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Effect of a Simultaneous Increase in Crude Protein and Total Digestible Nutrients Contents on Late Fattening Hanwoo Steers

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ABSTRACT

Background: Shortening the fattening period can reduce production costs and inedible fat, but may simultaneously negatively affect productivity and profitability. We hypothesized that increasing the crude protein and total digestible nutrients (TDN) in the formula feed could address the issues associated with shortened fattening periods without adversely affecting rumen fermentation. This study investigated the effect of a simultaneous increase of crude protein and TDN contents compared to conventional formula feed on growth performance, blood metabolites, carcass characteristics and meat composition of late fattening Hanwoo steers.

Methods: Thirty Hanwoo steers were randomly assigned to one of two dietary groups: control group (16.4% crude protein and 83.6% TDN based on dry matter) and treatment group (17.4% crude protein and 84.6% TDN based on dry matter).

Result: The rumen parameters were similar between the control and treatment groups. Likewise, the effect of additional crude protein and TDN contents in the formula feed showed no significant impact on the growth performance or blood metabolites. The carcass weight and yield index were slightly higher in the treatment group compared to the control, yet these differences were not statistically significant. Furthermore, the dietary treatment did not affect marbling score, meat color, fat color, texture, maturity, pH, surface colors, drip loss, cooking loss, sensory characteristics, or fatty acid composition in the longissimus muscle.

Key words: Crude protein, Hanwoo steers, Longissimus muscle, Total digestible nutrients.

INTRODUCTION

Recently, in Korea, efforts have been undertaken to shorten the fattening period to reduce feed costs and decrease the production of inedible fat in Hanwoo (Hong, 2016). However, shortening the fattening period may reduce the carcass weight and intramuscular fat (marbling), which may lower the farmer's income. Therefore, there are opinions that the crude protein and total digestible nutrients (TDN) in the formula feed should be increased during the late fattening period (Jeong *et al.,* 2010).

It was reported that an increase in the supply of crude protein through feed increased feed intake and an increase in intestinal amino acid absorption increased weight gain (Nagpal *et al.,* 2011). In addition, Trenkle (2003) reported that increasing the crude protein content of the feed had positive effects on the feed conversion ratio (FCR), carcass weight, marbling in the finishing steers. Moreover, previous studies (Kim *et al.,* 2013) reported that the increase in the crude protein content of the feed had a positive effect on growth performance and carcass characteristics during the late fattening period. Conversely, Chung *et al.* (2015) reported that the TDN level of feed was a major factor affecting the growth performance, carcass characteristics and fat deposition in beef cattle. In addition, increasing the TDN levels in the late fattening formula feed improves the dry matter (DM) digestibility, energy availability, average daily gain (ADG) and meat quality grade (Chung *et al.,* 2015; Jeong *et al.,* 2010).

Recent research has been centered on determining the optimal levels of crude protein and TDN for various growth stages. In addition, there are few studies on the effects of the simultaneous increase of crude protein and TDN contents in formula feed on growth performance, blood metabolites, carcass characteristics and meat composition during the late fattening period.

We hypothesized that increasing the levels of crude protein and TDN in the feed for late fattening Hanwoo steers could address the challenges in growth and meat quality, such as body weight and marbling, associated with shortened fattening periods, without adversely affecting the rumen fermentation characteristics. Therefore, this study evaluates the *in vitro* rumen fermentation characteristics following increases in crude protein and TDN in the formula feed and investigates the growth performance, blood metabolites, carcass characteristics and meat composition of late fattening Hanwoo steers.

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MATERIALS AND METHODS

Ethics statement

All procedures on animals were carried out in compliance with South Korea regulations (Animal and Plant Quarantine Agency·Ministry of Food and Drug Safety Joint Animal Testing and/or Laboratory Animal Related Committee (IACUC; 2020) Standard Operating Guidelines).

Animals, treatments and management

A cow equipped with rumen fistula was used for the *in vitro* experiment. In the field trial, 30 late fattening Hanwoo steers (694.3±71.7 kg and 25 months of age) were used. The steers were randomly assigned to one of the two dietary groups: control group (16.4% crude protein and 83.6% TDN based on DM) and treatment group (17.4% crude protein and 84.6% TDN based on DM). The control and treatment groups were fed experimental feeds for approximately 4 months.

The steers were allotted into six pens (5 m × 10 m) and the floor was covered with 20 cm of sawdust Formula feed was provided twice daily (08:30 and 17:00) using an automatic feeding system (SEOCHANG 65M/M, Seochang Co, Ltd, Cheonan, Korea). The 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 diets are listed in Table 1.

