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Effects of cold temperature and fat supplementation on growth performance and rumen and blood parameters in early fattening stage of Korean cattle steers

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ABSTRACT

We investigated the effects of cold ambient temperatures and the supplementation of rumen-protected fat (RPF) on the growth performance, rumen fermentation parameters, and blood metabolic parameters of Korean cattle steers during the early fattening stage. Twenty Korean cattle steers (body weight = 356.5 ± 3.72 kg, age = 16.8 ± 0.34 months) were divided into two groups: 10 steers were fed a conventional concentrate (control), and 10 steers were fed a concentrate supplemented with 8 g/kg RPF (treatment). Steers were fed a concentrate at 1.6% body weight and 3 kg tall fescue hay daily for 12 weeks in 2016: January 8 to February 2 (P1), February 3 to March 3 (P2), and March 4 to April 1 (P3). Body weight was measured, and blood and rumen fluid were collected four times from each steer: at the start of the experiment, and then at 4-week intervals. The mean indoor temperatures during P1 (-7.37 °C) and P2 (-2.96 °C) were lower ($P < 0.001$) than the mean P3 temperature (6.22 °C). These lower temperatures were within the range that causes mild to moderate cold stress (CS) in other cattle breeds. By contrast, the mean indoor temperature during P3 is considered thermoneutral. The average daily gain ($P = 0.39$ and $P = 0.16$) and gain-to-feed ratio ($P = 0.26$ and $P = 0.57$) were not affected by RPF supplementation or time-period. Total dry matter, crude protein, ether extract, neutral detergent fiber, and acid detergent fiber intakes per body weight were higher (all $P < 0.001$) in colder P1 or P2 compared to other time periods, but these variables were not affected ($P \geq 0.31$) by RPF supplementation, except ether extract intake. Ruminant total VFA concentrations were higher ($P = 0.001$) in a colder January than in other months, but these were not affected ($P = 0.26$) by RPF supplementation. The ruminal propionate proportion was higher ($P < 0.001$) in a colder January than in other months, whereas the butyrate proportion was lower ($P = 0.01$). Serum triglyceride ($P = 0.007$), total cholesterol ($P = 0.001$), and high-density lipoprotein ($P = 0.03$) concentrations were lower during colder months. RPF supplementation increased ($P = 0.008$) serum cholesterol concentrations. Our results indicate that mild to moderate CS may not affect Korean cattle, as cattle growth performance was not affected by colder temperatures, although

Abbreviations: NH₃-N, ammonia nitrogen; ADG, average daily gain; BW, body weight; CS, cold stress; CP, crude protein; EE, ether extract; G:F ratio, gain to feed ratio; HDL, high density lipoprotein; NEFA, non-esterified fatty acid; RPF, rumen-protected fat; TG, triglyceride; VFA, volatile fatty acids

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concentrate and forage intakes were increased, and some changes in blood metabolic and ruminal parameters were observed. The 8 g/kg RPF supplementation did not improve growth performance under cold conditions, although it did not decrease intake and ruminal total VFA concentration.

1. Introduction

In South Korea, winter lasts for approximately 3 months from December to February. The mean minimum temperatures in December and January in Korea over the last 30 years are -5.6 °C and -3.2 °C, respectively (Korea Meteorological Administration, 2017). These temperatures are considered to cause mild cold stress (CS; Grzych, 2010). In recent years, winter temperatures in Korea have been getting colder (Korea Meteorological Administration, 2017), and cattle may thus suffer from CS during winter. Cold exposure has been shown to alter metabolic rates, digestive capacities, immune responses, back-fat thickness, meat quality, and milk yield in several cattle breeds at different ages (Kennedy et al., 1976; Brouček et al., 1991; Park et al., 2018).

Supplementing the diet of beef cattle with fat could increase energy density effectively. Dietary supplementation with fat has been shown to increase both the levels of circulating glucose in lactating dairy cows and substrate availability for energy reserves (Kronfeld et al., 1980). Therefore, dietary fat supplementation could help to alleviate CS (Hess et al., 2008). However, fat supplementation can reduce rates of rumen fermentation and fiber digestion in ruminants (Palmquist and Jenkins, 1980). Thus, rumen-protected fat (RPF) is used to enhance energy intake without compromising bacterial activity in the rumen (Jenkins and Palmquist, 1984). Dietary supplementation with RPF has had positive effects on dairy cows and beef cattle (McNamara et al., 2003; Hill and West, 1991). We

Table 1

Ingredients in the control concentrate and rumen-protected fat (RPF)-supplemented concentrate and composition of the concentrates and fescue hay fed to Korean cattle steers.

