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Effect of nonfiber carbohydrate and crude protein levels in the formula of feed on in vitro rumen fermentation characteristics and growth performance of weaned Hanwoo calves

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Abstract: This study was conducted to determine the effect of nonfiber carbohydrates (NFC) and crude protein (CP) present in formula feed on the rumen fermentation characteristics and growth performance of weaned Hanwoo calves. Forty weaned calves were randomly assigned to four dietary groups based on NFC and CP levels as follows: high NFC (HN) at 38%–42%, low NFC (LN) at 33%–36%, high CP (HP) at 25%–26%, and low CP (LP) at 23%–24%. These groups were categorized as HN-HP, HN-LP, LN-HP, and LN-LP groups. Total digestible nutrients (TDN) and CP levels did not significantly affect the in vitro ruminal total gas, propionate, acetate, and NH_3-N concentrations after 12 h of incubation. Butyrate concentration was significantly higher in the LN-LP group than in the HN-HP group after 6 h of incubation ($p < 0.05$). Neutral detergent fiber degradability was significantly lower in the LN-LP group than in the HN-HP group ($p < 0.05$). The varying CP and NFC levels of formula feed did not significantly affect the growth performance of Hanwoo calves. Therefore, HN and HP levels of formula feed had no significant effect on rumen fermentation and growth performance. In summary, these results suggest that NFC at 33% and CP at 23% in formula feeds are sufficient in satisfying the energy and protein requirements of weaned Hanwoo calves.

Key words: Crude protein, nonfiber carbohydrates, formula feed, Hanwoo calf

1. Introduction

The nutritional status of weaned calves should be carefully monitored and managed to promote their normal growth and health. This is directly related to an increase in the production of meat and dairy products; thereby, contributing to overall economic advantages [1]. Moreover, nutritional management is essential in preventing stunted growth in calves from the time they are weaned until they become adult cattle.

The chemical composition of formula feed and final metabolites affect growth performance and rumen villi development of weaned calves [2]. Rumen microorganisms synthesize propionate and butyrate from formula feed, and these biomolecules stimulate the rumen epithelial tissue to form healthy villi [3,4]. In addition, the composition of formula feed and forage plays an important role in establishing a healthy rumen microbial population [2].

Assessing the nutrient levels of formula feed is vital for promoting optimal growth among weaned calves. Among the key nutrients to be assessed, the energy (carbohydrate) and protein levels of formula feed should be considered first because they are essential for the optimal growth of weaned calves, especially when forage quality is low [5,6]. However, excessively high carbohydrate and crude protein (CP) levels in formula feed can cause liver dysfunction or premature obesity. Therefore, adequate carbohydrate and CP levels in formula feed are essential for optimal growth and health of weaned calves; however, the optimum carbohydrate and protein levels to be present in formula feed for weaned Hanwoo calves have not yet been determined. We hypothesized that nonfiber carbohydrates (NFC) and CP levels of formula feed could affect the rumen fermentation characteristics and growth performance of weaned calves.

Therefore, this study was conducted to determine the effect of NFC and CP levels in formula feed on the in vitro rumen fermentation characteristics and growth performance of weaned Hanwoo calves.

2. Materials and methods

2.1. Animals, treatments, and management

Three steers (650.2 ± 31.2 kg, aged 28.5 ± 0.2 months) with rumen fistula were used for the in vitro experiment. The field trial of growth performance was conducted using forty Hanwoo calves (109.2 ± 11.8 kg, aged 3.1 ± 0.2 months). The calves were randomly assigned to four

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dietary groups based on NFC and CP levels as follows: high NFC (HN) at 38%–42%, low NFC (LN) at 33%–36%, high CP (HP) at 25%–26%, and low CP (LP) at 23%–24%. These groups were categorized as HN-HP, HN-LP, LN-HP, and LN-LP groups. The chemical composition, carbohydrate fractions, and protein fractions of the experimental feed are listed in Table 1.

