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Effects of oat hay and leguminous forage mixture feeding on enteric methane emission, energy utilization, and feed conversion efficiency in male crossbred Simmental beef cattle

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Abstract

Dietary manipulation has the potential to mitigate methane (CH_4) emission and to maintain or enhance livestock productivity. We conducted two experiments to investigate the effects of replacing oat hay by leguminous forages (alfalfa hay [AH], 0, 8, 16, and 24%, experiment 1; common vetch hay [CVH], 0, 10, 20, and 30%, experiment 2) on energy metabolism of crossbred Simmental cattle. In experiment 1, total volatile fatty acid (VFA) concentrations increased quadratically with increasing AH proportions ($p = .006$) with a forage-to-concentrate ratio of approximately 50:50, whereas the CH_4 energy to gross energy intake ratio (CH_4-E:GEI) was significantly lower with 16% AH compared with 24% AH diet ($p < .05$). In experiment 2, there were no differences in the total VFA concentrations among the four diet groups with a forage-to-concentrate ratio of around 60:40 ($p > .05$); however, CH_4-E:GEI was significantly lower in the 30% CVH diet compared with the 10% CVH diet ($p < .05$). There was no significant difference in feed conversion efficiency among the four diet groups in each experiment. The results suggest that substituting 16 and 30% oat hay by AH and CVH provide optimal diets with forage-to-concentrate ratios of 50:50 and 60:40, respectively, which may reduce CH_4 emission without compromising the feed conversion efficiency of crossbred Simmental cattle.

KEYWORDS

alfalfa, CH_4 emission, common vetch, dryland, Simmental cattle

1 | INTRODUCTION

Ruminant husbandry, a prominent source of anthropogenic methane (CH_4), has a considerable impact on global warming (Moss et al., 2000; Shibata & Terada, 2010). Globally, the emission of enteric CH_4 from ruminant livestock accounts for up to 28% of anthropogenic CH_4 emissions and an estimated 30%–40% of emissions from agricultural sources (Beauchemin et al., 2008; Moss et al., 2000). Global CH_4 emissions increased by almost 40% from 1970 to 2004 (IPCC, 2007), and they are estimated to increase by 60% on the basis of proportional CH_4 emissions from expected livestock populations in 2030 (FAO, 2003). In addition to its influence on climate change, the formation of enteric CH_4 also leads to a significant energy loss (2%–12%) of dietary gross energy intake (GEI) in ruminants (Pen et al., 2006). Supplementation of diets with leguminous forage is thought to mitigate CH_4 emissions from ruminants and improve

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the utilization efficiency of dietary GEI, which can consequently enhance livestock productivity (Kobayashi et al., 2017).

Condensed tannins or bioactive plant metabolites, such as essential oils, flavonoids, and saponins (Pen et al., 2006), extracted from leguminous forage, can reduce CH_4 emissions and also improve feed conversion efficiency (FCE) (Liu et al., 2018; Shibata & Terada, 2010). Changes in diet quality, in terms of reduced grass forage and increased leguminous forage such as alfalfa, or a higher grain diet, affect the diet in a similar way to the addition of leguminous forage extract (Beauchemin et al., 2008). However, too much leguminous forage in the diet may lead to adverse effects; for example, a low proportion of alfalfa hay (AH, 22%) for growing Simmental cattle slightly increased FCE and reduced CH_4 emission, whereas CH_4 emission significantly increased and FCE significantly decreased in cattle fed a high AH diet (44%) (Kobayashi et al., 2017). Hence, there is a need to identify a diet with an appropriate proportion of legumes for sustainable agronomic practice in dryland environments.