Items	Control concentrate	RPF concentrate	Tall-fescue hay
Ingredients, g/kg DM			
Steamed-flaked corn	153	136	
Wheat bran	200	200	
Rice bran	6.60	62.4	
Salt	5.90	3.50	
Soy hull	20.0	20.0	
Molasses	40.0	40.0	
Ammonium chloride	3.20	1.00	
Palm meal	100	100	
Condensed molasses soluble	15.0	11.0	
Coconut meal	100	100	
Live yeast	0.10	0.10	
Ground corn	80.0	80.0	
Corn gluten feed	200	200	
Beat pulp	25.7	8.60	
Limestone	28.6	27.6	
Bentonite	20.0	0.00	
Rumen protected fat (RPF)	0.00	8.00	
Mineral/vitamin premix ¹	1.90	1.90	
Total	1000	1000	
Chemical Composition, g/kg DM			
Concentrate diet			
Dry matter	886	883	891
Crude protein (CP)	145	145	65.6
Ether extract (EE)	36.3	53.8	15.8
Ash	99.8	79.0	58.7
Calcium	13.2	11.6	.20
Phosphorus	5.30	5.80	1.10
Neutral detergent fiber (aNDF)	273	276	658
Acid detergent fiber (ADF)	115	118	395
Non fiber carbohydrate (NFC)	343	340	
Starch	328	334	
Metabolizable energy (ME) ² , MJ/kg	12.1	12.9	
Net energy (NE) ³ , MJ/kg	5.41	5.94	

⁴Digestible energy = $0.04409 \times$ total digestible nutrient (%).

¹ Mineral and vitamin premix contained vit. A (2,650,000 IU), vit. D₃ (530,000 IU), vit. E (1,050 IU), niacin (10,000 mg), Mn (4,400 mg), Zn (4,400 mg), Fe (13,200 mg), Cu (2,200 mg), iodine (440 mg), and Co (440 mg/kg of Grobic-DC). Grobic-DC was provided by Bayer Health Care (Leverkusen, Germany).

² ME = $[1.01 \times (\text{digestible energy}^4) - 0.45] + 0.0046 \times (\text{EE} - 3)$ (NRC, 2016).

³ NE = $1.42 \text{ ME} - 0.174 \text{ ME}^2 + 0.0122 \text{ ME}^3 - 1.65$ (NRC, 2016).

previously conducted a study using 5 g/kg RPF concentrate, but supplementation at this level did not affect growth performance in Korean cattle steers, although RPF supplementation increased serum total cholesterol and high-density lipoprotein (HDL) concentrations (Kang et al., 2019a). In this study, the RPF concentration was increased to 8 g/kg. We investigated the effects of cold temperatures and 8 g/kg RPF supplementation on the growth performance, rumen fermentation, and blood biochemistry of Korean cattle steers during the early fattening stage.

2. Materials and methods

2.1. Animals, diet, and feeding

All experimental procedures were conducted as described in Kang et al. (2019a). Experimental procedures involving animals were approved by the Seoul National University Institutional Animal Care and Use Committee (SNU-IACUC), Republic of Korea, and conducted in accordance with the Animal Experimental Guidelines of the SNU-IACUC. This study was conducted at the University Animal Farm of the College of Agriculture and Life Sciences at the Pyeongchang campus of Seoul National University, South Korea. We used 20 Korean cattle steers aged 16.8 ± 0.34 months and a body weight (BW) of 356.5 ± 3.72 kg. All steers were fed a control concentrate (approximately 1.6% BW per animal) using an automatic feeding station (DeLaval Alpro System; DeLaval, Sweden) and tall fescue hay (3 kg/day/head) for 2 weeks prior to the experiment. Water was provided freely.

During the 12-week experimental period [January 8 to February 2 (P1), February 3 to March 3 (P2), and March 4 to April 1 (P3)], steers were either fed a concentrate with 8 g/kg RPF (treatment, approximately 1.6% BW per animal) or continued with the control concentrate (approximately 1.6% BW per animal) using an automatic feeding station. The pellet forms of the control concentrate and the RPF-supplemented concentrate were made by Cargill Agri Purina, Inc. (Seongnam-si, Republic of Korea). The RPF used in this study is a prilled form of palm oil with an energy density of 39.0 MJ/kg (Haneol Corp., Anseong, Korea); it is described in further detail in Naik (2013). The RPF comprises 996.3 g/kg free fatty acids, including 854.8 g/kg palmitic acid (C 16:0), 70.5 g/kg oleic acid (C 18:1), 34.5 g/kg myristic acid (C 14:0), 16.4 g/kg linoleic acid (C 18:2), and 10.4 g/kg lauric acid (C 12:0). The concentrate formula and chemical composition of the concentrates and fescue hay are shown in Table 1. Daily intake of concentrates was recorded online automatically using a computer running the DeLaval Alpro System software. Concentrate amounts were adjusted based on the BWs of individual animals at 4-week intervals. Steers were provided with two equal amounts of roughage at 08:00 h and 18:00 h daily. The residual roughage was weighed before each morning's feeding. Concentrate and tall fescue hay samples were collected weekly and stored at -20 °C until analysis. We calculated the intake of nutrients including dry matter (DM), crude protein (CP), ether extract (EE), neutral detergent fiber (aNDF), and acid detergent fiber (ADF) of each feed. The BW was measured before the morning feeding on the start day and at 4-week intervals thereafter.