The steers with rumen fistula were given a total mixed ratio (TMR) of 12 kg per steer. TMR was provided in divided twice daily at 08:30 h and 17:30 h, and the steers had free access to water and mineral blocks (Calcium: 14.5%, Phosphorus: 8.0%, Salt: 10.0%, Magnesium: 0.5%, Copper: 200 ppm, Selenium: 26 ppm, Zinc: 400 ppm, Vitamin A: 50,000 IU/lb, Vitamin D3: 10,000 IU/lb, and Vitamin E: 100 IU/lb). The calves were provided in divided with experimental formula feed (2.5–4.0 kg per calf) and timothy hay (2.5–3.0 kg per calf) twice daily at 08:30 h and 17:30 h. Experimental formula feed was increased by

Table 1. Chemical composition, carbohydrate, and protein fractions of the experimental feed.

Items	HN-HP	LN-HP	HN-LP	LN-LP
Chemical composition				
DM (%)	86.7	89.2	89.0	88.8
CP (%DM)	26.0	25.6	24.2	23.4
EE (%DM)	3.83	2.59	2.99	3.59
Ash (%DM)	7.80	8.02	7.16	7.89
NDF (%DM)	22.1	31.6	28.1	31.3
ADF (%DM)	9.7	15.9	16.1	13.1
SolP (%DM)	8.9	7.0	4.2	8.4
NDICP (%DM)	1.43	1.13	1.03	2.62
ADICP (%DM)	0.69	0.52	0.74	1.03
Lignin (%DM)	1.80	2.42	2.63	3.43
Sugar (%DM)	6.70	8.50	6.30	8.60
Starch (%DM)	29.7	27.4	22.5	20.6
NFC (%DM)	41.7	36.4	38.1	33.3
TDN (%)	76.8	71.4	73.1	71.2
NEm (Mcal/kg)	2.01	1.81	1.88	1.79
NEg (Mcal/kg)	1.35	1.17	1.24	1.17
Carbohydrate fraction				
CA (% CHO[14])	13.8	13.3	9.70	10.3
CB1 (%CHO)	47.6	32.3	34.5	42.1
CB2 (%CHO)	5.45	6.58	14.3	3.84
CB3 (%CHO)	26.2	38.7	31.9	31.4
CC (%CHO)	6.93	9.10	9.69	12.6
Protein fraction				
PA+PB1 (%CP)	34.3	27.2	17.0	35.7
PB2 (%CP)	60.3	68.2	78.8	52.9
PB3 (%CP)	2.90	2.40	1.20	6.80
PC (%CP)	2.60	2.00	3.00	4.40

DM: Dry matter, CP: Crude protein, EE: Ether extract, NDF: Neutral detergent fiber, ADF: Acid detergent fiber, SOLP: Soluble protein, NDICP: Neutral detergent insoluble protein, ADICP, Acid detergent insoluble protein, NFC: Nonfiber carbohydrate, TDN: Total digestible nutrient, NEm: Net energy for maintenance, NEg: Net energy for growth, CA: Fast fermented, CHO: Carbohydrate, CB1: Moderate fermented, CB2: Intermediately fermented, CB3: Slowly fermented, CC: Unfermentable, PA: Nonprotein nitrogen, PB1: Rapidly degradable protein, PB2: Intermediately degradable protein, PB3: Intermediately degradable protein, PC: Unavailable protein.

0.5 kg per month, and timothy hay was maintained after increasing by 0.5 kg in the first month. The calves had free access to water and mineral blocks. The ingredient and chemical composition of TMR and timothy hay are shown in Table 2.

2.2. Proximate analysis, carbohydrate, and protein fractions

The experimental feed was dried at 65 °C for 72 h, grounded to a particle size of 1 mm, and then analyzed. The DM, CP, ADF, and crude ash of the experimental feed were analyzed [7], and EE was analyzed [8]. CP content was calculated as follows: 6.25 × total nitrogen content. The total nitrogen content of the feed was analyzed using a Leco FP-528 nitrogen combustion analyzer (Leco Corporation, MI, USA).