Alfalfa occupies the largest planted area of perennial legume crops in the world, and common vetch is a primary source of annual forage legume for ruminants in the arid and cold areas of the world (Huang et al., 2017; Kobayashi et al., 2018). Previous studies have shown that alfalfa supplementation in a grass hay basal diet could increase the digestibility of dry matter (DM), crude protein (CP), and digestible CP in sheep diets (Haddad, 2000). Besides, the CP intake, digestible organic matter (OM) intake, and in vitro OM digestibility of the diet containing oat-common vetch mixture diet are significantly higher than with oat-only diets for cattle (Assefa & Ledin, 2001). Variation in livestock between studies, such as animal type, weight, gender, and age, leads to different results. Simmental cattle have historically been used for beef, are renowned for the rapid growth of their young, and have been studied extensively in various diets throughout the world (Kobayashi et al., 2017; Mc Parland et al., 2007). However, there is little information available in the literature of effects of oat-alfalfa and oat-common vetch mixture diets on CH_4 emission and energy utilization in growing male Simmental cattle. Therefore, the objective of the current study was to investigate the effects of different levels of oat-alfalfa and oat-common vetch forage mixture feed on enteric CH_4 emission and energy utilization efficiency in male Simmental cattle. A part of this experiment has been published but that focused on nitrogen utilization (Du et al., 2019).

2 | MATERIALS AND METHODS

This study was conducted at the Linze Grassland Agriculture Trial Station, Lanzhou University, China (latitude 39.24°N, longitude 100.06°E, 1,390 m above sea level). The environment is characterized by a typical temperature continental climate, with an average temperature of 7.7°C and annual precipitation of 121.5 mm, as derived from the agricultural meteorological station in Linze Grassland Agriculture Trial Station from 2006 to 2016 using a CR5000 datalogger (Campbell Scientific Inc.). In these two experiments, oat hay (OH) was purchased from a forage company (Sanbao Agricultural Company), and the ingredients of feed concentrate (wheat bran, maize, and soybean meal) were sourced locally. AH was the second harvest and common vetch was harvested at the podding stage and prepared as common vetch hay (CVH). The chemical composition of the ingredients of the concentrate and the forage was shown in our published article (table 1 of Du et al., 2019).

2.1 | Animals, treatments, and diets

The present study was conducted under the regulations of experimental field management protocols of Animal Ethics Committee of Lanzhou University (file No. 2010–1 and 2010–2) in accordance with the Guides for Management of Laboratory Animals in Gansu Province, China (Gansu Provincial Department of Science & Technology, 2005). The target forage-to-concentrate ratio of all experimental diets was fixed (60:40, DM basis) in these two experiments. In experiment (Exp) 1, 16 crossbred male Simmental cattle (Simmental × local cattle) with an initial body weight (BW) of 134 ± 7.9 kg [mean ± standard deviation (*SD*), 5 months of age] were assigned to four diets with different OH-to-AH ratios (60:0, AH-0; 52:8, AH-8; 44:16, AH-16; and 36:24, AH-24 on a DM basis of total feed supplied) in a randomized block design (four replicates per diet). In Exp 2, the same 16 crossbred male Simmental cattle with BW of 206 ± 16.5 kg (mean ± *SD*, 9 months of age) were also assigned to four diets with different OH-to-CVH ratios (60:0, CVH-0; 50:10, CVH-10; 40:20, CVH-20; and 30:30, CVH-30 on a DM basis of total feed supplied) in a randomized block design (four replicates per diet). There was a 2-month interval between the end of Exp 1 and the start of Exp 2, and the animals were allocated to their respective diets in Exp 1 and Exp 2 according to a completely independent randomized block design. There were no significant differences in the initial average BW among animals in the four diet groups in each experiment (table 3 of Du et al., 2019). The target daily body weight gain (BWG) for each animal was set at 1.0 and 1.3 kg/day for Exp 1 and 2, respectively. Both experiments lasted for 50 days, which included an initial 14 days of diet acclimation followed by 36 days of data collection.