2.2. Analysis of feed chemical composition

The dry matter (method 930.15), CP (Kjeldahl N \times 6.25, method 981.10), EE (method 920.39), ash (method 942.05), calcium (method 927.02), phosphorus (method 965.17), and starch (method 948.02) contents of the concentrates and tall fescue hay were determined using an analytical method provided by the Association of Official Agricultural Chemists (AOAC, 2000). The aNDF and ADF content of the concentrates and the tall fescue hay were analyzed using the sequential method in an ANKOM200 Fiber Analyzer (Ankom Technology Corp., Macedon, NY, USA). Details of the method and reagents used are described in Van Soest et al. (1991).

2.3. Measurement of ambient temperatures

Indoor and outdoor temperatures and relative humidity were recorded at 1-h intervals using four HOBO data loggers (Onset Computer Corp., Bourne, MA, USA). Mean, minimum, and maximum temperatures and relative humidity were recorded daily. Monthly data were obtained by averaging over 28 days each month. The experimental farm was covered with a roof and the animals were raised indoors; therefore, the animals were protected from rainfall and direct sunlight. Doors on both sides of the barn were kept open to allow the steers to be exposed to cold temperatures. As a result, low winds may have affected wind-chill temperatures.

2.4. Blood collection and analysis

Blood was collected before feeding at approximately 09:00 h (after 9 h of fasting) on day 1 of the experiment, and at 4-week intervals thereafter. Blood was collected via jugular venipuncture using a 20-mL non-heparinized vacutainer (Becton–Dickinson, Franklin Lakes, NJ, USA). The serum was then separated using centrifugation at $1,500 \times g$ at 4 °C for 15 min and stored at -80 °C.

Serum levels of glucose, triglyceride (TG), HDL, cholesterol, albumin, and non-esterified fatty acids (NEFAs) were determined using a biochemistry automatic analyzer (Hitachi 7180; Hitachi, Tokyo, Japan). The analytical reagents for glucose, TG, HDL, cholesterol, and albumin were purchased from JW Medical (Seoul, Korea), whereas the reagents used for determining the levels of NEFAs were purchased from WAKO (Osaka, Japan). All analytical methods were verified in a previous study (Kang et al., 2016).

2.5. Rumen-fluid collection and analysis

Rumen fluid was collected after 3 h of feeding using the oral stomach tube method, as described in Shen et al. (2012). The pH of

the rumen fluid was measured immediately using a pH meter (Ohaus Corp., Parsippany, NJ, USA). For the analysis of volatile fatty acids (VFAs), 1 mL of rumen fluid was mixed with 0.2 mL of 25% meta-phosphoric acid and stored at -20°C . Another 30 mL of rumen fluid was stored at -70°C for the analysis of ruminal ammonia nitrogen ($\text{NH}_3\text{-N}$). $\text{NH}_3\text{-N}$ concentrations were determined using a modified colorimetric method (Chaney and Marbach, 1962). VFA concentrations were determined using an Agilent Tech 7890A gas chromatograph (Hewlett Packard, Waldbronn, Germany) with a SUPELCOWAX 10 Capillary GC Column (length, 30 m; internal diameter, 0.25 mm; film thickness, 0.25 μm). The method was verified in a previous study (Kang et al., 2019a). Briefly, 1 μL of sample was injected with helium carrier gas. The inlet and detector temperatures were held at 220°C . An initial oven temperature of 80°C was maintained for 1 min, and afterward, the oven temperature was increased at $20^{\circ}\text{C}/\text{min}$ to 180°C , maintained for 1 min, and then increased at $10^{\circ}\text{C}/\text{min}$ to 200°C , followed by a final run time of 14 min.

2.6. Statistical analyses

Temperatures were compared among months using one-way analysis of variance by the R Studio for Windows software (R Studio, Boston, MA, USA). Data of growth performance and blood and rumen parameters were analyzed using a mixed-model with repeated-measure analysis by PROC MIXED of SAS 9.4 (SAS Institute, Cary, NC, USA). Fixed effects were month, diet, and their interaction, and random effects were animals within a diet group. Month was treated as a repeated measure. The best-fitted covariance structures were determined by the lowest value of Akaike information criterion from models with covariance structures of autoregressive, compound symmetry, unstructured, toeplitz, and variance components. Differences were considered significant at a level of $P \leq 0.05$, and a tendency was considered at $0.05 < P \leq 0.10$.

