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ANIMAL

Effects of the crude protein concentration on the growth performance and blood parameters in growing Hanwoo steers (*Bos taurus coreanae*)

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Abstract

The sufficient amount of protein supply is crucial for improving the growth performance of growing beef cattle. In addition, due to the improvement in the genetic potential of the carcass weight of Hanwoo steers, dietary protein requirements may be increased during the rapid growth period. Accordingly, the dietary crude protein (CP) level in growing Hanwoo steers has been increasing in the field. However, little scientific evidence is available in relation to this. Therefore, this study was conducted to test whether a higher dietary CP level than convention would improve the growth performance and body metabolism in growing Hanwoo steers. Fifty growing Hanwoo steers were randomly divided into two groups and fed either a commercial diet (CON) or a higher CP (HCP) concentrate mix, provided with a similar level of dietary energy. Tall fescue hay was provided *ad libitum*. The dietary CP level did not affect growth performance and blood metabolite. Nitrogen intake, predicted nitrogen excretion, and retained nitrogen were higher in the HCP group than in the CON group ($p < 0.01$). Although there was no difference in the nitrogen utilization efficiency, the growth efficiency per retained nitrogen decreased in the HCP group ($p = 0.02$). A higher dietary CP level may increase nitrogen retention in growing Hanwoo steers without improving growth performance, which leads to reduced growth efficiency per retained nitrogen. Furthermore, considering the high price of feed protein and increased nitrogen excretion to the environment, a further increase in the protein level may not be sustainable.

Keywords: blood metabolite, growth performance, Hanwoo, protein, steer

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Introduction

The supply of a sufficient amount of protein is crucial for improving the growth performance in growing beef cattle. Dietary protein is necessary for maintaining normal physiological functions (e.g., musculoskeletal maintenance and enzyme and immune cell production) and growth. Nutrient

Requirements of Beef Cattle (NASEM, 2016) estimates that crude protein (CP) contributes 15.6% of the gain weight when the shrunk body weight is 250 kg. Thus, lack of dietary protein can lead to a decrease in average daily gain (ADG), dry matter intake (DMI), gain to feed ratio, and carcass quality (Galyean, 1996; Hammond, 1997). On the other hand, sufficient dietary protein during the growing phase improves weight gain and carcass measurements, including carcass weight, dressing percentage, and marbling score (Owens and Gardner, 2000). Protein supply also improves weight gain and feed conversion ratio and decreases backfat thickness (Martin et al., 1979; Perry et al., 1983). Protein supply is more important for fast-growing cattle because both animal and rumen microbes require more protein to support rapid growth (Galyean, 1996).

Several studies showed that feeding a high-protein diet to growing cattle improves growth performance. For example, Huntington et al. (2001) indicated that an increase in dietary protein intake increased ADG linearly in the group fed a concentrate-rich diet. In a study by Anderson et al. (1988), there was no difference in carcass weight, but ADG was higher in the group that received the highest protein diet. In Hanwoo, a Korean native breed known for high-quality beef, total weight gain and ADG were also higher in the high CP-fed group than the control group (MAFRA, 2007). Due to the recent improvement in the genetic potential of Hanwoo, there may be an increase in the protein needs in growing Hanwoo steers during the fast-growing stage. Accordingly, a high level of dietary protein is regarded as beneficial, and the dietary CP concentration of the concentrate mix for growing cattle has been increasing in the field.

On the other hand, some other studies were against overfeeding dietary protein. They suggested an oversupply of CP does not improve animal performance or efficiency but rather raises an environmental pollution and energy cost for detoxifying excess ammonia (Kohn et al., 2005; Sinclair et al., 2014). Gleghorn et al. (2004) reported a quadratic effect of protein supplementation on ADG and DMI and no difference in feed efficiency with high CP contents. Increased dietary protein would rather decrease the protein deposition rate (McBride et al., 2003; Schroeder and Titgemeyer, 2008). Broderick (2003) concluded that excessive protein supplements would depress N utilization and are economically unbeneficial. Therefore, there is a need to know the upper level of dietary protein that positively affects the growth performance of growing cattle. However, to the best of our knowledge, there is no study on how much to increase the dietary protein in growing Hanwoo steers.

Therefore, this study was conducted to investigate the effect of high dietary protein on growth performance and body metabolism in growing Hanwoo steers. A 12-week feeding trial was performed with 50 growing Hanwoo steers. The steers were fed a commercial or higher protein concentrate mix, and their growth performance and blood metabolites 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-01129).

