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Concentrate supplementation: A way to mitigate enteric methane emissions in pregnant Hanwoo heifers

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Abstract

The objective of this study was to investigate the effect of supplementing concentrates in a forage-based diet on methane emissions of pregnant Hanwoo heifers. Twenty-one pregnant Hanwoo heifers (481 ± 42.4 kg) were divided into two groups: 1) a group receiving forage only (control, CON) and 2) the other group receiving forage with 4 kg of a concentrate mix (treatment, TRT). Methane (CH_4) concentration was measured using a laser methane detector, following an 18-d adaptation period, according to previously established protocols. Feed intake was recorded throughout the experimental period. Ruminal fluid was collected and analyzed for pH, ammonia-nitrogen (NH_3-N), and volatile fatty acid (VFA). The TRT exhibited higher dry matter and neutral detergent fiber intake than CON ($p < 0.05$) with elevated NH_3-N ($p < 0.001$) and total VFA concentrations ($p = 0.013$). The proportions of butyrate, valerate, and iso-valerate were higher in TRT than CON ($p < 0.05$). Notably, CH_4 concentrations per kg dry matter intake was lower in the TRT group, both from respiration and eructation ($p < 0.05$). In conclusion, supplementing concentrates in a low-quality forage-based diet for pregnant Hanwoo heifers fulfills nutrient requirements and reduces CH_4 emissions, suggesting a potential strategy to reduce environmental impact of Hanwoo production.

Keywords: eructation, Hanwoo, laser methane detection, respiration, rumen characteristics

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Introduction

Pregnant beef heifers are often fed only forage as their diet (Carlson et al., 2022). Feeding high-quality forage hay should supply sufficient nutrients to meet the nutrient requirements of beef heifers (Adams et al., 1996). Nevertheless, supplementation of corn, by-products, and crop residues can offer economic benefits, given the reduction in perennial grasslands and the high

cost of hay (Carlson et al., 2022). Moreover, in countries like Korea, which heavily rely on imported hay and domestic rice straw for cattle feeding (Chang, 2018), a forage-based diet is often found to be insufficient in providing necessary nutrients for cattle (Aquino et al., 2020). Inadequate nutrition during pregnancy potentially leads to complications such as calf mortalities and respiratory or intestinal dysfunction (Wu et al., 2006). Thus, maintaining body condition during gestation and lactation by providing sufficient nutrients is crucial for the development of the uterus, fetal growth, and the subsequent calf's weight gain (Funston et al., 2010). In this regard, concentrate supplementation is crucial and cost-effective for the productivity of low-quality forage-feeding heifers (Pritchard and Males, 1982).

In addition to its nutritional and economic advantages, concentrate supplementation may also offer environmental benefits. Dietary forage leads to higher production of acetate and hydrogen during anaerobic fermentation, resulting in increased methane (CH_4) production (Janssen, 2010). Methane is one of the greenhouse gases produced by livestock farming, which is reported to be responsible for 30% of global greenhouse gas emissions (FAO, 2023). Furthermore, methanogenesis in the rumen also represents an energy loss, ranging from 2 to 12% of the gross energy intake (Johnson and Johnson, 1995). Previous research has demonstrated that concentrate supplementation (forage : concentrate = 50 : 50) in dairy cows reduces CH_4 production per unit of milk production (Patel et al., 2011). Bayat et al. (2017) reported that a diet with a forage-to-concentrate ratio of 35 : 65 reduced CH_4 emissions, lowered pH levels, and increased volatile fatty acid (VFA) production by organic matter intake in lactating cows. In Jersey and Holstein cows, concentrate supplementation at levels of 70% and 91% led to a modified rumen VFA profile and reduced CH_4 gas emissions (Olijhoek et al., 2022). Based on these findings, it is hypothesized that concentrate mix supplementation can meet the nutrient requirements of pregnant heifers while simultaneously reducing CH_4 emissions. However, this has not yet been studied in pregnant Hanwoo heifers.

Furthermore, CH_4 emissions from cattle in farm settings should be assessed with a large number of animals in practical conditions. The laser methane detection (LMD) method has been proven effective for this purpose (Chagunda et al., 2013). The LMD utilizes infrared absorption spectroscopy and incorporates a semiconductor laser as a focused excitation source. It employs the technique of second harmonic detection in wavelength modulation spectroscopy to determine the concentration of CH_4 (Chagunda et al., 2009). Kang et al. (2022) have developed protocols and further validated the LMD's capability to distinguish CH_4 emissions in a large number of cattle under practical conditions. Therefore, this study aimed to assess CH_4 emissions using LMD in a large population of pregnant Hanwoo heifers under practical field conditions when a low-quality forage-based diet was supplemented with a concentrate mix to provide more nutrients.