3. Results

3.1. Ambient temperatures

The mean (-7.37°C , -2.96°C , and 6.22°C) and minimum (-12.52°C , -8.18°C , and -2.11°C) indoor temperatures differed ($P < 0.001$) by period in the order $P1 < P2 < P3$ (Table 2). Mean temperatures were also recorded on days that blood and rumen fluid were collected. These temperatures were -7.92°C on January 8, -3.81°C on February 4, 2.87°C on March 4, and 11.84°C on April 1, 2016 (Table 4).

3.2. Growth performance

No interaction between RPF supplementation and month for all the parameters was observed. The mean BW was higher ($P < 0.001$) during P3 than P1 (Table 3). Total daily DM ($P = 0.01$) and CP ($P = 0.04$) intakes (kg/d) were higher in February compared to other months (Table 3). Total EE intake (kg/d) was not affected ($P = 0.15$) by month. Total aNDF and ADF intakes (kg/d) were higher ($P < 0.001$) during colder months. Concentrate DM intake (kg/d) tended to be lower ($P = 0.10$) in January compared to other months. In contrast, forage DM intake (kg/d) was affected ($P < 0.001$) by month and was higher in colder January.

Total EE intake (kg/d) was higher ($P < 0.001$) in the RPF supplementation group than in the control group. In contrast, total DM, CP, aNDF, and ADF intakes (kg/d) were not affected ($P \geq 0.70$) by RPF supplementation. Both concentrate and forage DM intakes (kg/d) were not affected ($P \geq 0.78$) by RPF supplementation.

Total nutrient intakes per body weight (kg/body weight) including DM, CP, EE, aNDF, and ADF were affected (all $P < 0.001$) by month, being higher in colder months, i.e., January or February. Both concentrate and forage DM intakes per body weight were higher ($P < 0.001$) in colder months. Total DM, CP, aNDF, and ADF intakes per body weight were not affected ($P \geq 0.31$) by RPF supplementation, but EE intake per body weight was increased ($P < 0.001$) by RPF supplementation. Both concentrate ($P = 0.30$) and forage ($P = 0.53$) DM intakes per body weight were not affected by RPF supplementation.

Table 2

Mean, maximum, and minimum indoor and outdoor temperatures from January to March 2016.

Items	January (P1) ¹	February (P2) ²	March (P3) ³	SEM ⁴	P value
Indoor temperature, $^{\circ}\text{C}$					
Mean	-7.37^{a}	-2.96^{b}	6.22^{c}	0.45	< 0.001
Maximum	-1.80^{a}	2.62^{b}	12.4^{c}	0.86	< 0.001
Minimum	-12.5^{a}	-8.18^{b}	-2.11^{c}	0.59	< 0.001
Outdoor temperature, $^{\circ}\text{C}$					
Mean	-8.79^{a}	-4.30^{b}	5.75^{c}	0.41	< 0.001
Maximum	-2.72^{a}	1.71^{b}	11.8^{c}	0.75	< 0.001
Minimum	-13.6^{a}	-9.94^{b}	-3.29^{c}	0.60	< 0.001

^{a-c}Mean values with different letters differ ($P < 0.05$).

¹ January 8 – February 2 (4 weeks).

² February 3 – March 3 (4 weeks).

³ March 4 – April 1 (4 weeks).

⁴ SEM = standard error of the mean.

Table 3

Growth performance of Korean cattle steers fed either the control concentrate or the concentrate supplemented with rumen-protected fat (RPF) from January to March 2016.

Items	January (P1) ¹		February (P2) ²		March (P3) ³		SEM ⁴	P value		
	Control	RPF	Control	RPF	Control	RPF		Diet	Month	Interaction
Age, month	16.9	16.8	17.8	17.7	18.8	18.7	0.40	0.87	< 0.001	0.70
Initial body weight, kg	358	355	375	376	394	393	4.57	0.93	< 0.001	0.46
Final body weight, kg	375	376	394	393	413	415	4.89	0.36	< 0.001	0.12
Average daily gain, kg/d	0.65	0.78	0.64	0.57	0.67	0.83	0.06	0.39	0.16	0.26
Total intake, kg/d										
DM	6.69	6.80	6.99	7.08	6.80	6.63	0.14	0.89	0.01	0.20
Crude protein	0.78	0.80	0.85	0.86	0.83	0.82	0.02	0.93	0.04	0.25
Ether extract	0.20	0.27	0.21	0.30	0.21	0.29	0.01	< 0.001	0.12	0.18
aNDF	2.72	2.80	2.72	2.77	2.60	2.54	0.05	0.75	< 0.001	0.15
ADF	1.42	1.47	1.39	1.42	1.33	1.29	0.03	0.70	< 0.001	0.15
Concentrate DM intake, kg/d	4.36	4.39	4.89	4.95	4.87	4.79	0.08	0.97	0.10	0.38
Forage DM intake, kg/d	2.33	2.41	2.10	2.13	1.93	1.84	0.06	0.78	< 0.001	0.17
Total intake, g/kg of body weight										
DM	18.3	18.3	17.6	18.1	16.5	14.1	0.38	0.33	< 0.001	0.14
Crude protein	2.15	2.13	2.14	2.19	2.01	1.73	0.04	0.31	< 0.001	0.16
Ether extract	0.53	0.74	0.53	0.77	0.50	0.61	0.02	< 0.001	< 0.001	0.21
aNDF	7.42	7.52	6.87	7.09	6.30	5.38	0.16	0.44	< 0.001	0.11
ADF	3.88	3.95	3.53	3.65	3.21	2.74	0.09	0.50	< 0.001	0.11
Concentrate DM intake, g/kg of body weight	11.9	11.8	12.3	12.6	11.8	10.2	0.25	0.30	< 0.001	0.15
Forage DM intake, g/kg of body weight	6.33	6.49	5.33	5.49	4.70	3.93	0.18	0.53	< 0.001	0.17
Feed efficiency (gain/feed)	0.097	0.115	0.092	0.081	0.098	0.125	0.009	0.26	0.57	0.49