Animals, housing, and dietary treatments

A total of 50 Hanwoo growing steers (body weight [BW], 256 ± 22 kg; 9 months old) participated in this three-month feeding trial. Animals, blocked based on their DMI as a percentage of BW during a preliminary feeding, were housed in pens. Each pen (10 m × 10 m) was equipped with one automatic concentrate feed bin and four forage feed bins. The feed bins automatically measured individual feed intake by recognizing each animal using the radio-frequency identification tag attached to them (Dawoon Co., Incheon, Korea).

A commercial concentrate mix containing 195 g·kg^{-1} CP was used for the control group (CON). A higher CP concentrate mix containing 222 g·kg^{-1} CP and the same energy level (iso-caloric) as CON was prepared and fed to the higher CP group (HCP). The feed composition of the concentrate mix is given in Table 1, and the analyzed chemical composition of the experimental concentrate mix and roughage (tall fescue) are shown in Table 2. Randomization was done through the random allocation of the treatments to each pen (Seo et al., 2018). The forage was fed *ad libitum* twice a day at 07:00 h and 17:30 h, and a commercial concentrate mix was provided through an automated concentrate feeder. Each day was divided into four periods of 6 hours; within each period, steers were able to consume up to one-fourth of the amount of daily allowable concentrate mix. If steers did not consume the amount of concentrate mix allowed during each period, they could consume the rest in the next period. The amount of concentrate mix fed was gradually increased from 3 to 5 kg, depending on the BW. The drinking water and mineral block were freely accessible to the animals throughout the experiment.

Table 1. Characteristics of participants (N = 1,046).

Item	Treatment[y]	
	CON	HCP
Corn (flaked)	192	193
Wheat (ground)	99	157
Corn (ground)	8	7
Lupin (flaked)	31	31
Coconut oil meal	56	57
DDGS	-	121
Soybean meal	96	96
Rapeseed meal	30	28
Palm kernel meal	71	57
Corn gluten feed	164	82
Wheat bran	118	69
Beet pulp pellet	20	20
Rice bran	21	-
Cottonseed hull	9	-
Limestone	34	34
Molasses	22	22
MSG-CMS	11	11
Salt	8	8
Sodium bicarbonate	6	6
Vitamin and mineral mix[z]	3	3

DM, dry matter; DDGS, dried distillers' grains with solubles; MSG-CMS, monosodium glutamate-condensed molasses solubles.
[y] CON, control diet contained 195 g·kg^{-1} of crude protein (CP); HCP, higher crude protein diet contained 222 g·kg^{-1} of CP.
[z] Vitamin and mineral mix: 33,330,000 IU·kg^{-1} vitamin A, 40,000,000 IU·kg^{-1} vitamin D, 20.86 IU·kg^{-1} vitamin E, 20 mg·kg^{-1} Cu, 90 mg·kg^{-1} Mn, 100 mg·kg^{-1} Zn, 250 mg·kg^{-1} Fe, 0.4 mg·kg^{-1} I, and 0.4 mg·kg^{-1} Se.

Sample collection and measurements

Daily feed intake was measured throughout the 12-week feeding trial. Every four weeks, daily intakes (i.e., forage and concentrate intakes) with 2.5 times the standard deviation above or below the mean were treated as outliers and omitted. BW was measured every four weeks before morning feeding. The concentrate and roughage were sampled regularly and stored at 4℃ until subsequent chemical analysis.

Table 2. Analyzed chemical composition (g·kg-1 DM or as stated) of the experimental diets.

Item	Treatment[z]		Tall fescue
	CON	HCP	
DM ($g\cdot kg^{-1}$ as fed)	868	867	914
OM	898	901	920
CP	195	222	75
SOLP	62	75	35
NDICP	26	25	8
ADICP	9	11	7
aNDF	295	268	656
ADF	127	128	428
ADL	31	32	56
Ether extract	35	36	11
Ash	102	99	80
Ca	17	16	3
P	7	6	1
K	13	13	24
Na	5	5	1
Cl	9	8	6
S	4	4	1
Mg	4	4	2
TDN	711	722	549
NEm ($MJ\cdot kg\ DM^{-1}$)	6.8	7.0	5.2
NEg ($MJ\cdot kg\ DM^{-1}$)	4.3	4.4	2.8
Total carbohydrates	668	643	834
NFC	403	401	195
Carbohydrate fraction ($g\cdot kg^{-1}$ carbohydrate)			
CA	72	89	86
CB1	400	428	16
CB2	132	108	132
CB3	290	259	615
CC	112	119	162
Protein fraction ($g\cdot kg^{-1}$ CP)			
PA + B1	318	338	467
PB2	548	550	425
PB3	86	63	13
PC	49	49	95

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; NEm, net energy for maintenance; NEg, 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.