In vitro rumen fermentation

Ruminal fluid was collected from the ruminal fistula of a cow prior to morning feed administration. 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 and Van Soest, 1970) at a ratio of 1:3. To maintain anaerobic conditions, O_2-free CO_2 was bubbled through the diluted rumen fluid until transferred into serum bottles. 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 aluminum cap. The sealed serum bottles were incubated in an incubator at 39°C for 24 h.

The *in vitro* ruminal pH was measured using a pH meter (FP20, Mettler Toledo). The total gas production was measured using a pressure transducer (EA-6, SunBee Instrument, Seoul, Korea) according to the method of Theodorou *et al*. (1994).

The ammonia concentration was determined using the method of Chaney and Marbach (1962) and the volatile fatty acid (VFA) concentration was measured using gas chromatography (Agilent 7890A, Agilent Technology, CA, USA).

The DM degradability was calculated by washing the filter bag (F57, Ankom Technology, NY) with distilled water after incubation for 24 h drying it at 60°C for 72 h and measuring the weight.

Growth performance and blood metabolites

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

Every two months, 3 mL of blood was drawn from the jugular vein of the steers using an 18-gauge needle attached to a heparin-coated Vacutainer (Becton-Dickinson, Franklin Lakes, NJ, USA). Immediately after collection, samples were chilled and transported to the lab within 6 hours. Following centrifugation at 1,250 × g for 10 minutes to extract plasma, samples were processed with an automatic blood analyzer (Hitachi 7020, Hitachi Ltd., Tokyo, Japan) for assessing levels of metabolites.

Carcass characteristics

All steers were slaughtered to assess the meat yield traits (carcass weight, back-fat thickness and rib-eye area) and quality traits (marbling score, meat color, fat color, texture and maturity) of the carcasses at the local slaughterhouse. Carcass characteristics were determined from the sirloins of each carcass. Meat graders evaluated the carcass traits according to the Korean carcass grading system (MAFRA, 2018).

Beef quality measurements

Muscle pH was assessed after carcass quality grading using a portable pH meter (Testo 206-pH2, Testo AG, Lenzkirch, Germany). After 30 min of blooming at 4°C, the meat surface color was measured using a chromameter (CR400, Minolta Camera, Osaka, Japan) and values were expressed as lightness (L*), redness (a*) and yellowness (b*) (CIE, 1978).

The drip and cooking losses were measured according to the procedure described by Honikel (1998). Following the measurement of cooking loss, the same samples were subsequently utilized for the Warner-Bratzler shear (WBS) analysis, employing a method adapted from AMSA (2015).

Sensory quality evaluation

Beef steaks were heated until the core temperature reached 71°C using an oven (180°C) and then stored in a water bath (54°C) until the evaluation. Ten trained panelists were used for sensory quality analysis in this study. Sensory panel training was performed for at least six months (1-2 times a week, 1 hour per session) according to the AMSA guidelines (2015) and all items were evaluated using a 9-point scale.

Fatty acid composition

The fatty acid composition of the longissimus muscle was determined using a method adapted from Folch *et al.,* (1957), analyzed with a chromatography-mass selective detector (GC, Agilent 7890N, USA; MSD, Agilent 5975A, USA). The GC setup included a DB-WAXetr column (300 m × 0.25 mm ID, 0.25 μm film), helium as the carrier gas at a flow rate of 1 ml/min and temperatures set at 210°C for the injection port, 260°C for the detector and 200°C for the oven, with a split ratio of 10:1.

Statistical analysis

Statistical analysis to obtain averages and standard differences were performed using IBM SPSS (Statistical Package for the Social Sciences, SPSS Inc. Chicago, IL, USA). Statistically significant differences between the treatments were determined using the t-test, with P<0.05 indicating significance.

RESULTS AND DISCUSSION

In vitro rumen fermentation

Table 2 the effect of a simultaneous increase in crude protein and TDN contents of the formula feed on the rumen parameters. The pH and ammonia concentrations were similar between the two groups. Gas production was slightly, but insignificantly, higher in the treatment group than in the control group. There was no effect of crude protein and TDN contents in the formula feed on the VFA concentrations and DM degradability.

Table 1: Ingredients and chemical compositions of experimental diets.

