a clot activator (BD Vacutainer Systems, BD, Franklin Lakes, NJ, USA). The serum tubes were placed on ice and then immediately transferred

**Table 3.** Effects of dietary crude protein content on intake and growth performance of Hanwoo finishers

Item	Diet <sup>1)</sup>		SEM	Gender			SEM	p-value		
	Control	HCP		Cow	Heifer	Steer		Diet	Gender	Interaction
BW (kg)										
Initial BW	628.5	616.4	14.89	662.9 <sup>a</sup>	555.2 <sup>b</sup>	649.3 <sup>a</sup>	21.9	0.57	< 0.01	0.64
Final BW	679.7	669.6	15.50	701.4 <sup>a</sup>	622.5 <sup>b</sup>	700.0 <sup>a</sup>	22.8	0.65	0.02	0.85
ADG (g/d)	538.5	558.5	50.65	406.2 <sup>b</sup>	705.3 <sup>a</sup>	534.0 <sup>ab</sup>	89.3	0.78	0.02	0.41
DMI (kg/d)	9.1	8.5	0.28	8.9 <sup>a</sup>	10.1 <sup>a</sup>	7.4 <sup>b</sup>	0.5	0.17	< 0.01	0.47
CPI (kg/d)	1.4	1.5	0.05	1.5 <sup>a</sup>	1.7 <sup>a</sup>	1.2 <sup>b</sup>	0.11	0.38	< 0.01	0.50
MEI (Mcal/d)	2.3	2.3	0.09	2.4	2.5	2.0	0.16	0.70	0.01	0.40
FCR	1.8	1.9	0.29	2.6	1.5	1.6	0.5	0.87	0.04	0.70

<sup>1)</sup>Control, containing 156 g/kg DM of CP; HCP, high level of crude protein, containing 173 g/kg DM of CP.

<sup>a,b</sup>Means that do not significantly differ with common superscripts within treatments ( $p < 0.05$ ).

BW, body weight; ADG, average daily gain; DMI, dry matter intake; CPI, crude protein intake; MEI, metabolizable energy intake; FCR, feed conversion ratio.

to the analytical laboratory to analyze the blood metabolites. The serum was analyzed for glucose, total cholesterol, triglycerides, blood urea nitrogen (BUN), creatinine, glutamic oxaloacetic transaminase (GOT), glutamate pyruvate transaminase (GPT), calcium (Ca), inorganic phosphate (IP), magnesium (Mg), albumin, and total protein using kits purchased from Wako Pure Chemical Industries (Osaka, Japan) and a clinical auto-analyzer (Toshiba Accute Biochemical Analyzer-TBA-40FR, Toshiba Medical Instruments, Tokyo, Japan).

### Statistical analysis

The experiment was conducted using a completely randomized block design, and the data were analyzed using the GLM procedure of SAS (SAS Institute, Carey, NC, USA) [20] according to the following linear model:

$$y_{ijk} = \mu + \tau_i + \rho_j + (\tau\rho)_{ij} + e_{ijk}$$

Where  $y_{ijk}$  is  $k^{\text{th}}$  observation in  $i^{\text{th}}$  treatment and the  $j^{\text{th}}$  gender,  $\mu$  is the overall mean,  $\tau_i$  is the fixed effect of  $i^{\text{th}}$  treatment ( $i=1$  to  $2$ ),  $\rho_j$  is the fixed effect of the  $j^{\text{th}}$  gender ( $j=1$  to  $3$ ),  $(\tau\rho)_{ij}$  is the fixed effect of the interaction between  $i^{\text{th}}$  treatment and  $j^{\text{th}}$  gender, and  $e_{ijk}$  is the unexplained random effect on  $k^{\text{th}}$  observation in  $i^{\text{th}}$  treatment and  $j^{\text{th}}$  gender.

Differences between the treatments were also compared with Tukey's test when there was a significant overall treatment effect [21]. Statistical significance was declared at  $p < 0.05$  and a trend was discussed at  $0.05 \leq p < 0.1$ .

## RESULTS AND DISCUSSION

Feeding a sufficient amount of dietary protein during the fattening period could help feed utilization and nutrient absorption, thereby producing high-quality beef cattle [2,3]. Based on this belief, the commercial diet's dietary CP level for beef cattle has been increasing. However, studies on how much to increase the protein level are scarce. Therefore, the present study aimed to investigate whether a higher CP level than a conventional diet could improve the growth performance, body metabolism, and carcass traits in finishing beef cattle.