[z] CON, control diet contained 195 $g\cdot kg^{-1}$ of CP; HCP, higher crude protein diet contained 222 $g\cdot kg^{-1}$ of CP.

Blood samples were taken from randomly selected 24 steers (12 steers per treatment) on the day before the onset of the trial and the last day of the experiment before measuring BW. Approximately 30 ml of blood was taken from each cattle's jugular vein and stored in a serum tube containing a clot activator (BD Vacutainer Systems, Oxford, UK). The serum tubes were placed on ice and then immediately transported to the analytical laboratory for the blood metabolite. Serum was obtained by centrifugation at 1,300 × g for 15 min at 4℃ and frozen at -80℃ until later analysis.

Nitrogen excretion (urine, fecal, and total) was calculated separately based on the following equations provided by Reed et al. (2015) for steers:

$$\text{Urinary nitrogen excretion } (g \cdot d^{-1}) = 6.80 + 0.405 \times \text{nitrogen intake } (g \cdot d^{-1}) \quad (1)$$
$$\text{Fecal nitrogen excretion } (g \cdot d^{-1}) = 0.506 + 0.352 \times \text{nitrogen intake } (g \cdot d^{-1})$$
$$\text{Total nitrogen excretion } (g \cdot d^{-1}) = 6.91 + 0.759 \times \text{nitrogen intake } (g \cdot d^{-1})$$

Chemical analysis

The feed samples were dried at 60℃ for 96 h and ground through a cyclone mill (Foss, Hillerød, Denmark) fitted with a 1 mm screen before chemical analysis. Nutrient compositions of samples were analyzed at Cumberland Valley Analytical Services Inc. (Waynesboro, PA, USA). The detailed procedure for the chemical analysis and the calculation for nutrient fractionation were followed as described by Jeon et al. (2016).

The serum was analyzed for glucose, total cholesterol, triglycerides, blood urea nitrogen (BUN), aspartate transaminase (AST), alanine transaminase (ALT), calcium (Ca), inorganic phosphate (IP), magnesium (Mg), albumin, creatinine, and total protein, using kits purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan) and a clinical auto-analyzer (Toshiba Accute Biochemical Analyzer-TBA-40FR, Toshiba Medical Instruments, Japan).

Statistical analysis

The experiment was conducted using a completely randomized block design, and the growth performance and nutrient intake data were analyzed using the GLIMMIX procedure of SAS (2015) according to the following linear model:

$$y_{ij} = \mu + \tau_i + b_j + e_{ij} \quad (2)$$

Where y_{ij} is jth observation in ith treatment, μ is the overall mean, τ_i is the fixed effect of ith treatment (i = 1 to 2), b_j is the random effect associated with the jth block (j = 1 to 2), and e_{ij} is the unexplained random effect on jth observation in ith treatment. Statistical significance was declared at $p < 0.05$, and a trend was discussed at $0.05 \leq p < 0.1$.

Similarly, blood data were analyzed using the GLIMMIX procedure of SAS, but initial blood metabolite data were used for covariate adjustment as follows:

$$y_{ij} = \mu + \tau_i + b_j + COV + e_{ij} \quad (3)$$

Where y_{ij} is jth observation in ith treatment, μ is the overall mean, τ_i is the fixed effect of ith treatment (i = 1 to 2), b_j is the random effect associated with the jth block (j = 1 to 2), COV is the covariate based on initial blood metabolite data, and e_{ij} is the unexplained random effect on jth observation in ith treatment. Statistical significance was declared at $p < 0.05$, and a trend was discussed at $0.05 \leq p < 0.1$.

Results

Intake, growth performance, and nitrogen excretion

There was no significant difference in final BW, ADG, DMI, and feed conversion ratio between the two treatments ($p > 0.05$; Table 3). As expected, CP intake was higher in the HCP group than in the CON group owing to a higher CP content in the concentrate mix in HCP ($p < 0.01$). Urinary, fecal, and total nitrogen excretions, calculated according to Reed et al. (2015), were higher in the HCP group than in the CON group ($p < 0.01$). Retained nitrogen (RN), calculated by subtracting the total nitrogen excretion from the nitrogen intake, was also higher in the HCP group than in the CON group ($p < 0.01$). However, the ratio of ADG to RN was higher in the CON group than in the HCP group ($p = 0.02$). The amount of nitrogen excretion per ADG was numerically higher in the HCP group than in the CON group, but it did not differ significantly.