N = 10/group.

¹ January 8 – February 2 (4 weeks).

² February 3 – March 3 (4 weeks).

³ March 4 – April 1 (4 weeks).

⁴ SEM = standard error of the mean.

Table 4

Ruminal parameters of each sampling day in Korean cattle steers after 3 h of feeding on the control concentrate or the concentrate supplemented with rumen-protected fat (RPF).

Items	January 8		February 4		March 4		April 1		SEM ¹	P value		
	Control	RPF	Control	RPF	Control	RPF	Control	RPF		Diet	Month	Interaction
Temperature ² , °C	-7.92		-3.81		2.87		11.84					
pH	6.14	6.29	6.31	6.10	6.56	6.54	5.97	6.15	0.56	0.34	0.57	0.80
NH ₃ -N, mg/dl	5.64	5.89	6.81	5.99	6.54	6.18	5.78	6.15	0.87	0.65	0.48	0.87
Total VFA, mM	133	133	90.5	80.4	85.3	79.9	99.4	86.9	3.27	0.26	0.001	0.31
VFA proportions, mol/100mol												
Acetate	65.2	65.8	67.1	67.6	67.6	67.4	67.2	67.3	0.31	0.80	0.28	0.98
Propionate	21.6	22.1	17.6	17.6	15.3	15.4	16.2	19.6	0.48	0.44	< 0.001	0.13
Iso-butyrate	0.46	0.40	0.84	0.77	0.77	1.67	0.77	0.72	0.14	0.37	< 0.001	0.42
Butyrate	11.0	10.3	12.0	12.2	14.3	13.6	13.8	10.5	0.33	0.03	0.01	0.09
Iso-valerate	0.63	0.54	1.27	0.91	0.80	0.78	0.77	0.75	0.04	0.23	< 0.001	0.65
Valerate	1.08	0.95	1.21	0.97	1.19	1.07	1.26	1.18	0.04	0.24	0.64	0.90
Acetate to propionate ratio	3.05	3.04	3.89	3.96	4.49	4.41	4.20	3.64	0.54	0.76	0.21	0.74

N = 10/group.

¹ SEM = standard error of the mean.

² Average indoor temperatures of four sampling times of each day.

The average daily gain and gain-to-feed ratios were not affected ($P \geq 0.16$) by month or RPF supplementation.

3.3. Ruminal fermentation parameters

Ruminal pH and NH₃-N concentration were not affected ($P \geq 0.34$) by month or RPF supplementation (Table 4). Total VFA concentrations were affected ($P < 0.001$) by the month, being higher in the colder month of January compared to other months.

The propionate proportion was higher ($P < 0.001$) in colder January, whereas the butyrate proportion was lower ($P = 0.01$). Cold temperatures did not affect ($P \geq 0.21$) the acetate and valerate proportions or the acetate:propionate ratio. The iso-butyrate and iso-valerate proportions were affected ($P < 0.001$) by the month, but this difference was not related to temperature.

The total VFA concentrations were not affected ($P = 0.26$) by RPF supplementation. The butyrate proportion was lower ($P = 0.03$) in the RPF supplementation group than in the control group in March and April, but not in January and February. Other VFA proportions, including acetate ($P = 0.80$), propionate ($P = 0.44$), iso-butyrate ($P = 0.37$), iso-valerate ($P = 0.23$), and valerate ($P = 0.24$), and the acetate:propionate ratio ($P = 0.76$) were not affected by RPF supplementation.