Item	Formula feed		Rice straw
	Control	Treatment	
		Ingredients composition	
Corn (%)	30.84	43.77	-
Wheat (%)	10.00	7.00	-
Rice (%)	3.00	2.00	-
Wheat flour (%)	2.00	1.50	-
Wheat bran (%)	6.00	3.00	-
Corn gluten feed (%)	20.80	11.00	-
Soybean meal (%)	5.00	10.40	-
Palm kernel meal (%)	9.00	6.00	-
Corn-DDGS (%)	2.60	2.60	-
Lupin (%)	2.00	2.00	-
Cottonseed (%)	2.00	3.00	-
Cane molasses (%)	3.00	3.60	-
Rumen protected fat (%)	-	0.40	-
Salt dehydrated (%)	0.60	0.70	-
Limestone (%)	2.26	2.03	-
Vitamin premix1 (%)	0.10	0.10	-
Mineral premix2 (%)	0.20	0.10	
Sodium bicarbonate (%)	0.50	0.70	
Probiotic (%)	0.10	0.10	
		Chemical composition (DM basis)	
Dry matter (%)	88.52	88.59	89.51
Crude protein (%)	16.38	17.41	4.05
Ether extract (%)	4.00	4.22	1.81
Crude fiber (%)	5.92	4.68	36.20
Neutral detergent fiber (%)	24.95	22.33	71.33
Acid detergent fiber (%)	9.82	8.47	21.79
Crude ash (%)	5.90	6.57	10.28
Ca (%)	1.05	1.10	0.29
P (%)	0.47	0.48	0.10
Non-fibrous carbohydrate (%)	53.19	53.84	25.45
Total digestible nutrients (%)	83.59	84.64	38.34

Corn-DDGS: corn dried distiller's grains with solubles.

[1]Vitamin premix: vitamin premix provided the following quantities of vitamins per kilogram of diet: vitamin A, 10,000 IU; vitamin D3, 1,500 IU; vitamin E, 25 IU.

[2]Mineral premix: mineral premix provided the following quantities of minerals per kilogram of diet: Fe, 50 mg; Cu, 7 mg; Zn, 30 mg; Mn, 24 mg; I, 0.6 mg; Co, 0.15 mg; Se, 0.15 mg.

Based on the findings, it can be concluded that as the availability of nutrients in the feed for the rumen increases, there is an elevation in the production of volatile fatty acids, ammonia, gases and the DM degradability (Dey *et al*., 2022). However, an excessive increase in soluble carbohydrates leads to a decrease in rumen acidity and negatively affects rumen metabolism (Keles and Demirci, 2011). Nevertheless, this study found no differences in such rumen fermentation characteristics, suggesting that the cause might be the minimal difference in non-fibrous carbohydrate content (Table 1) and a similar level of nutrients available in the rumen between the two groups.

Growth performance and blood metabolites

Table 3 shows the effect of a simultaneous increase in crude protein and TDN contents of the formula feed on the growth performance and blood metabolites concentrations of the late fattening Hanwoo steers. No differences were observed in ADG, formula feed intake, rice straw intake and FCR between the control and treatment groups. The concentrations of the blood metabolites were similar between the two groups.

This study showed that the protein content in the formula feed had little effect on the ADG of late fattening Hanwoo steers, similar to the report by MAFRA (2007). Furthermore, Jeong *et al*. (2010) and Chung *et al*. (2015) also reported no significant difference in ADG and FCR with an increase in crude protein or TDN (approximately 2%) in the formula feed. Similarly, this study also found that increasing crude protein and TDN levels in the compound feed did not affect growth performance, which is consistent with previous research findings. Similar to this study, Paek *et al*. (2005)

Table 2: Effect of simultaneous increase in crude protein and TDN contents of the formula feed on rumen parameters at 24 h *in vitro* incubation.

Items	Control	Treatment	Sem
pH	6.72	6.70	0.019
Gas (mL/g DM)	239.21	250.03	7.929
NH_3-N (mg/dL)	26.40	26.21	1.204
Acetate (mmol/mol)	575.23	571.86	6.469
Propionate (mmol/mol)	258.52	263.31	8.621
Butyrate (mmol/mol)	120.14	118.42	2.510
Total-VFA (mM)	47.70	49.24	1.004
A:P ratio	2.23	2.18	0.096
Dry matter degradability (%)	75.91	79.80	1.701

SEM- Standard error of mean.

Table 3: Effect of simultaneous increase in crude protein and TDN contents of the formula feed on growth performance of the late fattening Hanwoo steers.