### Intake behavior and growth performance

DMI, daily CP intake (CPI), and ME intake (MEI) did not differ by the dietary CP levels (Table 3). There is controversy over whether dietary CP content changes intake levels in cattle. Several studies with feedlot cattle showed no difference in feed intake with increasing CP content in the feed. In the Gleghorn et al. [11], who fed low-, standard-, and high-level CP diets to finishing cattle, there was no difference in DMI among the treatments. Providing the same energy level and different CP levels (12% and 14%) to the finishing steers, there was no difference between treatments [6]. In the pre- and post-partum dairy cattle, there was no difference in DMI when fed 16% or 19% CP contained diet [22]. There were no differences in the finishing beef cattle according to dietary CP levels (13.2% to 18.6%, 13.7% to 20.2%) by varying the dried distiller's grain with solubles (DDGS) in the feed [23,24]. On the contrary, a few studies showed an increase in feed intake by increasing dietary CP level. When CP content was increased from 15.1% to 18.4%, feed intake increased linearly in the lactating cow [25]. DMI increased linearly with dietary CP content (13.6% to 32.7%) by increasing the diet's DDGS content during the 130 days before slaughter [26]. Interestingly, several studies also reported a decrease in feed intake as the dietary CP level increased. In lactating cattle, DMI decreased linearly as the dietary CP risen from 15.6% to 18.0% [27].

Also, in steers, a high protein diet increased intake, but feed intake decreased with a further protein increase [28]. Oltjen et al. [29] suggested that a supply of protein that exceeds the animal's ability to convert ammonia into urea caused a decrease in feed intake. In the current study, both treatments fed high dietary protein, but it is presumed that the CP content was not high enough to reduce intake.

According to the diet treatment, there was no significant difference in the final BW, ADG, and feed conversion ratio (FCR), even though growth performance differed by gender as expected (Table 3). These results were consistent with other studies. In finishing cattle, the dietary protein level did not affect ADG and FCR [30,31]. In the study in which CP content was differed by different DDGS contents, there was no difference in ADG according to CP content [26]. There was also no effect on ADG and feed efficiency according to the dietary CP level in the growing-finishing beef cattle [32]. According to the dietary protein level, there was no difference in the BW gain in lactating dairy cows [25]. In the study of Buckner et al. [24], there was no difference in feed efficiency, but ADG increased until 14.4% of CP and then decreased (14.4% to 18.6%). They argued that an increase in dietary CP promoted growth as long as energy supply was not limited, but the growth rate was reversed as the amount of available protein exceeded available energy since the energy that is required to discharge excessive nitrogen. In the present study, however, additional dietary CP levels did not reduce the cattle's growth rate. The dietary CP levels might not be high enough to retard the cattle's growth although both were high. Initial BW was the highest in adult cows, and steer had a higher weight than heifers despite the same age. At the end of the experiment, cows and steers showed similar BW, while heifers had lower weight than the other two genders ( $p < 0.05$ ).

### Blood metabolites

No statistically significant difference was seen in any of the energy and protein metabolism indicators in the blood (Table 4). The most common observation with an increase in dietary CP is elevated concentrations of total protein, BUN, or both in the blood [25,33]. However, we did not observe this phenomenon in this study, although BUN concentration was numerically higher in the HCP group than in CON (19.5 mg/dL vs. 17.8 mg/dL, Table 4). This is probably because the actual CPI between the two treatments did not significantly differ because of numerical reduction in feed intake in the HCP group (Table 3).

Total protein concentration in the current study was similar to the typical range of values in Hanwoo, 6.5–7.5 g/dL, reported by Cho et al. [34]. However, the concentration of BUN in this study was higher than a typical range of values in Hanwoo, 8–14 mg/dL [34]. This was expected because BUN concentration is related to the protein-to-energy ratio [35], and the dietary protein-to-energy ratio in this study was higher than in typical situations. This study's protein-to-energy ratios were 58 g/Mcal and 67 g/Mcal for CON and HCP, respectively, which were higher than a typical value (42–46 g/Mcal) in Hanwoo [36]. Nonetheless, it is not uncommon to have a BUN concentration higher than 18 mg/dL in Hanwoo beef cattle [37].

### Carcass characteristics

The backfat thickness was lower in HCP than in CON, although carcass weight, rib eye area, marbling score, and the meat and fat color scores did not significantly differ (Table 5). Due to a lower backfat thickness, the yield score was higher in HCP than in CON ( $p < 0.05$ ), but the yield score did not increase enough to improve the yield grade ( $p > 0.05$ ). However, when we apply the recently revised grading system of beef carcasses, which is the most sensitive to backfat thickness [38], both the yield score and grade improved in HCP than CON ( $p < 0.05$ ). The underlying