3.4. Blood metabolites

Serum glucose concentrations were affected ($P = 0.008$) by the month, being higher in colder January and February compared to other months (average values for each month: 80.6 mg/dL in January, 79.8 mg/dL in February, 75 mg/dL in March, and 77.7 mg/dL in April; Table 5). Serum TG ($P = 0.007$), total cholesterol ($P = 0.001$), and HDL ($P = 0.03$) concentrations were lower during the colder months, but serum albumin and NEFA concentrations were not affected ($P \geq 0.70$) by the month. Serum cholesterol concentrations were higher ($P = 0.008$) in the RPF supplementation group than in the control group. However, serum metabolites, including glucose ($P = 0.11$), TG ($P = 0.86$), HDL ($P = 0.59$), albumin ($P = 0.34$), and NEFA ($P = 0.59$) concentrations, were not affected by RPF supplementation.

4. Discussion

4.1. Ambient temperatures at the experimental farm

Cattle with dry winter coats are considered to experience mild CS at temperatures of 0 °C to -6.7 °C, moderate CS at -7.2 °C to -13.9 °C, and severe CS at < -13.9 °C (Grzych, 2010). Thus, the mean temperatures during P1 (-7.37 °C) and P2 (-2.96 °C) could be associated with moderate or mild CS, whereas P3 (6.22 °C) temperatures were considered thermoneutral. However, temperature ranges associated with CS remain to be defined for Korean cattle breeds. Thus, there are likely differences in the temperature ranges for various states of CS such as moderate, mild, and severe for Korean cattle compared with other cattle breeds. The CS tolerance varies depending on the breed; for example, robust and slow-growing cattle such as Scottish Highlander, Galloway, Hereford, and Aberdeen Angus are assumed to be relatively resistant to CS, whereas cold conditions do not seem well suited for raising highly productive cattle such as Jersey, Holstein, Limousin, Charolais, and Belgian Blue (Wallis de Vries, 1994).

4.2. Growth performance

Under cold conditions, farm animals not only consume more feed, but also grow slower and produce less milk, because additional energy is required for metabolic maintenance, and so less energy from food goes towards productive processes (Young, 1981). Nevertheless, growth performance (average daily gain and the gain-to-feed ratio) was not affected by cold temperatures in this study. In Korean cattle steers reared under thermoneutral conditions and of a similar age (16–18 months) as those in this study, the average daily gain was between 0.58 and 0.81 kg/d (Kwon et al., 2005), similar to the weight gain in the current study (0.57–0.83 kg/d). In this study, the forage DM intake (kg/d) was higher in colder January than in other months, and the concentrate DM intake tended to be lower in colder January. However, the daily intake (kg/d) was affected by both temperature and the age of the animal, as both body weight and intake increased with age. Thus, we calculated feed intake/body weight. Both concentrate and forage intakes (kg/body weight) were higher in the colder months of January or February. Our study indicates that the increase in concentrate and forage intakes during the colder months contributed to the increase in total feed intake. Our results are consistent with a previous study that showed increased feed intake during colder months (Young, 1981). Therefore, increased nutrient intakes likely provided the additional nutrients needed by the animals during the cold period, resulting in no changes in weight gain and feed efficiency. Furthermore, increased forage intake may contribute to alleviating CS through the generation of metabolic heat; for example, a high-fiber diet produced greater metabolic heat in growing beef heifers (Reynolds et al., 1991). One possible explanation for the increased

Table 5

Fasting serum parameters for each sampling day in Korean cattle steers fed either the control concentrate or the concentrate supplemented with rumen-protected fat (RPF).

Items	January 8		February 4		March 4		April 1		SEM ¹	P value		
	Control	RPF	Control	RPF	Control	RPF	Control	RPF		Diet	Month	Interaction
Glucose, mg/dL	72.3	88.0	78.9	80.7	73.3	76.7	74.8	80.6	1.23	0.11	0.008	0.67
Triglyceride, mg/dL	10.7	12.5	16.4	17.0	20.2	19.6	17.4	19.1	0.74	0.86	0.007	0.65
Cholesterol, mg/dL	109	137	122	158	137	158	161	197	5.48	0.008	0.001	0.52
HDL ² , mg/dL	68.4	73.8	83.8	75.8	81.8	82.3	89.0	91.7	2.12	0.59	0.03	0.31
Albumin, mg/dL	2.99	3.25	3.50	3.15	3.03	3.00	3.37	3.44	0.06	0.34	0.80	0.22
NEFA ³ , mg/dL	229	279	194	132	196	215	234	294	14.8	0.59	0.70	0.41

N = 10/group.

¹ SEM = standard error of the mean.

² HDL = high density lipoprotein.