The same amount of experimental diets was provided in each Exp, and the total amount of supplied diets was calculated based on the target BWG, the BW of cattle in each Exp, the published values and equations of the Agricultural and Food Research Council (AFRC, 1993), and the Chinese Feeding Standard for Beef Cattle (CFSBC, 2004, Ministry of Agriculture of the People's Republic of China). All experimental diets were designed to supply adequate metabolizable energy (ME) and metabolizable protein (MP) to meet the target BWG for each animal according to AFRC (1993) and BW of cattle (measured every 9 days). The CP, ME, and MP levels of all diets in each Exp were shown in our published article (table 2 of Du et al., 2019). Throughout the experimental period, all cattle were supplied with free access to water and a mineral mixture. The daily mixed forage was divided into two equal parts and offered as separate meals twice a day (08:00 and 20:00 hr in Exp 1, 08:00

and 18:00 hr in Exp 2). The mixed concentrate was fed once a day (14:00 hr in Exp 1 and 13:00 hr in Exp 2).

2.2 | Measurements and sampling procedures

The amount of feed offered and all leftovers were recorded three times per day prior to feeding (08:00, 14:00, and 20:00 hr in Exp 1; 08:00, 13:00, and 18:00 hr in Exp 2, respectively) throughout the experimental period. The difference between the feed refusals and the feed supplied was used to calculate forage daily DM intake (DMI) and concentrate DMI for each animal. After the acclimation period for experimental diets (14-day period), the cattle were moved to individual respiration chambers for 9 days. Within the 9 days of measurements in the chamber, the cattle were acclimatized for the first 2 days. Digestibility data were collected over the following 4 days and gas exchange data [oxygen (O_2) consumption, CH_4, and carbon dioxide (CO_2) emissions] were collected over the remaining 3 days. The BWG (kg/day) was calculated based on the difference between the beginning and the end of data collection period (36 days), and FCE (kg DMI/kg BWG) was calculated by total DMI (kg/day) divided by BWG (kg/day). The total weight of daily excreted feces and urine was recorded during the 4 days' data collection period in the chamber. Feces, which were excreted on a plastic mat placed under the cattle, were collected immediately with a shovel, placed in a plastic container, weighed, mixed, and sampled once per day. Ten percent of each feces sample was stored at −20°C for later chemical analysis. Total urine was collected through a handmade urine bag into a bucket and was acidified by addition of 10% v/v sulfuric acid (H_2SO_4) to ensure a pH < 3.0 by a portable pH instrument (PHBJ-260; Shanghai INESA Scientific Instrument Co., Ltd.). Twenty percent of the daily urine was stored at −20°C for later chemical analysis.

Emissions of CH_4 were measured using cattle multi-channels open respiration metabolic chamber [MORMC, Grassland-Livestock-Interaction Research Institute (GLIRI), Lanzhou University] as described by Du et al. (2019). Four indirect open-circuit respiration chambers (GLIRI-MORMC-001) were used, with one cattle housed per chamber. The respiration chambers were made with double Perspex walls fitted in aluminum frames, with a total volume of approximately 18 m^3 (4.2 m long, 1.95 m wide, and 2.2 m high), equipped with a computer-controlled air-handling system with air conditioning units set to a temperature of 18 ± 1°C and relative humidity of 60 ± 10%. Each chamber was equipped with a gas flow meter (GFM57; Aalborg) at the outflow site to record total airflow, and an engine to ensure a slight negative pressure within each chamber. All chambers were ventilated by suction pumps with a flow rate of 45–50 m^3/h. The exhaust air was removed from each chamber separately for the measurement of volume, temperature, and humidity. The concentrations of CO_2, CH_4, and O_2 during the gas exchange measurements (3-day period) to determine atmospheric air entering and exhaust gas leaving each chamber, were measured every 20 min (4 min for each chamber and/or ambient air) using a multi-gas analyzer (VA-3000; Horiba Ltd.) in a general control room. The analyzer was calibrated using standard gases [O_2-free nitrogen (N_2) and a known quantity of CH_4, CO_2, and O_2 (span gas); Dalian Special Gases Co., Ltd.,] at the beginning of the gas exchange collection period in each Exp. This determined the absolute range 0–500 μl/L for CH_4, 0–2,000 μl/L for CO_2, and 0%–25% v/v for O_2, and the linearity within this range. The rate of CH_4 recovery was determined by comparing the amount of CH_4 loss from a gas cylinder in the bottom of the chambers, and the CH_4 accumulations passing through the chambers (Livestock Research Group of the Global Research Alliance, 2014). The purpose of the calibrations was to ensure a gas recovery rate was approximate 100 ± 2% for all chambers, as highlighted by Gerrits et al. (2018). CH_4 emission was expressed as the average CH_4 emission (g/day) from 3 days' measurement. Ruminal digestible organic matter intake (RDOMI, kg/day) was calculated by organic matter (OM) intake (kg/day) × OM digestibility (fraction) × 0.65 (ARC, 1980). CH_4:RDOMI (g/kg) was calculated by CH_4 emission (g/day) divided by RDOMI (kg/day).