To measure the fiber content, NDF, and acid detergent lignin (ADL) were analyzed [9]. Heat-stable α-amylase was used for NDF analysis. Among the carbohydrate fractions, ethanol-soluble carbohydrate (ESC) and starch were analyzed [10]. Soluble protein (SOLP) was analyzed [11]. Neutral detergent insoluble crude protein (NDICP) and acid detergent insoluble crude protein (ADICP) were analyzed [12]. The energy value of the feed as TDN was evaluated [5].

Based on the measured carbohydrate and protein contents, the carbohydrate and protein fractions of the Cornell Net Carbohydrate and Protein System (CNCPS) were evaluated [13] with modifications [14]. The amount of sugar and organic acids, which belong to carbohydrate A fraction (CA), was estimated by measuring the ESC content; carbohydrate B1 fraction (CB1) was estimated by measuring the starch content using the feed component analysis. Carbohydrate B2 (CB2) and B3 (CB3) fractions were calculated using the following formulas: CB2 = NFC – CA – CB1 and CB3 = (NDF – NDICP) – (2.4 × ADL). Carbohydrate C fraction (CC) was calculated as follows: 2.4 × ADL content.

The CNCPS protein fractions were determined as follows. Nonprotein nitrogen and soluble true protein were grouped together and labeled as protein A + B1 fractions (PA + PB1). The amount of PA + PB1 was similar to the SOLP content in the feed analysis. PA + PB1 represents proteins that are immediately lysed and degraded in the rumen. Protein B2 fraction (PB2) was calculated as follows: PB2 = 100 – NDICP – SOLP. PB2 is a group of proteins that are not immediately soluble but are rapidly digested in the rumen (intermediate degradable CP). Protein B3 fraction (PB3), representing a slowly degradable fiber-bound CP that is gradually digested in the rumen, was calculated as follows: PB3 = NDICP – ADICP. Protein C fraction is an indigestible protein in the rumen; it was determined by measuring the ADICP content of the feed.

Table 2. Ingredient and chemical composition of TMR and timothy hay.

Items	TMR	Timothy
Ingredient composition (%)		
Concentrated feed	15.0	-
Corn flake	12.0	-
Soybean meal	4.0	
Corn gluten feed	13.0	-
Cottonseed	4.0	
Lupin flake	4.0	-
Barley brewers grains	14.0	-
Annual ryegrass straw	8.0	-
Tall fescue straw	3.0	
Oat hay	3.0	-
Cane molasses	2.5	
Sodium bicarbonate	0.4	
Limestone	0.5	
Vitamin-mineral premix	0.1	
Water	16.5	
Chemical composition (%)		
Dry matter	64.05	92.50
Crude protein	11.84	9.80
Ether extract	2.98	1.86
Crude fiber	7.51	26.42
Crude ash	5.41	7.96
NDF	29.14	55.70
ADF	12.46	38.10

Concentrated feed contained the following percentage of ingredients: corn, 21.0%; cane molasses, 4.0%; cassava residue, 6.0%; wheat bran, 9.0%; corn gluten feed, 15.0%; soybean meal, 13.5%; rapeseed meal, 7.0%; coconut meal, 11.0%; palm kernel meal, 9.9%; animal fat, 0.3%, salt dehydrate, 0.5%; limestone, 1.9%; calcium sulfate, 0.2%; sodium bicarbonate, 0.5%, vitamin-mineral premix, 0.20%.

Vitamin-mineral premix provided the following quantities of vitamins and minerals per kg of diet: vitamin A, 10,000 IU; vitamin D3, 1500 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.

NDF: Neutral detergent fiber, ADF: Acid detergent fiber.