**Table 4.** Effects of dietary crude protein contents on blood metabolites

Item	Diet <sup>1)</sup>		SEM	Gender			SEM	p-value		
	Control	HCP		Cow	Heifer	Steer		Diet	Gender	Interaction
Glucose (mg/dL)	65.62	83.28	9.054	62.76	89.17	71.42	13.307	0.18	0.24	0.06
Total cholesterol (mg/dL)	166.99	159.39	9.433	133.05 <sup>b</sup>	188.83 <sup>a</sup>	167.70 <sup>ab</sup>	13.864	0.57	< 0.01	0.31
Triglycerides (mg/dL)	16.26	20.78	2.181	20.68 <sup>a</sup>	23.33 <sup>a</sup>	11.56 <sup>b</sup>	3.206	0.15	0.02	0.25
BUN (mg/dL)	17.80	19.47	0.803	17.34 <sup>b</sup>	21.80 <sup>a</sup>	16.76 <sup>b</sup>	1.180	0.16	0.01	0.12
Creatinine (mg/dL)	1.35	1.38	0.061	1.28	1.44	1.38	0.090	0.77	0.26	0.62
GOT (IU/L)	116.98	123.89	8.399	122.04	138.92	100.35	12.344	0.57	0.07	0.25
GPT (IU/L)	17.73	17.75	0.749	17.38	18.79	17.03	1.100	0.99	0.45	0.06
Ca (mg/dL)	8.88	8.91	0.271	8.76	9.03	8.89	0.398	0.93	0.83	0.82
IP (mg/dL)	6.19	6.32	0.234	5.52 <sup>b</sup>	6.77 <sup>a</sup>	6.48 <sup>a</sup>	0.344	0.70	< 0.01	0.29
Mg (mg/dL)	2.10	2.08	0.083	1.87 <sup>b</sup>	2.25 <sup>a</sup>	2.15 <sup>ab</sup>	0.122	0.84	0.02	0.58
Albumin (g/dL)	3.13	3.19	0.099	3.10	3.27	3.10	0.145	0.69	0.61	0.70
Total protein (g/dL)	6.46	6.58	0.240	6.69	6.52	6.36	0.353	0.73	0.67	0.69
Total bilirubin (mg/dL)	0.02	0.02	0.009	0.03	0.03	< 0.01	0.014	0.87	0.18	0.29

<sup>1)</sup>Control, containing 156 g/kg DM of CP; HCP, high level of crude protein, contained 173 g/kg DM of CP.

<sup>a,b</sup>Means that do not significantly differ with common superscripts within treatments ( $p < 0.05$ ).

BUN, blood urea nitrogen; GOT, glutamic oxaloacetic transaminase; GPT, glutamate pyruvate transaminase; IP, inorganic phosphate.

**Table 5.** Effects of dietary crude protein content on carcass characteristics of Hanwoo cattle

Item	Diet <sup>1)</sup>		SEM	Gender			SEM	p-value		
	Control	HCP		Cow	Heifer	Steer		Diet	Gender	Interaction
Final SBW (kg)	717.7	707.9	13.75	718.8	698.3	721.3	20.20	0.62	0.64	0.95
Carcass weight (kg)	432.7	421.3	8.70	424.9	421.5	434.5	12.79	0.36	0.70	0.97
Marbling score	61.6	57.4	4.98	58.2	54.3	66.0	7.33	0.55	0.45	0.93
Meat color	5.00	4.93	0.103	5.06	4.83	5.00	0.151	0.61	0.45	0.45
Fat color	3.07	3.16	0.132	3.22	3.00	3.13	0.194	0.66	0.60	0.81
Quality grade	2.26	2.45	0.221	2.44	2.50	2.13	0.325	0.54	0.59	0.88
Back fat (mm)	21.9	17.0	1.49	22.4 <sup>a</sup>	23.5 <sup>a</sup>	12.5 <sup>b</sup>	2.19	0.03	< 0.01	0.79
Rib eye area (cm <sup>2</sup> )	95.8	96.3	2.40	95.3	98.2	94.6	3.53	0.90	0.73	0.16
Yield score <sup>2)</sup>	59.7	63.3	0.97	59.6 <sup>b</sup>	59.3 <sup>b</sup>	65.5 <sup>a</sup>	1.42	0.01	< 0.01	0.69
Yield grade <sup>2)</sup>	2.67	2.37	0.156	2.89 <sup>a</sup>	2.67 <sup>ab</sup>	2.00 <sup>b</sup>	0.230	0.19	< 0.01	0.55
Yield score_2019 <sup>3)</sup>	58.7	60.1	0.38	58.7 <sup>b</sup>	58.8 <sup>b</sup>	60.8 <sup>a</sup>	0.56	0.02	0.01	0.52
Yield grade_2019 <sup>3)</sup>	2.71	2.18	0.147	2.67 <sup>a</sup>	2.67 <sup>ab</sup>	2.00 <sup>b</sup>	0.216	0.02	0.02	0.91

<sup>1)</sup>Control, contained 156 g/kg DM of CP; HCP, high level of crude protein, contained 173 g/kg DM of CP.