2.3 | Collection and chemical analysis of ruminal fluid

Rumen fluid samples were taken from each animal 4 hr post-forage feeding in the morning using a stomach tube on the last day of each experiment. The pH of the ruminal samples was measured immediately using a portable pH meter (model is above). Then, the samples were strained through two layers of muslin (mesh size 1 mm^2) and then stored at −20°C for subsequent volatile fatty acid (VFA) analysis. The rumen filtrate was thawed and centrifuged at 10,000*g* for 30 min at a temperature of 4°C. 0.5 ml of supernatant of ruminal fluid was mixed with 0.5 ml of 20 mmol/L internal standard (Crotonic acid), and then 0.01 ml of 85% ortho-phosphoric acid was added to acidify the mixed liquid. The mixed samples was kept for overnight at 4°C and then centrifuged at 3,000*g* for 15 min at a temperature of 4°C, supernatant of which was transferred to gas chromatograph tubes for VFA analysis by a gas chromatograph (Trace1300; Thermo Ltd.) fitted with a polar capillary column.

2.4 | Chemical analysis

The stored feces samples were thawed and feces samples obtained from each animal over the 4 days were mixed. After that, samples were oven dried at 65°C for 48 hr to measure the DM percentage and then ground to pass through a 1 mm screen. A portion of each dried feces sample, mixed forage, and concentrate samples was used for analysis of ash (method 942.05; AOAC, 1990). Another portion of each dried feces sample was used to determine gross energy (GE) by an automatic isoperibol calorimeter (6,400; PARR Instrument Company), and neutral detergent fiber (NDF) with an ANKOM 2000 fiber analyzer (ANKOM Technology, Fairport, NY, USA) following the protocol described by Van Soest et al. (1991). The ash was included to provide NDF analyses of all forages, concentrates,

and feces samples. The α-amylase for NDF analysis was used only for concentrate samples. Urinary energy (UE) was also determined by the automatic isoperibol calorimeter (see above), and N was determined by the Kjeldahl procedure as described previously by the AOAC (1990). For UE measurement, 4 ml of fully mixed urine was taken and absorbed on filter paper of known weight, and then the total energy of the filter paper with the urine sample was measured by automatic isoperibol calorimeter after drying. A further five samples of the same filter paper (known weight) were measured to determine energy content (MJ/kg), which was used to calculate the UE. GE and NDF were measured in the forage and concentrate of the diets using the above methods and instruments.

2.5 | Energy balance

Digestible energy intake (DEI) was calculated as the difference between GEI and excreted feces energy (FE). ME intake (MEI) was calculated as the difference between DEI, and the sum of UE and CH_4 energy (CH_4-E) output. Retained energy (RE) was calculated using the equation: MEI − heat production (HP). CH_4-E was calculated from CH_4 emission (L/day) and the conversion coefficient (39.54 kJ/L; Brouwer, 1965). CH_4 emission (grams) was also calculated from CH_4 emission (L/day) and the conversion coefficient (0.716 g/L; Brouwer, 1965). HP (kJ/day) was calculated with the equation: HP (kJ/day) = 16.18 × O_2 consumption (L/day) + 5.02 × CO_2 production (L/day) − 2.17 × CH_4 production (L/day) − 5.99 × N excretion (urinary N, g/day) (Brouwer, 1965).