Materials and Methods

This study was conducted at the Center for Animal Science Research, Chungnam National University (CNU), Korea. The use of animals and protocols for this experiment were reviewed and pre-approved by the CNU Animal Research Ethics Committee (202203A-CNU-058).

Animals, experimental design, and diet

A total of twenty-one Hanwoo heifers (481 ± 42.4 kg) were used in this experiment. All the Hanwoo heifers were bought when they were of similar age (8 mo) from a local commercial Hanwoo market (Korea). They were vaccinated

upon arrival at the CNU Animal Science Research Centre and were raised here until the experiment. Initially, there were 25 heifers, of which 4 were excluded because they were not pregnant. The experiment lasted for 24 d, consisting of 18 d of adaptation and 6 d of measuring periods. Each two heifers of similar BW were housed in a pen (5 m × 5 m) equipped with a forage feed bin, which enabled us to automatically determine individual forage intake (Dawoon Co., Korea). Heifers were divided into two groups: 1) a group receiving forage only (n = 11; control, CON) and 2) the other group receiving forage supplemented with 4 kg as-fed of a concentrate mix (n = 10; treatment, TRT). The formulation and chemical composition of the experimental diets are shown in Tables 1 and 2, respectively. The heifers were fed twice daily at 08:00 and 18:00 h. Forage and drinking water were freely accessible to the animals throughout the experiment. For the TRT group, each heifer was individually offered a concentrate mix of 4 kg per day. These pregnant heifers had a 30-min to consume the concentrate mix, after which any remaining amount was measured to determine actual intake. Roughage intake was measured daily using an automatic feed intake measurement device (Dawoon Co., Korea). Consequently, this allowed for the measurement of dry matter intake (DMI) during the experimental period.

Table 1. Diet formulation of the experimental diet.

Ingredients ($g \cdot kg^{-1}$ DM)	Concentrate mix
Corn, ground	130
Wheat, ground	90
Bakery byproduct	30
Copra meal	70
Corn gluten feed	120
Rice bran	30
Wheat bran	65
Almond shell	70
Palm kernel meal, expeller	160
Palm kernel meal, solvent extraction	60
Rapeseed meal	40
Sunflower meal with hull	30
Limestone	31
Molasses	65
Salt	6
Sodium bicarbonate	1
Vitamin and mineral mix[z]	2

[z] 33,330,000 $IU \cdot kg^{-1}$ vitamin A; 40,000,000 $IU \cdot kg^{-1}$ vitamin D; 20.86 $IU \cdot kg^{-1}$ vitamin E; 20 $mg \cdot kg^{-1}$ Cu; 90 $mg \cdot kg^{-1}$ Mn; 100 $mg \cdot kg^{-1}$ Zn; 250 $mg \cdot kg^{-1}$ Fe; 0.4 $mg \cdot kg^{-1}$ I; and 0.4 $mg \cdot kg^{-1}$ Se.

Table 2. Analyzed chemical composition ($g \cdot kg^{-1}$ DM or as stated) of the experimental diets (Continued).

Items	Concentrate mix	Tall fescue
DM ($g \cdot kg^{-1}$ as fed)	895	893
OM	886	930
CP	155	72
SOLP	59	28
NDICP	39	14

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

Items	Concentrate mix	Tall fescue
ADICP	16	9
aNDF	418	716
ADF	246	426
ADL	70	59
Ether extract	22	11
Ash	114	70
Ca	17	3
P	6	2
K	12	23
Na	4	1
Cl	9	5
S	4	2
Mg	4	2
TDN	604	528
NEm (MJ·kg^{-1} DM)	5.8	4.4
NEg (MJ·kg^{-1} DM)	3.3	2.1
Total carbohydrates	709	847
NFC	309	144
Carbohydrate fraction (g·kg^{-1} carbohydrate)		
CA	87	63
CB1	253	2
CB2	96	106
CB3	299	660
CC	235	168
Protein fraction (g·kg^{-1} CP)		
PA + B1	378	386
PB2	368	415
PB3	149	73
PC	105	126

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).