Table 3. Effect of the dietary protein concentration on growth performance and nutrient intake in Hanwoo steers (n = 50).

Item	Treatment[y]		SEM	p-value
	CON	HCP		
Initial BW (kg)	256	256	4.6	0.99
Final BW (kg)	341	344	5.2	0.70
ADG ($g \cdot day^{-1}$)	950	982	26.5	0.40
DMI ($kg \cdot day^{-1}$)				
Concentrate	3.50	3.50	0.027	0.87
Forage	2.81	2.79	0.084	0.88
Total	6.30	6.29	0.102	0.94
CPI ($g \cdot day^{-1}$)				
Concentrate	682	778	5.4	< 0.01
Forage	211	209	6.3	0.86
Total	892	987	10.5	< 0.01
Nitrogen intake ($g \cdot d^{-1}$)	143	158	1.7	< 0.01
Nitrogen excretion ($g \cdot d^{-1}$)[z]				
Urine	64.6	70.7	0.69	< 0.01
Fecal	50.7	56.1	0.59	< 0.01
Total	115.3	126.8	1.28	< 0.01
Retained nitrogen ($g \cdot d^{-1}$)	27.5	31.2	0.42	< 0.01
ADG/RN ($g \cdot g^{-1}$)	34.7	31.6	0.93	0.02
Nitrogen excretion/ADG ($g \cdot g^{-1}$)	0.12	0.13	0.003	0.16
FCR	6.77	6.48	0.188	0.28

BW, body weight; ADG, average daily gain; DMI, dry matter intake; CPI, crude protein intake; RN, retained nitrogen; FCR, feed conversion ratio-DMI (kg)/ADG (kg).

[y] CON, control diet contained 195 $g \cdot kg^{-1}$ of crude protein (CP); HCP, higher crude protein diet contained 222 $g \cdot kg^{-1}$ of CP.

[z] Urine excretion = 6.80 + 0.405 × nitrogen intake, fecal excretion = 0.506 + 0.352 × nitrogen intake, total excretion = 6.91 + 0.759 × nitregen intake (Reed et al., 2015).

Blood metabolite

Among blood metabolites, the levels of glucose, AST, ALT, cholesterol, and triglycerides, known as energy metabolism indicators, did not significantly differ depending on the CP level ($p > 0.05$; Table 4). Blood glucose levels tended to be higher in the HCP group than in the CON group ($p = 0.07$). Total protein, BUN, albumin, and creatinine, known as protein metabolism indicators, levels were not altered by treatment. There was also no significant difference in the blood concentrations of Ca, IP, and Mg, which are related to mineral metabolism. However, IP tended to be higher in the HCP group than in the CON group ($p = 0.08$).

Table 4. Effect of the dietary protein concentration on blood metabolites in Hanwoo steers (n = 24).

Item	Treatment[z]		SEM	p-value
	CON	HCP		
Total protein ($g \cdot dL^{-1}$)	4.88	5.00	0.219	0.70
AST ($IU \cdot L^{-1}$)	76.90	74.30	5.190	0.73
ALT ($IU \cdot L^{-1}$)	27.60	28.00	1.740	0.88
BUN ($mg \cdot dL^{-1}$)	17.10	18.30	0.530	0.14
Ca ($mg \cdot dL^{-1}$)	7.74	7.89	0.286	0.71
IP ($mg \cdot dL^{-1}$)	7.01	7.50	0.185	0.08
Cholesterol ($mg \cdot dL^{-1}$)	92.40	95.50	6.290	0.73
Triglycerides ($mg \cdot dL^{-1}$)	21.10	22.80	1.520	0.43
Glucose ($mg \cdot dL^{-1}$)	68.80	74.00	1.910	0.07
Albumin ($IU \cdot L^{-1}$)	2.76	2.86	0.096	0.45
Creatinine ($mg \cdot dL^{-1}$)	1.18	1.17	0.028	0.74
Mg ($mg \cdot dL^{-1}$)	1.78	1.86	0.070	0.47

AST, aspartate transaminase; ALT, alanine transaminase; BUN, blood urea nitrogen; IP, inorganic phosphate.
[z] CON, control diet contained 195 $g \cdot kg^{-1}$ of crude protein (CP); HCP, higher crude protein diet contained 222 $g \cdot kg^{-1}$ of CP.