2.6 | Statistical analyses

One-way analysis of variance (ANOVA) was used to analyze the effects of diets on CH_4 emission, energy balance, and energy utilization efficiency. Differences among means were considered significant at the $p \leq .05$ level based on Tukey's test. Data on energy balance and energy utilization efficiency obtained from each experiment were subjected to the general linear models procedure for orthogonal polynomial analysis. Statistical Package for the Social Sciences (SPSS), version 19.0 (SPSS Inc.), was used for all analyses.

3 | RESULTS

3.1 | Effects of diets on enteric CH_4 emissions

In Exp 1, CH_4 emission (g/day, Figure 1a), CH_4:DMI (Figure 1b), and CH_4:RDOMI (Figure 1c) did not differ between the AH-0 and AH-16 groups ($p > .05$); however, emissions were significantly higher in the AH-24 group compared with the AH-16 group, regardless of whether CH_4 was expressed as a proportion of DMI or RDOMI ($p < .05$, Figure 1b and c). In Exp 2, there were no differences in CH_4 emission per day (Figure 1e) or CH_4:RDOMI (Figure 1g) among the four diets ($p > .05$). However, CH_4:DMI was significantly lower in the CVH-0 and CVH-30 groups than in the CVH-10 group (Figure 1f). CH_4 emission, expressed as milligrams per min per kilogram BW over 24 hr post-feeding, is shown in Figure 2a (Exp 1) and 2b (Exp 2). There were intermittent peaks throughout the day for both experimental groups, which occurred a short time after feed supply. The peak of CH_4 emission (mg/kg BW/min) was higher following concentrate supply than following forage supply (Figure 2a and b).

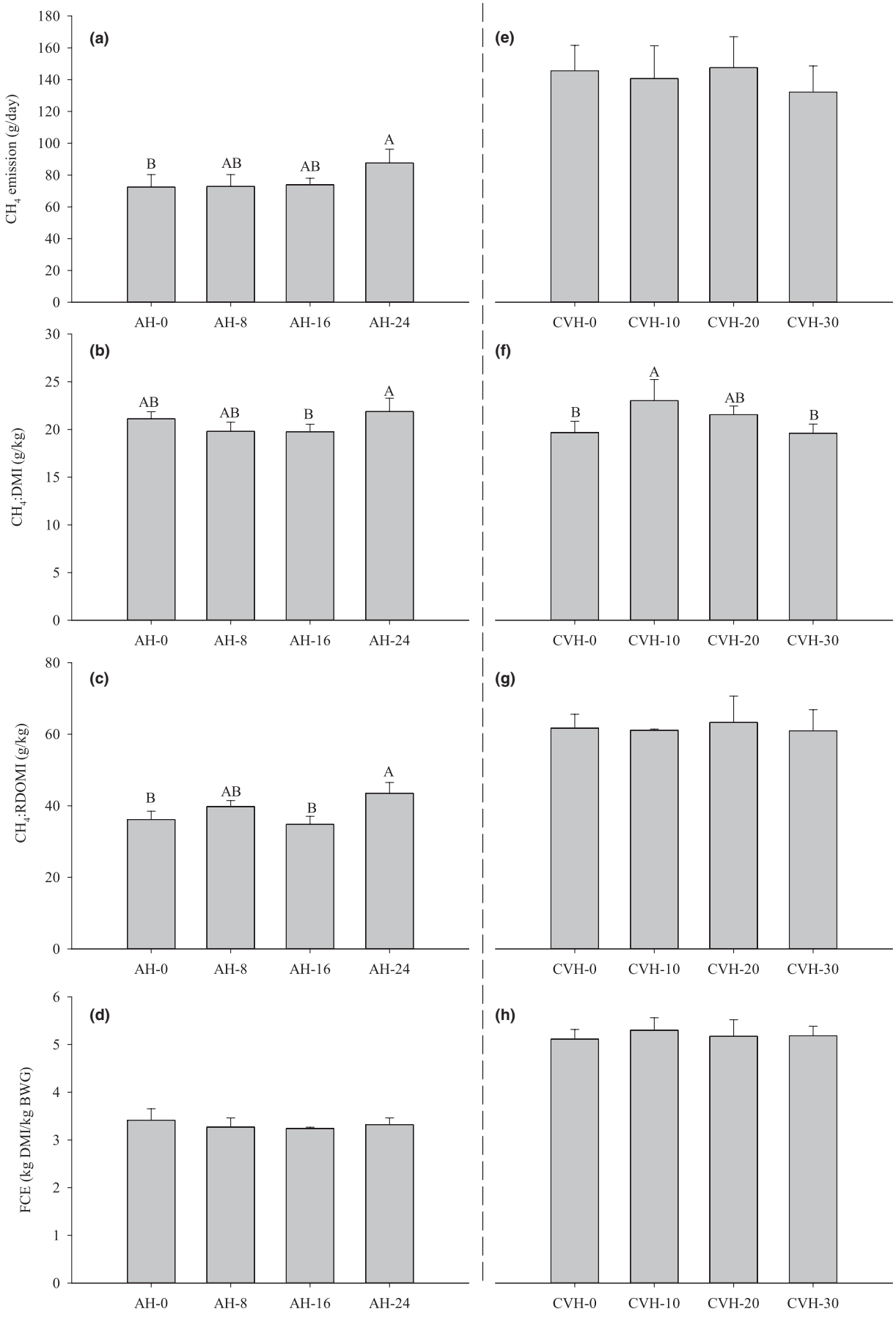
3.2 | Effects of diets on ruminal fermentation