Measuring CH_4 emissions using LMD

The CH_4 concentration in the exhaled gas of the animals was measured using the LMD according to Kang et al. (2022). Briefly, the LMD was installed on a tripod, aiming at the animal's nostril from a distance of 1 m. The CH_4 concentrations were measured every 0.5 s for 6 min. The measurements were performed four times (-2, -1, and +1, +2

h after the morning feed) daily for all heifers, which were duplicated over 2 d. For data analysis, the peaks of the CH_4 concentration measured by the LMD were detected using the automatic multiscale-based peak detection (AMPD) R package. The peaks were divided into two pathways (respiration and eructation) by fitting a double normal distribution using the mixdist R package. A larger number of peaks belonged to respiration, but they were of lower value. The mean of the normal distribution was assumed to be the representative CH_4 concentration of the exhaled gas from the pathway for the hour. The four-time values of a day were averaged to represent the mean CH_4 concentration of the day, and the 2 d' mean values were averaged.

Feed analysis

The diet samples were dried at 60℃ for 96 h and ground through a cyclone mill (Foss, Denmark) and screened through a 1 mm screen before chemical analysis. The nutrient composition of the samples was analyzed at Cumberland Valley Analytical Services Inc. (USA). Feed analysis procedures are described in detail in another study (Jeon et al., 2016). The contents of DM (#934.15), CP (#990.03), ether extract (#920.39), acid detergent fiber (#973.18), and ash (#942.05) were determined according to AOAC International (2006). The total nitrogen was measured by the Dumas method using a Leco FP-528 Nitrogen Combustion Analyzer (Leco Inc., USA). CP was calculated as 6.25 times the nitrogen content. A heat-stable amylase was used to assess the acid detergent lignin (ADL) content and the neutral detergent fiber (NDF) content, which was then expressed inclusive of residual ash (aNDF). The soluble protein, neutral detergent insoluble crude protein (NDICP), and acid detergent insoluble crude protein (ADICP) contents were also determined. The contents of ethanol soluble carbohydrates (ESC), starch, and macro and micro-mineral content were determined.

The content of total digestible nutrient (TDN), net energy for maintenance, and net energy for growth were estimated using NRC (2001) equations. The dietary carbohydrate and protein fractions were estimated according to the Cornell Net Carbohydrate and Protein System (Fox, 2003) with the following modifications. The different carbohydrate fractions were classified as follows: Carbohydrate A fraction (CA) represented sugars and organic acids and was assumed to be equivalent to ESC. Carbohydrate B1 fraction (CB1) referred to starch. Carbohydrate B2 fraction (CB2) was calculated as the soluble fiber, obtained by subtracting CA and CB1 from NFC (non fiber carbohydrates). Carbohydrate B3 fraction (CB3) represented the available NDF, estimated by subtracting 2.4 times ADL from aNDF. Carbohydrate C fraction (CC) indicated the unavailable carbohydrate, estimated as 2.4 times ADL. Concerning the protein fractions, PA + B1 (protein A and B1 fractions) denoted the soluble protein, which was identical to soluble CP (crude protein) or soluble CP (SOLP). PB2 referred to the intermediate degradable CP, estimated as 100-NDICP-SOLP. Protein B3 fraction (PB3) represented the slowly degradable fiber-bound CP, estimated as NDICP-ADICP. Lastly, protein C fraction (PC) indicated the unavailable CP, which was equal to ADICP. All the carbohydrate and protein fractions were expressed as grams per kilogram of total carbohydrate or CP, respectively. Minerals were analyzed according to AOAC International (2006) method.

Rumen sampling and analysis

Rumen samples were collected from all heifers over three consecutive days to ensure the acquisition of representative samples for each feeding cycle: at 14:00 (6 h post-feeding) on day 1, 11:00 (3 h post-feeding) on day 2, and 07:00

(before feeding) on day 3. The collection was performed using esophageal stomach tubing, following the method described by Lee et al. (2019). Briefly, after discarding the initially obtained ruminal fluid (approximately 200 mL), 500 mL of ruminal fluid was collected in a glass flask. The rumen contents were instantly subjected to pH measurement (EcoMet P25, Istek Inc., Korea), and the remaining samples were stored at -20℃.