Items	Control	Treatment	SEM
Growth performance			
Initial body weight (kg)	683.0	705.6	13.150
Final body weight (kg)	750.2	770.5	13.842
Average daily gain (kg/d)	0.81	0.78	0.032
Formula feed intake (kg dry matter)	7.99	8.00	0.071
Rice straw intake (kg dry matter)	1.06	1.06	0.030
Feed conversion ratio	12.18	12.38	0.594
Blood metabolites			
Glucose (mg/dL)	70.00	71.07	1.149
Cholesterol (mg/dL)	180.13	180.90	5.055
Non-esterified fatty acid (uEq/L)	140.10	172.74	10.356
Creatinine (mg/dL)	1.47	1.48	0.019
Blood urea nitrogen (mg/dL)	18.68	19.46	0.423
Total protein (g/dL)	6.89	6.75	0.092
Albumin (g/dL)	3.83	3.80	0.041
Total glyceride (mg/dL)	21.27	21.00	1.080
Calcium (mg/dL)	8.96	8.92	0.068
Phosphorus (mg/dL)	7.91	7.55	0.117
Magnesium (mg/dL)	2.35	2.32	0.024

SEM- Standard error of mean.

reported that an increase in the TDN content (increased from 72% to 74%) in the formula feed during the late fattening period did not affect the ADG or FCR and suggested further studies (more than 3% TDN difference). Conversely, some studies (Kim, 2015; Jin *et al*., 2012) reported that the increase in TDN level in the formula feed increased the DMI and ADG. This difference might be due to the differences in TDN content in the formula feed among studies.

It was reported that there was no difference in the concentrations of blood metabolites after an increase in TDN in the formula feed during the late fattening period (Cho *et al.,* 2019; Chung *et al.,* 2015). Similarly, in this study, the simultaneous increase in TDN and crude protein contents of the formula feed had no effect on the concentrations of blood metabolites during the late fattening period.

Carcass characteristics

Table 4 shows the effect of a simultaneous increase in crude protein and TDN contents of the formula feed on the carcass characteristics of the late fattening Hanwoo steers. The carcass weight and yield index were higher in the treatment group than in the control group, but there was no significant difference between the groups. The back-fat thickness was slightly, but not significantly, lower in the treatment group than in the control group. There was no effect of dietary treatment on marbling score, meat color, fat color, texture, or maturity.

Kim *et al*. (2013) reported that an increase in the crude protein content in the formula feed during the late fattening period positively affected the back-fat thickness and marbling score. Chung *et al*. (2015) reported that the back-fat thickness of Hanwoo steers slaughtered at 26 and 30 months of age was higher in the high energy treatment than in the control. However, this study showed no correlation between TDN in the formula feed and back-fat thickness. This might be because there was no difference in DMI due to limited feeding the formula feed in all groups (Table 2). Similar to this study, previous studies (Cho *et al.,* 2019; Chung *et al.,* 2015) showed that the difference in TDN levels in formula feed during the late fattening period affected the carcass weight.

Beef quality measurements, sensory evaluation and fatty acid composition

Table 5 shows the effect of a simultaneous increase in crude protein and TDN contents of the formula feed on the meat quality characteristics of the Hanwoo steers. Between the control and treatment groups, no differences were observed in pH, surface color, drip loss and cooking loss in the *longissimus muscle*. The shear force was slightly, but not

Table 4: Effect of simultaneous increase in crude protein and TDN contents of the formula feed on carcass characteristics of late-fattening Hanwoo steers.

Items	Control	Treatment	Sem
Yield traits			
Carcass weight (kg)	480.4	499.5	8.631
Rib-eye area (cm^2)	102.5	101.4	1.753
Back-fat thickness (mm)	13.67	11.33	0.675
Yield index (%)	64.67	65.53	0.478
Quality traits			
Marbling score	5.27	5.07	0.324
Meat color	4.00	4.20	0.057
Fat color	3.00	3.00	0.001
Texture	1.07	1.13	0.057
Maturity	2.53	2.47	0.094

SEM- Standard error mean.

Table 5: Effect of simultaneous increase in crude protein and TDN contents of the formula feed on pH, surface color, drip loss, cooking loss and shear force in longissimus muscle of Hanwoo steers.

Items	Control	Treatment	Sem
pH	5.71	5.75	0.014
Color			
Lightness (L*)	33.9	34.5	0.403
Redness (a*)	17.5	16.8	0.347
Yellowness (b*)	11.7	11.5	0.278
Drip loss (%)	1.30	1.39	0.188
Cooking loss (%)	19.2	20.2	0.757
Shear force (N)	62.5	58.6	1.334

SEM- Standard error mean.

significantly, lower in the treatment group than in the control group.