The mean ruminal pH was 6.60 and 6.28 across treatments in Exp 1 and 2, respectively (Table 2). The total VFA concentration increased in a quadratic manner from the AH-0 group to the AH-24 group in Exp 1 ($p = .006$, Table 2), and was significantly higher in the AH-8 and the AH-24 groups than in the AH-0 group ($p < .05$, Table 2); there was no significant difference in the total VFA concentrations between groups in Exp 2. There was no significant difference in the mean acetate:propionate ratio between groups in either experiment; however, in Exp 1, the molar proportions of propionate in cattle fed diets including AH were significantly lower than in those fed diets without AH ($p < .05$, Table 2). In Exp 2, the molar proportion of butyrate in cattle was significantly lower in the CVH-30 group than in the CVH-20 group ($p < .05$, Table 2). Additionally, in Exp 1, the molar proportion of iso-valerate was significantly lower in the AH-0 and the AH-8 groups than in the AH-16 group ($p < .05$, Table 2). In Exp 2, the molar proportion of iso-valerate followed a parabolic trend from the CVH-0 group to the CVH-30 group ($p = .005$, Table 2), and was significantly higher in the CVH-20 group than in the CVH-0 group ($p < .05$, Table 2).

3.3 | Effects of diets on energy metabolism and FCE

Energy intake, output, and utilization efficiency are presented in Table 1. In Exp 1, GEI and FE increased quadratically from the AH-0 group to the AH-24 group ($p < .05$), and were significantly higher in the AH-24 group than in the AH-0 group ($p < .05$). The highest MEI was observed in the AH-16 group, and the lowest CH_4-E was observed in the AH-0 group ($p < .05$). There were no differences in DEI, UE, HP, and RE among the four diet groups ($p > .05$). Regarding

FIGURE 1 Methane (CH_4) emission (g/day), CH_4:dry matter intake [(DMI), g/kg], CH_4:ruminal digestible organic matter intake [(RDOMI), g/kg], and feed conversion efficiency [(FCE), kg DMI/kg body weight gain (BWG)] among the four diets in Exp 1 and 2. Values are the means and standard deviations. Uppercase letters without common letters are significantly different ($p < .05$) in each Exp (Exp 1, a, b, c, and d; Exp 2, e, f, g, and h). The absence of letters indicates there was no significant difference. RDOMI was calculated from organic matter intake (OMI) × organic matter (OM) digestibility × 0.65 (ARC, 1980)