Discussion

According to the results of this study, a higher CP concentration in the diet did not affect the total DMI, growth rate, or feed efficiency. Unlike the current study, positive responses due to an increase in dietary protein have often been reported. Feed efficiency tends to increase as the dietary CP concentration increases in an isocaloric diet. When an isocaloric diet was fed to steer, DMI, as well as ADG, increased according to the dietary CP level increased (9.7 - 13.0%) (Tritschler et al., 1984). When bulls in growing-finishing period were fed 10, 12, and 14% CP diets, ADG was increased in 12 and 14% CP treatment groups (Anderson et al., 1988). In growing steer and heifer fed with 11.0, 12.5, and 14.0% CP feed, there was no difference in DMI, but ADG increased linearly (Bailey et al., 2008).

On the other hand, some literature reported no difference in intake and growth performance according to CP level. In particular, the positive impact of increased CP was diminished when the CP level of the control diet was already high. In a study on feeding grass silage with different protein contents, BW gain did not differ by dietary CP levels (11.1 - 15.5%) (Martin et al., 1978). Some studies did not show a difference in ADG regarding the dietary protein level, which contained different urea and distiller by-product levels in the beef cattle (Shain et al., 1998; Gibb et al., 2008). In a study in which the protein feeding level was adjusted by varying the amount of soybean meal added to the grass silage, there was no difference in BW gain according to RDP intake (Steen, 1996). There was no difference in final BW and ADG according to protein levels in the study by Prado et al. (2014), which consisted of 14.6% and 16.8% dietary protein. In our study, the CP level of CON, a commercial diet, was even higher than that of the high CP diets in most previous studies. Thus, no further improvement in growth performance may not be expected by providing additional dietary CP.

An increase in dietary CP level resulted in significantly greater nitrogen excretion into the environment. When CP intake increases, nitrogen excretion through urine and feces increases (Reed et al., 2015). Nitrogen retention was also greater in the HCP group than in the CON group. However, the ratio of ADG to RN was higher in the CON group than in the HCP group. In other words, the growth efficiency per RN was reduced in the HCP group, implying that additional absorption of nitrogen did not result in body mass accumulation. This is consistent with the study by Menezes et al. (2019) that also found no difference in ADG and carcass weight, although NI and RN were higher in the high protein diet group (12.2 and 14.2%) than the low protein diet group (10.3%). Since nitrogen excretion is a cause of environmental pollution, an increase in nitrogen excretion without a difference in productivity, as in this study and previous studies, is not favorable.

Higher CP intake did not affect the blood concentration of most metabolites. Only a tendency of higher blood IP and glucose concentrations was observed in the HCP group. Blood metabolite concentrations were within the normal ranges in Hawoo steers (Kim et al., 2005; MAFRA, 2007; Chung et al., 2015). Feeding a high CP diet often increases BUN, total protein, and albumin concentrations (Rusche et al., 1993; Ndlovu et al., 2007; Law et al., 2009). However, in the present study, additional dietary CP did not increase the serum BUN level mainly because its concentration in the control group was already higher than the normal BUN level (12.3 mg·dL^{-1}) in Hanwoo (Cho et al., 2008). Consistently with our study, there was a study that showed no changes in the blood protein-related metabolites by providing additional dietary CP (Bahrami-Yekdangi et al., 2014).

A tendency of higher blood glucose in the HCP group may simply be due to a higher level of carbohydrate A and B1 fractions, which refer to sugars and starch, respectively (Fox et al., 2004). Another possibility is that an increased dietary protein level may improve starch digestion and absorption from the small intestine and increase blood insulin, as suggested in previous studies (Owens et al., 1986; Pethick et al., 2000; Berger and Pyatt, 2005).

Overall, the results of this study suggest that the additional CP level increase in the conventional concentrate mix for growing Hanwoo steers may not improve productivity and may decrease environmental sustainability. However, because the experiment was conducted over only three months of the growing period, the long-term effects of a high-protein diet on growth performance and carcass characteristics may need to be further investigated.

Conclusion

A higher CP concentrate (222 g CP·kg DM^{-1}) than that in the conventional concentrate mix (195 g CP·kg DM^{-1}) had no significant effect on growth performance or body metabolism in growing Hanwoo steers. On the other hand, the increased CP intake is expected to increase both urinary and fecal nitrogen excretion, which may contribute to environmental pollution. Considering the extra cost for increasing feed protein and nitrogen utilization efficiency, further increasing the CP levels higher than 200 g CP·kg DM^{-1} in the concentrate mix for growing Hanwoo steers might not be economically and environmentally sustainable.

Conflict of Interests

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

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