Samples were then transferred to the laboratory, where they were thawed and centrifuged at 14,000 × g for 10 min at 4℃ for VFA and ammonia-N analysis, as described in detail by Lee et al. (2019). Briefly, for the VFA analysis, ruminal fluid supernatant (1 mL) was mixed with 0.2 mL of metaphosphoric acid (250 $g \cdot L^{-1}$) and kept at 4℃ for 30 min. Following centrifugation of the mixture at 14,000 × g for 10 min at room temperature, the supernatant was injected into a gas chromatograph (HP 6890, Hewlett-Packard Co., USA) equipped with a flame ionization detector and capillary column (Nukol Fused silica capillary column 30 m × 0.25 mm × 0.2 μm, Supelco Inc., USA). The temperature of the oven, injector, and detector was 90℃ to 180℃, 185℃, and 210℃, respectively. Nitrogen was used as the carrier gas at a flow rate of 40 $mL \cdot min^{-1}$. For the NH_3-N analysis, the stored ruminal fluid underwent re-centrifugation at 21,000 × g for 15 min, and 20 μL of the supernatant was mixed with 1 mL of phenol color reagent and 1 mL of alkali-hypochlorite reagent. The mixture was then incubated in a water bath for 15 min at 37℃. After being mixed with 8 mL of distilled water, the optical density of the mixture was measured at 630 nm, using a spectrophotometer (UV-1800, Shimadzu Corp., Japan).

Statistical analysis

The animals were used as unit per treatment where animal number was within power range. The data were checked for normality and homoscedasticity before further analysis. All statistical analysis was performed using SAS software (SAS Institute Inc., USA). The data was analyzed using PROC TTEST. Significance was declared at $p < 0.05$, and a trend was discussed at $0.05 \leq p < 0.1$.

Results and Discussion

The metabolic demands for energy and protein escalate during the latter stage of gestation in pregnant heifers, a critical period for sustaining both maternal health and fetal growth (Patterson et al., 2003; Gionbelli et al., 2016). Considering the nutritional requirement, it might be necessary to supplement energy and protein, especially when the diet is based on low-quality forage (Schillo et al., 1992).

The results of concentrate supplementation on feed intake are shown in Table 3. In the TRT group, DMI increased by 2.4 kg per day compared to CON ($p < 0.001$). This rise was mainly due to the voluntary intake of concentrate mix at the rate of 3.5 kg DM. Furthermore, concentrate supplementation resulted in a 24% decrease in forage intake in the TRT group ($p = 0.002$). However, despite this reduction in forage consumption, there was a 38% increase in total NDF intake (NDFI) due to the concentrate supplementation ($p = 0.014$).

In cattle, when a nutrient-deficient diet is supplemented with concentrate, DMI increases, which aligns with our findings (DelCurto et al., 2000). Stafford et al. (1996) indicated a 16% increase in total DMI when poor quality tallgrass was supplemented with a concentrate mix (CP 17.5%) at 0.15% of BW in growing beef steers. In their study, the concentrate supplementation did not alter forage intake. However, Loy et al. (2007) reported decreased forage DMI in beef heifers when forage was supplemented with concentrates. In their study (Loy et al., 2007), concentrate

supplementation at 0.40% of BW increased total DMI by 12%, while forage feed intake was reduced by 10% in heifers. Conversely, protein supplementation could increase forage intake (DelCurto et al., 2000). The impact of concentrate supplementation in forage and total DMI appears to depend on nutritional balance, frequency of feeding, and the types of forage and concentrates in the diet (Huston et al., 1999; Loy et al., 2007).

Table 3. Effect of different forage to concentrate ratio diet on feed intake of Hanwoo heifers.

Items (kg)	Treatment		SEM	p-value
	CON	TRT		
Initial BW	480	483	19.2	0.851
fDMI	4.2	3.2	0.36	0.002
cDMI	-	3.5	-	-
Total DMI	4.2	6.8	0.44	< 0.001
Total NDFI	2.9	3.8	0.30	0.014

CON (control), receiving forage only; TRT (treatment), receiving forage with 4 kg concentrate mix; SEM, standard error of the mean; BW, body weight; fDMI, forage dry matter intake; cDMI, concentrate dry matter intake; DMI, dry matter intake; NDFI, neutral detergent fiber intake.

Supplementation of concentrate slightly reduced ($p < 0.05$) the pH of ruminal fluid in the TRT group (Table 4). The concentrate supplementation significantly elevated rumen NH_3-N concentration ($p < 0.001$), maintaining it above 5 $mg \cdot L^{-1}$, which is the minimum concentration for optimal rumen microbial protein synthesis (Firkins et al., 2007). There was also a 12% increase in the total VFA concentration in the rumen of TRT group animals ($p = 0.013$). In the TRT group, rumen acetate concentration was decreased compared to CON ($p < 0.001$). On the other hand, concentrate supplementation increased the concentrations of butyrate ($p < 0.001$), valerate ($p < 0.001$), and iso-valerate ($p = 0.041$) in the rumen. Propionate concentration did not differ between the groups ($p > 0.05$). Finally, concentrate supplementation tended to decrease the acetate : propionate ratio ($p = 0.074$).