(a)
CH4 emission (g/day)
180
160
140
120
100
80
60
40
20
0
B
AB
AB
A
AH-0
AH-8
AH-16
AH-24
(e)
CVH-0
CVH-10
CVH-20
CVH-30
(b)
CH4:DMI (g/kg)
30
25
20
15
10
5
0
AB
AB
B
A
(f)
B
A
AB
B
(c)
CH4:RDOMI (g/kg)
80
60
40
20
0
B
AB
B
A
(g)
(d)
FCE (kg DMI/kg BWG)
6
5
4
3
2
1
0
(h)

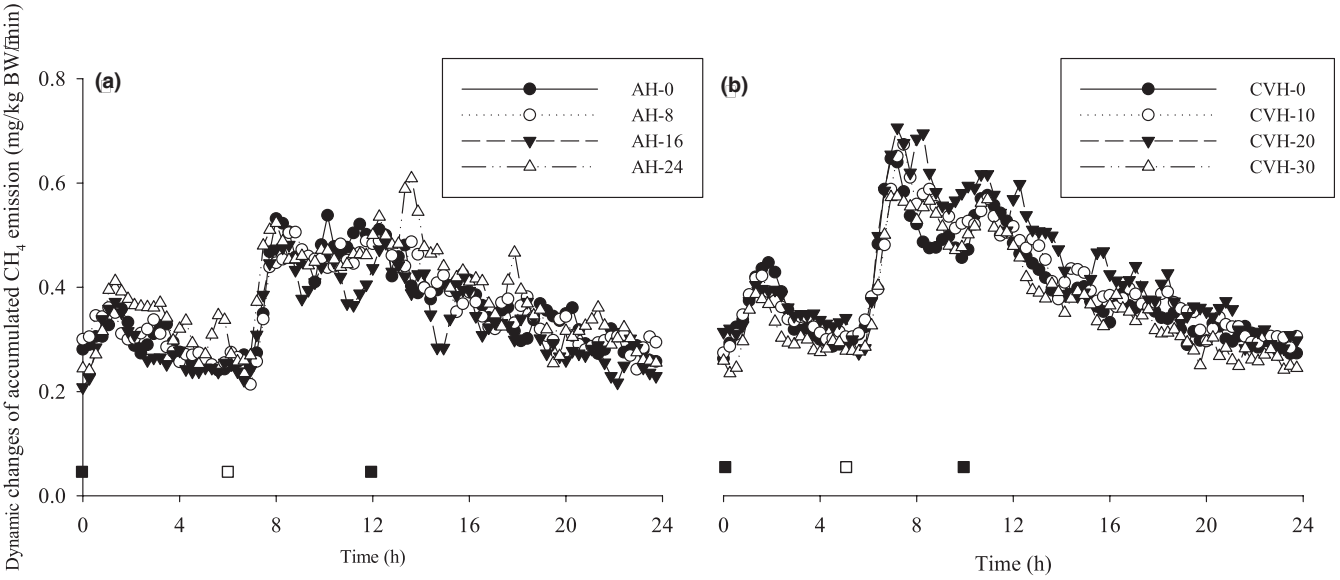


FIGURE 2 Dynamic changes in accumulated CH_4 emission [mg/kg body weight (BW)/min] during a 24 hr period (starting from forage offered at 08:00 hr and ending at 8:00 hr the next day) in Exp 1 (a) and 2 (b). The spots [■ and □ in (a) and (b)] represent the time when the forage and concentrate were supplied, respectively

the energy utilization efficiency, no differences in DEI:GEI, MEI:GEI, UE:GEI, HP:GEI, or RE:GEI ($p > .05$) were found between groups; however, CH_4-E loss (CH_4-E:GEI) and FE:GEI were significantly higher in the AH-24 group than in the AH-16 and AH-8 groups, respectively ($p < .05$).