2.3. In vitro rumen fermentation

Ruminal fluid was collected from the ruminal fistula of steers prior to morning feed administration. The collected ruminal 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 [15]

at a ratio of 1:3. To maintain anaerobic conditions, oxygen-free carbon dioxide was bubbled through the diluted ruminal fluid until it was transferred into serum bottles. Under completely anaerobic conditions, each 60 mL of the ruminal fluid was dispensed into 125 mL serum bottles containing 1 g of the experimental feed and completely sealed with a butyl rubber stopper and aluminum cap. The sealed serum bottles were incubated at 39 °C for 24 h.

In vitro ruminal pH was measured using a pH meter (FP20, Mettler Toledo International, Inc., Ohio, USA). Gas production was measured using a pressure transducer (EA-6, SunBee Instrument, Inc., Seoul, Korea) according to the method of Theodorou et al. [16].

To determine the ammonia concentration, 10 mL of the culture solution was centrifuged (3000 × g for 15 min at 4 °C). Then, 5 mL of the supernatant and 0.05 mL of $HgCl_2$ were mixed and centrifuged (3000 × g for 15 min at 4 °C), and 1 mL of the supernatant was collected from this procedure. The ammonia concentration was estimated [17].

To determine the volatile fatty acid (VFA) concentration, 10 mL of the culture solution was collected, then 1 mL of 20% HPO_3 and 0.5 mL of saturated $HgCl_2$ were added, and the mixture was centrifuged (1250 × g for 15 min at 4 °C). The supernatant was collected and the VFA concentration was measured using gas chromatography (Agilent 7890A, Agilent Technologies, Inc., CA, USA). VFA standard solutions (Volatile Free Acid Mix-CRM46975, Sigma-Aldrich Co., SL, USA) containing acetate, propionate and butyrate were diluted 0, 1, 2, 3, 5, and 6 times. The diluted standard solution was analyzed using GC, and individual VFAs were calculated by creating a standard curve based on the analyzed area value.

To calculate the DM degradability, the filter bag (F57, ANKOM Technology Corporation, NY, USA) was washed with distilled water after incubation for 24 h, dried at 60 °C for 72 h, and the weight of the bag was measured.

2.4. Growth performance

Body weight (BW) was measured at the beginning and end of the experimental period; the average daily gain (ADG) was calculated by dividing the total weight gain by the number of experimental days. Dry matter intake (DMI) was calculated by measuring the amount of residual feed before feeding in the morning. The feed conversion ratio (FCR) was calculated by dividing DMI by ADG.

2.5. Statistical analysis

All statistical analyses were performed using IBM SPSS Statistics for Windows, version 24 (IBM Corp., NY, USA). The means of treatment groups were compared using one-way analysis of variance (ANOVA), followed by Duncan's multiple range test. Differences were considered statistically significant at $p < 0.05$.

3. Results

3.1. In vitro rumen fermentation characteristics

The effects of varying NFC and CP levels in formula feed on the in vitro rumen fermentation characteristics are shown in Figure 1 and Table 3. Although gas production increased in all treatment groups as the incubation time increased; however, NFC and CP levels of formula feed did not markedly affect gas production among the treatment groups. DM degradability after 6 and 12 h of incubation was similar among the treatment groups. After 6 h of incubation, NDF degradability was higher in the LN-HP and HN-HP groups than in the LN-LP group ($p < 0.05$). In addition, NDF degradability after 12 h of incubation was significantly higher in the HN-HP group than in the LN-LP group ($p < 0.01$).

The pH and NH_3-N concentration showed similar tendencies among the treatment groups, regardless of incubation time. At 6 h of incubation, the total VFA, acetate, and propionate concentrations did not differ among the treatment groups, but butyrate concentration was significantly higher in the LN-HP group than in the HN-HP group ($p < 0.05$). At 12 h of incubation, the total VFA, acetate, propionate, and butyrate concentrations were similar among the treatment groups.

3.2. Growth performance of weaned calves

Table 4 shows the effect of varying NFC and CP levels in formula feed on the growth performance of weaned Hanwoo calves. There were no differences in the final BW and DMI of male calves among the treatment groups, and the ADG was similar among the treatment groups. A small difference of 5.15–5.64 was observed in the FCR in male calves. In the case of female calves, the final BW, ADG, DMI, and FCR were similar among the treatment groups.