Table 4. Effect of different forage to concentrate ratio diet on ruminal fluid characteristics of Hanwoo heifers.

Items	Treatment		SEM	p-value
	CON	TRT		
pH	6.90	6.72	0.084	0.032
NH_3-N ($mg \cdot dL^{-1}$)	3.12	6.79	0.789	< 0.001
Total VFA (mM)	65.7	73.9	2.98	0.013
Acetate ($mmol \cdot mol^{-1}$)	691	656	4.9	< 0.001
Propionate ($mmol \cdot mol^{-1}$)	187	185	3.1	0.517
Butyrate ($mmol \cdot mol^{-1}$)	73	105	2.9	< 0.001
Iso-butyrate ($mmol \cdot mol^{-1}$)	19	19	0.9	0.615
Valerate ($mmol \cdot mol^{-1}$)	14	18	0.6	< 0.001
Iso-valerate ($mmol \cdot mol^{-1}$)	16	18	0.8	0.041
Acetate : Propionate	3.69	3.55	0.072	0.074

CON (control), receiving forage only; TRT (treatment), receiving forage with 4 kg concentrate mix; SEM, standard error of the mean; NH_3-N, ammonia nitrogen; VFA, volatile fatty acid.

In our study, ruminal NH_3-N concentration in the CON group was only 3.12 mg·dL^{-1}, which might limit the metabolizable protein (MP) supply to the animals. A ruminal NH_3-N concentration below 5 mg·dL^{-1} is generally considered insufficient for adequate ruminal microbial protein synthesis (Firkins et al., 2007), which is crucial for meeting the MP requirements of ruminants. This lower NH_3-N concentration in the CON group might be an indicator of nutrient deficiency of a forage-based diet. With high-quality forage, this might not be an issue, as it can provide sufficient NH_3-N for effective microbial protein synthesis (Broderick, 1995). However, low-quality forage often requires concentrate supplementation, recognizing the importance of nitrogen availability.

Concentrate supplementation altered the rumen ecosystem as expected. It increased total VFA production and reduced pH in the rumen, which is consistent with previous studies (Sun et al., 2010; Jiang et al., 2022). Highly fermentable carbohydrate supply due to concentrate supplementation can reduce rumen pH by increasing total VFA and other organic acid production (Stone, 2004). Furthermore, consistent with existing research, we observed a reduction in acetate production. Concentrate supplementation reduces the proportion of insoluble carbohydrates and NDF, which are primary producers of acetate (Sairanen et al., 2005). Typically, a high level of concentrate supplementation (around 70% of dietary DM) increases propionate production in the rumen (Olijhoek et al., 2018). However, our study diverged from this trend, showing no difference in propionate concentration. The reason may be attributed to the limited quantity of concentrate supplementation, constituting approximately 50 - 60% of the total diet. The non-fiber carbohydrate (NFC) of the concentrate might not have been sufficiently high enough to stimulate propionate production (Sairanen et al., 2005; Moorby et al., 2006). Furthermore, our study corroborated previous findings indicating an increase in butyrate concentration following concentrate supplementation (Sairanen et al., 2005; Miguel et al., 2019). Butyrate production, however, is not solely contingent on the diet, as acetate and butyrate are interconvertible between each other in the rumen. A portion of acetate may be converted to butyrate before absorption (Kristensen, 2001). Supplementation of concentrate can act as a stimulant for butyrate-producing microorganisms and promote the conversion of acetate to butyrate (Hackmann and Firkins, 2015). Similarly, the observed increase in valerate concentration in our study was aligned with previous study (Bayat et al., 2017). Within the rumen, acetate and propionate can be elongated to valerate and iso-valerate (Chen et al., 2011).

Concentrate supplementation reduced CH_4 concentration per kg DMI in pregnant Hanwoo heifers (Table 5). In the TRT group, the CH_4 concentration per kg DMI during respiration was 32% lower than CON ($p = 0.002$). Similarly,

Table 5. Effect of different forage to concentrate ratio diet on methane emissions of Hanwoo heifers.