<sup>2)</sup>Yield score =  $68.184 - 0.625 \times \text{back fat} + 0.130 \times \text{rib eye area} - 0.024 \times \text{carcass weight} + 3.23$ ; A grade,  $> 67.20$ ; B grade,  $63.30 \sim 67.20$ ; C grade,  $< 63.30$ .

<sup>3)</sup>Based on the revised beef grading system in 2019; Yield score for cow and heifer =  $(6.90137 - 0.9446 \times \text{back fat} + 0.31805 \times \text{rib eye area} + 0.54952 \times \text{carcass weight}) / (\text{carcass weight} \times 100)$ ; Yield score for steer =  $(11.06398 - 1.25149 \times \text{back fat} + 0.28293 \times \text{rib eye area} + 0.56781 \times \text{carcass weight}) / (\text{carcass weight} \times 100)$ ; A grade,  $> 61.83$  (cow and heifer) and  $> 62.52$  (steer); B grade,  $59.70 \sim 61.83$  (cow and heifer) and  $60.40 \sim 62.52$  (steer); C grade,  $< 59.70$  (cow and heifer) and  $< 60.40$  (steer).

<sup>a,b</sup>Means that do not significantly differ with common superscripts within treatments ( $p < 0.05$ ).

SBW, shrunk body weight; Quality grade, numerical conversion of quality grades (1, 2, 3, 4, and 5 correspond to the 1++, 1+, 1, 2, and 3 grades, respectively); Yield grade, numerical conversion of yield grades (1, 2, and 3 correspond to the A, B, and C grades, respectively).

mechanism of reducing backfat thickness due to increased dietary CP needs to be deciphered. In several studies, the backfat thickness was not affected by the dietary protein level [11,30]. In a study in which the dietary protein content was varied from 14% to 20% by increasing the inclusion rate of DDGS in the diet from 0% to 75%, there was no difference in backfat thickness [39]. Some studies even showed that the higher CP could increase the backfat thickness. The review by Galyean



[5] indicated that a diet with 14% CP tended to have thicker backfat than that with 11% CP. On the contrary, some studies reported a thinner backfat due to a higher level of dietary CP. The high protein (14% CP) fed group had thinner backfat compared to the control (12% CP) group in Hanwoo steers [6]. Gibb et al. [26] also showed that the high CP (20.0% CP) diet decreased backfat thickness compared to the low CP (13.6% of CP) diet. In their study, the dietary CP level was increased by increasing the level of DDGS content in the diet as our study. In some cases, DDGS caused thinner backfat thickness due to the DDGS itself or increased the ether extract (EE) contents [26,40,41]. However, the studies that fed iso-nitrous with different DDGS contents to lambs showed no difference in the backfat thickness according to the DDGS level or the EE contents [40,41].

A high protein diet did not increase the degree of marbling in our study, although Kim et al. [9] reported a higher marbling score in the high protein diet than in the control group. It has been argued that an increase in dietary protein level can improve starch digestion and absorption in the small intestine, which results in an increase in the blood insulin and glucose levels [2,42]. In this study, however, the starch level of HCP (281 g/kg DM) was 11.7% lower than that of the control (318 g/kg DM), and thus an improvement in marbling by increasing starch supply did not likely occur.

Overall, feeding HCP for the last three months of the finishing period did not affect DMI and BW gains in Hanwoo beef cattle. However, HCP decreased the backfat thickness significantly, resulting in a higher carcass yield score and yield grade according to the revised beef grading system. Thus, it is possible to expect a better beef grade by providing additional dietary CP than a conventional diet in the finishing period. However, feeding a high protein diet may increase the feeding cost. Besides, if dietary CP increases, the CP not used for the body protein deposition is excreted as feces and urine. Not only does the animal have to spend additional energy for nitrogen excretion, but also nitrogen excretion to the environment is undesirable economically and environmentally. Thus, the economic analysis and assessment of environmental impacts need to be done to evaluate the effects of increasing dietary CP in the finishing beef cattle. Further research is also necessary to elucidate the underlying mechanism by which a high CP diet reduces backfat thickness.

We concluded that increasing the amount of dietary protein did not affect DMI, growth performance, and carcass quality indexes. However, a diet containing higher protein than a conventional one decreased the backfat thickness, resulting in higher carcass yield indexes. Therefore, feeding a high CP diet may be beneficial in the farm income. However, it may not be a sustainable practice since it may increase feed cost and nitrogen excretion to the environment.

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