4. Discussion

In this study, the varying NFC and CP levels of formula feed did not affect the in vitro rumen fermentation characteristics. In general, rumen pH is affected by starch content and digestion patterns [18]. However, there was no difference in pH among the treatment groups in this study. Such a result could be attributed to the starch source and content of the formula feed. The starch content of formula feed prepared from four starch sources (barley, corn, oat, and wheat) was at a similar level (24.90%–25.60%), but the rumen pH was the highest with corn supplementation [19]. In this study, the rumen pH was maintained above 6.5 in most treatment groups. Therefore, there was no difference in rumen pH among the treatment groups because the starch source for the formula feed used in this study was similar and the starch content was not high enough to affect rumen pH.

Besides NH_3-N concentration, carbohydrate content also affects protein intake and degradability [20]. When

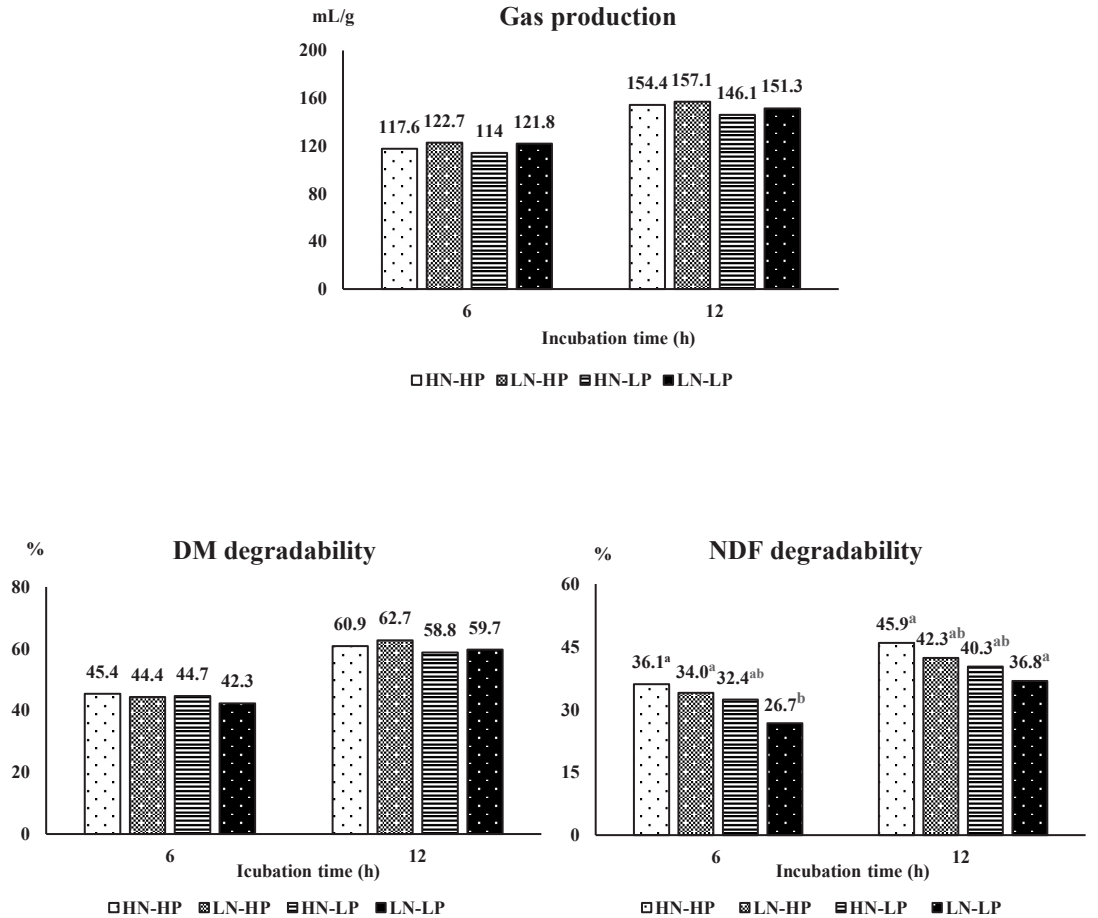

Figure 1. Effects of varying NFC and CP levels in formula feed on weaned calves in vitro rumen gas production, DM, and NDF degradability. [a,b]Means followed by different letters in the same row are significantly different ($p < 0.05$).