Items	Treatment		SEM	p-value
	CON	TRT		
CH_4 from respiration				
ppm	7.8	8.6	0.48	0.108
ppm per kg DMI	1.9	1.3	0.20	0.002
ppm per kg NDFI	2.8	2.3	0.30	0.139
CH_4 from eructation				
ppm	48.1	54.2	4.49	0.196
ppm per kg DMI	12.2	8.1	1.45	0.011
ppm per kg NDFI	17.1	14.6	2.20	0.272

CON (control), receiving forage only; TRT (treatment), receiving forage with 4 kg concentrate mix; SEM, standard error of the mean; DMI, dry matter intake; NDFI, neutral detergent fiber intake.

concentrate supplementation reduced the CH_4 concentration per kg DMI during eructation by 34% in the TRT group compared to CON ($p = 0.011$). Methane concentration in the exhaled gas from respiration and eructation per kg of NDFI were not different between the groups ($p > 0.05$).

Laser Methane Detector does not give a total CH_4 measurement for a day. However, short time measurements by LMD as ppm and ppm per kg DMI can correlate positively to the 24-h measurements of CH_4 (Kobayashi et al., 2021; Kang et al., 2022). Increasing concentrates in the diet at a level of 40% or more is widely acknowledged as a way to mitigate CH_4 production (Knapp et al., 2014). In our study, the concentration of CH_4 per kg DMI, was reduced through a diet supplemented with 50 - 60% concentrate. This aligns with the results from Bayat et al. (2017), where a 35 : 65 forage-to-concentrate ratio diet in cows resulted in a similar outcome. Similarly, a diet comprising 70% concentrate demonstrated a reduction in CH_4 per kg of DMI in beef cattle (Doreau et al., 2011). Beef heifers on a 90% concentrate diet and cows on a 61% concentrate diet also exhibited a comparable decrease in CH_4 per kg of DMI (Lovett et al., 2003; Olijhoek et al., 2018). Aguerre et al. (2011) supplemented a 47 : 53 forage-to-concentrate ratio diet and found a linear reduction of CH_4 per kg of DMI emissions in cows. The decrease in CH_4 production appears to be associated with a shift in rumen fermentation from acetate to other VFA like propionate, butyrate and valerate, along with a reduction of rumen pH (Martin et al., 2010). Propionate, butyrate and valerate production, as opposed to acetate production, serves as an alternative H_2 sink, diminishing its availability for methanogenesis (Moss et al., 2000). Additionally, high-concentrate diets, which are characterized by elevated starch levels, are linked to a decline in the population of ciliated protozoa (Morgavi et al., 2010). This reduction in protozoa, combined with a high starch diet and reduced pH, can contribute to decreased CH_4 production, especially since certain methanogens harboring ciliated protozoa are pH sensitive (Newbold et al., 1995). However, contrasting results exist. A low-level increase in the concentrate content in the diet does not consistently lead to a reduction in CH_4. Lovett et al. (2003) observed an increase in CH_4 g per kg DMI when feeding heifers a diet with a 40 : 60 forage-to-concentrate ratio. Similarly, an 8 $kg \cdot d^{-1}$ concentrate supplementation did not impact CH_4 emissions from Jersey cows (Van Wyngaard et al., 2018). Another study comparing 1 $kg \cdot d^{-1}$ vs. 5 $kg \cdot d^{-1}$ concentrate supplementation in cows revealed no difference in CH_4 per kg DMI but did show an increase in daily CH_4 production (Muñoz et al., 2015). The observed variations in CH_4 production could be attributed to factors such as forage quality, the *ad libitum* supply of forage, DMI from forage and concentrate and variations in CH_4 measuring methods (Muñoz et al., 2015). In our study, concentrate supplementation led to an increase in soluble carbohydrates in the diet, a reduction in rumen pH, and the promotion of alternative H_2 sinks by diverting VFA production.

Conclusion

Supplementing pregnant Hanwoo heifers with 4 kg of as-fed concentrate where average 3.5 kg of DMI from concentrate mix per animal effectively increased their DMI and ensured the provision of essential nutrients necessary for maintaining optimal rumen conditions. Additionally, this supplementation contributed to a notable reduction in CH_4 yield. Therefore, concentrate supplementation for pregnant heifers offers economic benefits, fulfills nutritional requirements, and presents environmental advantages. These collective benefits highlight its potential as a suitable strategy in Hanwoo cattle production.

Data Availability Statement

The data presented in this study are available on request from the corresponding author.

Conflict of Interests

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

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