Kim and Jung (2007) reported that the better the marbling score, the lower the shear force and cooking loss, but the higher the water holding capacity. Also, it is reported that the cooking loss of beef decreases as the meat quality level and intramuscular fat level increases (Ozutsumi, 1994). Kim *et al.* (2013) reported that the cooking loss of beef was lowered due to an increase in marbling after an increase in the crude protein content in the formula feed. However, this study showed no difference in drip loss, cooking loss, or shear force because there was no change in marbling from the change in crude protein and TDN contents of the formula feed.

Table 6 shows the effect of a simultaneous increase in crude protein and TDN contents of the formula feed on the sensory quality characteristics in the beef of the Hanwoo steers.

No differences were observed in tenderness or sensory characteristics of the longissimus muscle between the control and treatment groups

The simultaneous increase in crude protein and TDN in the formula feed during the late fattening period did not affect the sensory quality characteristics of cooked beef in this study, presumably due to the similar marbling scores between the two groups (Table 4). This is supported by previous studies (Bown *et al.,* 2016; Schaefer *et al.,* 1986) showing that there were generally no differences in juiciness, flavor and off-flavor in beef at the same marbling degree.

Table 7 shows the effect of a simultaneous increase in crude protein and TDN contents of the formula feed on the fatty acid composition in the beef of the Hanwoo steers.

Table 6: Effect of simultaneous increase in crude protein and TDN contents of the formula feed on sensory characteristics in longissimus muscle of Hanwoo steers.

Items	Control	Treatment	Sem
Softness	6.16	6.20	0.223
Initial tenderness	5.90	6.12	0.235
Chewiness	5.74	5.82	0.278
Rate of breakdown	5.62	5.81	0.188
Amount of perceptible residue	5.96	6.17	0.180
Total tenderness	5.93	6.03	0.230
Juiciness	6.07	6.12	0.240
Flavor intensity	6.55	6.72	0.170
Off-flavor intensity	7.38	7.35	0.213
Mouth coating	5.87	5.92	0.228
Overall acceptability	5.79	6.03	0.193

SEM- Standard error of mean.

Table 7: Effect of simultaneous increase in crude protein and TDN contents of the formula feed on fatty acid composition in longissimus muscle of Hanwoo steers.

Items	Control	Treatment	Sem
C12:0 (Lauric, %)	0.18	0.17	0.012
C14:0 (Myristic, %)	2.62	2.64	0.051
C16:0 (Palmitic, %)	25.0	24.8	0.533
C16:1n-9 (Palmitoleic, %)	5.41	5.59	0.285
C18:0 (Stearic, %)	11.6	12.0	0.261
C18:1n-9 (Oleic, %)	50.8	49.7	0.570
C18:2n-6 (Linoleic, %)	3.56	4.20	0.282
C18:3n-3 (á-linolenic, %)	0.10	0.13	0.024
C18:3n-6 (Linolenic, %)	0.16	0.10	0.030
C20:4n-6 (Arachidonic, %)	0.31	0.29	0.043
C20:5n-3 (Eicosapentaenoic, %)	0.19	0.15	0.052
C22:6n-3 (Docosahexaenoic, %)	0.01	0.01	0.004
SFA	39.5	39.8	0.388
UFA	60.5	60.2	0.382
MUFA	56.2	55.3	0.460
PUFA	4.33	4.90	0.271

SEM- Standard error of mean; SFA- Saturated fatty acid; UFA- Unsaturated fatty acid; MUFA- Mono-unsaturated fatty acid; PUFA- Poly-unsaturated fatty acids

The overall fatty acid compositions were similar between the two groups.

Jeong *et al.* (2010) reported that the contents of stearic, myristoleic and oleic acids was increased by a simultaneous increase of crude protein and TDN content in the formula feed during the late fattening period. However, this study found no difference in the fatty acid composition of the longissimus muscle after the change in crude protein and TDN content of the formula feed, presumably because there was no difference in the marbling level and meat quality grade. Previous studies (Kim, 2006) support the results of this study by indicating that the fatty acid composition of beef affects the marbling degree and meat quality grade.

CONCLUSION

In this study, increasing the crude protein and TDN in the formula feed to compensate for the shortened fattening period did not negatively affect the rumen fermentation characteristics. However, it also did not exhibit a positive impact on growth performance, carcass characteristics and meat quality, which are crucial for productivity and profitability. To address these issues, further research is suggested, potentially extending the experimental period (starting in the growing stage or early fattening stage) or increasing the crude protein and TDN levels beyond the current study's parameters.

Conflict of interest

All authors declare that they have conflict of interest.

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