the content of digestible starch in the rumen is increased, the efficiency of microbial protein synthesis is improved, which can increase milk protein content [21]. The rumen NH_3-N concentration decreased as starch degradability increased [22]. Therefore, carbohydrate and protein degradability affect microbial protein synthesis [23]; NH_3-N concentration decreases as microbial protein synthesis efficiency improves [24]. In this study, there was no difference in NH_3-N concentration between the LP and HP groups that could be attributed to the effect of carbohydrates, as the NFC/protein ratio of the formula feed was 1.3–1.6. This assumption could be related to the decomposition rate of NDF. The proliferation of fibrinolytic microorganisms is promoted when the following conditions in the rumen are satisfied: high carbohydrate and protein levels, and pH maintained at 6.5 or higher. It is presumed that NDF degradability increased in the HN-HP group compared to the LN-LP group. However, further studies on microbial properties are needed for a clearer interpretation.

Rumen development in calves is influenced by the intake of formula feed and nutrient composition [18,25]. In addition, formula feed induces microbial proliferation and stimulates VFA production contributing to rumen development [26]. In this study, the total VFA concentration was not affected by the varying carbohydrate and protein levels of the formula feed. However, butyrate concentration significantly increased in the LN-LP group after 6 h of incubation. It has been reported in several studies that among the VFAs, butyrate is most effective in promoting the development of rumen villi [27–29]. Lactose in formula feed is metabolized by butyrate fermentation in the rumen [30]. The concentration of butyrate in the rumen increased as more lactose was added [31]. Butyrate levels increased as the intake of formula feed increased. Because the same amount of formula feed was provided to the in vitro cultures in this study, the difference in butyrate concentration in the treatment groups could be due to varying lactose levels. The LN-LP formula feed had the highest concentration of milk replacer; the amount

Table 3. Effects of varying NFC and CP levels in formula feed on weaned calves in vitro rumen pH, NH_3-N, and VFA concentrations.

Items	Time (h)	HN-HP	LN-HP	HN-LP	LN-LP	SEM	p value
pH	6	6.84	6.85	6.84	6.81	0.02	0.36
	12	6.59	6.65	6.63	6.58	0.03	0.09
NH_3-N (mg/dL)	6	18.8	19.3	19.1	20.0	0.65	0.37
	12	27.6	28.3	25.9	25.9	1.71	0.41
Total VFA (mM)	6	35.7	38.6	37.4	38.7	1.56	0.25
	12	50.1	52.7	51.6	47.6	3.04	0.40
Acetate (mmol/mol)	6	398.0	411.0	404.3	418.5	8.69	0.15
	12	433.8	427.0	427.8	427.3	4.57	0.43
Propionate (mmol/mol)	6	234.3	231.3	240.5	234.0	4.68	0.29
	12	241.8	251.0	255.3	241.8	7.38	0.22
Butyrate (mmol/mol)	6	138.5[b]	146.5[ab]	142.5[ab]	147.0[a]	2.74	0.05
	12	152.5	154.0	150.3	147.3	2.90	0.16

[a,b]Means followed by different letters in the same row are significantly different ($p < 0.05$). SEM: Standard error of the mean, VFA: Volatile fatty acids.

Table 4. Effect of varying NFC and CP levels in formula feed on the growth performance of weaned Hanwoo calves.