³ NEFA = non-esterified fatty acid.

forage intake during colder conditions is an increase in the passage rate in the reticulo-rumen (Kennedy et al., 1976). Meanwhile, the optimum temperature range for raising Korean cattle has not yet been established. Another possible explanation for the lack of a decrease in growth performance during cold temperatures is that the temperatures experienced by the cattle during our study period may not have been cold enough to affect their growth performance.

The growth of steers decreased under CS, as the ME requirement for maintenance increased (Delfino and Mathison, 1991). Nutrients with high energy density such as fats may be used to alleviate CS. When 40–60 g/kg of unprotected dietary fats were added to the finishing diets of cattle to increase dietary energy density under thermoneutral conditions, the average daily gain increased, but feed intake decreased (Zinn and Plascencia, 1996; Zinn, 1989). RPF could be supplemented instead of unprotected fat, as the use of RPF may mitigate the inhibitory effect of fat supplementation on feed intake in ruminants (Haddad and Younis, 2004). In our previous study, supplementation with 5 g/kg RPF during winter did not affect the growth performance of Korean cattle steers (Kang et al., 2019a). In this study, we increased the supplementation level to 8 g/kg, but did not observe changes in growth performance. In a study with beef cattle, supplementation with 45 g/kg RPF comprising the calcium salts of fatty acids in a corn-based diet also did not improve growth performance (Hill and West, 1991). The authors suggested that the absence of change in growth performance was due to decreased dry matter intake with fat supplementation. However, concentrate DM intake was not affected by RPF supplementation in the current study, although EE intake was increased. As described above, the average daily gain of steers of a similar age under thermoneutral conditions in another study (Kwon et al., 2005) was similar to those in our study. Collectively, our results indicate that neither a cold environment nor RPF supplementation affected animal growth. A possible explanation is that energy intake levels with the control concentrate were sufficient to support maintenance and growth, and there was no increase in growth due to the additional energy supplied through RPF supplementation.

4.3. Rumen fermentation parameters

Ruminal pH and $\text{NH}_3\text{-N}$ concentrations were not affected by the month. Rumen pH was elevated in cold-exposed wethers (Kennedy, 1985; Kelly and Christopherson, 1989), in contrast to our results. Ruminal ammonia concentrations in the wethers did not differ between warm and cold conditions (Kennedy et al., 1976), but these were lower in cold-exposed wethers (Kennedy and Milligan, 1978). The different responses of ruminal pH and ammonia concentrations under cold conditions may be due to species differences (cattle vs. sheep) or cold intensity. Another explanation for the lack of changes in ruminal pH and ammonia concentrations is that the cold conditions were not severe enough to have an effect on these parameters.

Higher total VFA concentrations were observed during colder January than in the other months. In our previous study, cold temperatures from early February (mean ambient temperature = -0.26 °C) to early May (mean ambient temperature = 14.0 °C) in 2015 did not affect total VFA concentrations (Kang et al., 2019a), in contrast to the findings of the present study. In this study, VFA concentrations were measured starting in early January 2016, which had a mean indoor temperature of -7.92 °C. Measurements were taken until early April, which had a mean indoor temperature of 11.4 °C. Thus, the temperature on the first day that VFA concentrations were measured was lower in this study than that in the 2015 study (Kang et al., 2019a). A study of sheep also showed no change in total VFA concentrations due to cold exposure (Kennedy and Milligan, 1978). However, in other studies, cold-exposed sheep had lower total VFA concentrations in the rumen (Kennedy et al., 1976; Kelly and Christopherson, 1989; Kennedy, 1985), possibly associated with an increased digesta passage rate and, consequently, a reduced rumen fermentation rate (Kennedy, 1976).

The propionate proportion was higher in colder January than in other months. Similarly, the propionate proportion increased when sheep were exposed to cold (Kennedy et al., 1976; Kennedy and Milligan, 1978; Kennedy, 1985; Kelly and Christopherson, 1989). This increased propionate proportion may be due to the reduced fermentation rates of cellulose and hemicellulose during cold weather, as reported in cold-exposed sheep (Kennedy and Milligan, 1978). In contrast, the butyrate proportion was lower in colder January than in other months, similar to the results of the sheep study (Kennedy and Milligan, 1978). Cold temperatures did not affect the proportions of acetate and valerate and the acetate:propionate ratio. The finding showing no difference in the acetate proportion during the cold months is not consistent with other studies, which found lower acetate proportions in cold-exposed sheep (Kennedy and Milligan, 1978; Kennedy, 1985; Kelly and Christopherson, 1989), possibly associated with the reduced rumen fermentation rate through the increased digesta passage rate (Kennedy, 1976). The inconsistencies in total VFA concentrations and individual VFA proportions under cold conditions may be due to differences in animal species (cattle vs. sheep), age, or cold intensity.