Items	HN-HP	LN-HP	HN-LP	LN-LP	SEM	p value
Male calves						
Initial BW (kg)	115.4	116.8	117.5	115.9	1.41	0.76
Final BW (kg)	213.6	211.4	207.4	208.2	2.46	0.66
ADG (kg)	1.07	1.03	0.98	1.00	0.02	0.55
Formula feed intake (kg)	2.87	2.87	2.87	2.87	-	-
Timothy intake (kg)	2.58	2.61	2.63	2.67	-	-
DMI (kg)	5.45	5.48	5.50	5.54	-	-
FCR	5.15	5.42	5.64	5.63	0.14	0.42
Female calves						
Initial BW (kg)	101.7	102.5	102.2	101.6	1.56	0.91
Final BW (kg)	182.8	185.2	189.8	191.6	2.98	0.43
ADG (kg)	0.88	0.90	0.95	0.98	0.02	0.18
Formula feed intake (kg)	2.87	2.87	2.87	2.87	-	-
Timothy intake (kg)	2.47	2.55	2.43	2.57	-	-
DMI (kg)	5.34	5.42	5.30	5.44	-	-
FCR	6.24	6.18	5.61	5.63	0.16	0.39
Male and female calves						
Initial BW (kg)	108.6	109.7	109.9	108.8	1.87	0.99
Final BW (kg)	198.2	198.3	198.6	199.9	3.26	0.85
ADG (kg)	0.97	0.96	0.96	0.99	0.02	0.95
Formula feed intake (kg)	2.87	2.87	2.87	2.87	-	-
Timothy intake (kg)	2.53	2.58	2.53	2.62	-	-
DMI (kg)	5.40	5.45	5.41	5.49	-	-
FCR	5.69	5.80	5.62	5.63	0.15	0.25

SEM: Standard error of the mean, ADG: Average daily gain, DMI: Dry matter intake, FCR: Feed conversion ratio.

of lactose in the formula feed had an effect on butyrate concentration (data not shown). There was no difference in butyrate concentration among the treatment groups at 12 h of incubation because water-soluble carbohydrates, such as lactose, are easily and rapidly fermented in the rumen [32]. For a more detailed evaluation, it is necessary to subdivide the incubation time. Furthermore, the ratio is more important than the energy and protein levels of formula feed, suggesting that the use of a specific raw material may affect the production of VFAs in the rumen.

To minimize nutritional stress in weaned calves, it is necessary to provide a sufficient amount of feed that meets the energy and protein requirements of the calves [33,34]. In the present study, there was no difference in the growth performance of the weaned calves despite the varying NFC and CP levels in the formula feed. This suggests that the energy and protein levels of the LN-LP diet can meet the energy and protein requirements for the maintenance and growth of calves under limited formula feed-feeding conditions. The varying energy and protein intake affect calf growth: the amount of body fat increased in the high intake group, and the difference in DMI was more than twice between the low and high intake groups [35]. However, in this study, the difference in ADG among the treatment groups was small because the DMI required corresponding to the weight and age of the calf was provided equally to all treatment groups, following the Korean feeding standard [36]. The difference in ADG was small when the protein content of the formula feed for calves was increased from 18.0% to 21.0% [37]. Furthermore, the weight and intake of calves showed similar trends when formula feed with increased nonstructural carbohydrates was fed [38]. Therefore, the influence of DMI on the growth performance of calves may be of greater significance than the energy and protein levels of formula feed.

In this study, the varying NFC and CP levels of formula feed did not affect the rumen fermentation characteristics and growth performance of weaned Hanwoo calves. The results suggest that formula feed with NFC at 33% and CP at 23% can meet the energy and protein requirements for the growth of weaned Hanwoo calves when the appropriate DMI based on the weight and age of the calf is provided. In addition, the results indicate that instead of the NFC and CP levels of formula feed, specific raw materials (type and addition ratio) could affect rumen fermentation characteristics.

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Conflict of interest

The authors declare no conflict of interest.

Ethical approval

The experimental animals used in this study were approved by the Animal Experiment Ethics Committee of Kangwon National University (No: KW-210716-1).

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