RPF has been used to increase dietary energy density without affecting rumen fermentation (Beede and Collier, 1986). In the current study, RPF supplementation did not affect the major ruminal VFA characteristics, including total VFA concentrations, proportions of acetate, propionate, iso-butyrate, iso-valerate, and valerate, and the acetate:propionate ratio. Similarly, no differences in VFA concentrations were reported for 5 g/kg RPF supplementation under cold conditions (Kang et al., 2019a) or 8 g/kg RPF supplementation during the hot season (Kang et al., 2019b). During digestion, RPF may be moved into the small intestine without affecting rumen fermentation. RPF supplementation decreased the butyrate proportion in the rumen. Similar results were reported for dairy cows, which had a lower butyrate proportion in the rumen when levels of protected lipids in their dietary supplement were increased (Bines et al., 1978).

4.4. Blood metabolites

Consistent with our previous study (Kang et al., 2019a), serum glucose concentrations were higher during colder months of January and February compared to the other months. Higher glucose levels during the cold season are associated with increased metabolic or heart rates (Young, 1975). Serum glucose concentrations were unaffected by RPF supplementation. Similarly,

supplementation with 50 g/kg unprotected fat of DM intake did not affect circulating glucose concentrations in lactating beef cows (Lake et al., 2005). By contrast, the intake of protected fat, of which 25% of the metabolizable energy is fat, increased plasma glucose concentrations in dairy cows (Kronfeld et al., 1980). Kronfeld et al. (1980) suggested that the additional fat supply may spare glucose utilization, resulting in increased glucose levels. The varying glucose levels in response to fat supplementation may be due to differences in levels of fat supplementation, and cattle breeds and age. Thus, RPF supplementation at 8 g/kg may be insufficient to change glucose concentrations significantly.

TG, total cholesterol, and HDL concentrations were lower in January and February (CS conditions) than in March (thermo-neutral). Acute cold exposure has been shown to increase the oxidation of free fatty acids in the arterial blood of young steers by causing the steers to shiver (Bell and Thompson, 1979). Collectively, these results suggest that lipid metabolism under cold conditions may be adapted to mobilize fat to provide energy for heat generation and the maintenance of body temperature.

Total cholesterol concentrations increased when cattle were supplemented with RPF in this study. Similarly, in our previous study, 5 g/kg RPF supplementation under cold conditions led to increased total cholesterol concentrations (Kang et al., 2019a). Holstein calves that were supplemented with approximately 30 g/kg fat from 3 days of age exhibited higher total cholesterol concentrations in the plasma at age 70 days, but not at age 35 days, during the cold season (Ghasemi et al., 2017). These increases in cholesterol concentrations following RPF supplementation indicate that higher levels of dietary fat were transferred into the blood (Park and Rafalowski, 1983). RPF supplementation could be used to provide high energy levels in animals experiencing CS, considering that total cholesterol levels decreased under cold conditions and that RPF supplementation increased cholesterol levels. Meanwhile, HDL concentrations were not affected by RPF supplementation.

Cold temperatures did not affect circulating NEFA concentrations in this study. Similarly, no changes in NEFA concentrations were observed under cold conditions in our previous study (Kang et al., 2019a). On the other hand, plasma NEFA concentrations were higher when sheep were exposed to cold (Sano et al., 2007). Plasma NEFA concentrations were not affected by RPF supplementation in this study. Similarly, no changes in NEFA concentrations were observed with 5 g/kg RPF (Kang et al., 2019a). However, RPF supplementation increased plasma NEFA concentrations in lactating dairy cows (Duske et al., 2009). These inconsistent results for plasma NEFA concentrations following exposure to cold conditions or RPF supplementation may be due to differences in animal species (cattle vs. sheep), cattle breed or age, or cold intensity. Another possible explanation is that the cold conditions and RPF supplementation at 8 g/kg may have been insufficient to affect plasma NEFA concentrations in this study.

5. Conclusion

Our results reveal that mild or moderate CS does not affect the growth performance of Korean cattle steers at the early fattening stage. However, cold temperatures increase concentrate and forage intakes and affect rumen fermentation and blood metabolite levels. Supplementing the concentrate with 8 g/kg RPF did not improve growth performance under cold environments, although it did not decrease nutrient intakes except for EE and ruminal total VFA concentrations.

CRedit authorship contribution statement

Hyeok Joong Kang: Investigation, Formal analysis, Conceptualization, Data curation, Formal analysis, Writing - original draft. **Jinoh Lee:** Investigation, Methodology. **Seung Ju Park:** Investigation, Methodology. **Dajinsol Jung:** Investigation, Methodology. **Sang Weon Na:** Investigation, Methodology. **Hyun Jin Kim:** Investigation, Methodology. **Myunggi Baik:** Conceptualization, Investigation, Supervision, Writing - review & editing, Funding acquisition.

Declaration of Competing Interest

The authors declare that there are no conflicts of interest.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:<https://doi.org/10.1016/j.anifeedsci.2020.114624>.

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