In Exp 2, there were no differences in GEI between groups, however, DEI and MEI were significantly higher in the CVH-0 group than in the CVH-10 group ($p < .05$). In addition, FE was significantly higher in the CVH-30 group than in the CVH-10 group ($p < .05$). There were no differences in other energy balance components (UE, CH_4-E, HP, and RE) between groups. The DEI:GEI and MEI:GEI were significantly lower in the CVH-30 group than in the CVH-0 group ($p < .05$), whereas FE:GEI was significantly higher in the CVH-30 group than in the CVH-0 group ($p < .05$). The lowest value of CH_4-E:GEI was observed in CVH-30 group, which was significantly lower than that observed in the CVH-10 group ($p < .05$).

There was no difference in FCE among the four diet groups ($p > .05$, Figure 1d and h) no matter in Exp 1 or 2.

4 | DISCUSSION

4.1 | Enteric CH_4 emissions and ruminal fermentation

Total DMI is the critical driver of daily CH_4 production (Wang et al., 2019; Yan et al., 2000). In Exp 1, the change in CH_4 emission (g/day) corresponded with the total DMI from the AH-0 group to the AH-24 group which supported the previous finding (Wang et al., 2019; Yan et al., 2000). In addition, the higher CH_4 emission (g/day) in Exp 2 than in Exp 1 also indicated that a higher DMI would lead to a higher CH_4 production.

In general, an appropriate proportion of leguminous forage in a diet is considered to be effective for mitigating CH_4 emission in beef cattle (Hess et al., 2004). This is because legumes are rich in secondary metabolites, such as saponins and tannins, which have potential to inhibit the activity of protozoa and methanogen in the rumen (Beauchemin et al., 2008). Common vetch and alfalfa are saponins-containing plants (Evidente et al., 2011; Liu et al., 2018). The absence of significant changes in CH_4:GEI (Table 1) in most leguminous forage treatments in these two experiments except AH-16 treatment relative to the control (AH-0) in Exp 1 indicate no inhibition effect of saponins on methanogenesis with substitution of legume for grass. This is probably due to the sun-dried process of AH and CVH (the ambient air temperature was around 35°C and ground temperature could be up to 50°C during the drying processes according to datalogger), which could modify the structure of saponins and damage its antimethanogenic and antiprotozoal properties (Guyader et al., 2015). In Exp 1, the slight decrease in CH_4:DMI (Figure 1b) and CH_4:GEI (Table 1) from the AH-0 to the AH-16 groups could be attributed to an increasing feeding level [ME intake/ME requirement for maintenance; AFRC (1993), Table 1]. The higher feeding level could lead to a faster outflow rate of feedstuff from the rumen (AFRC, 1993), therefore, shifts part of the digestion from rumen to hindgut. The hindgut fermentation has a lower CH_4 production than the ruminal fermentation (Fievez et al., 1999; Hess et al., 2004). However, the CH_4:DMI (Figure 1b) in the AH-24 group was higher than that in the AH-16 group under the same feeding level (Table 1). When low ruminal available N results in a limitation of microbial growth, more ruminal fermented energy will be used for CH_4 production by methanogens (Hess et al., 2004). The relative lower ruminal ammonia *N* (table 5 of Du et al., 2019) and higher total VFA concentration (Table 2) could lead to a higher CH_4 production in the AH-24 group than the AH-16 group (Figure 2a and b, Table 1).

TABLE 1 Effects of diet on energy intake, energy excretion, and energetic utilization in Simmental crossbred cattle (four replicates per diet) for experiments 1 (Exp 1) and 2 (Exp 2)

	Exp[‡,†] 1						Polynomial contrast[¶]		Exp[‡,†] 2						Polynomial contrast[¶]	
Item	AH-0	AH-8	AH-16	AH-24	*SEM*[¶]	*p* Value	*L*	Q	CVH-0	CVH-10	CVH-20	CVH-30	*SEM*[¶]	*p* Value	*L*	Q
Actual forage-to-concentrate ratio	48:52	45:55	49:51	50:50	0.018	.054	0.154	0.077	59:41	50:50	56:44	55:45	0.032	.098	0.561	0.257
Feeding level[§]	1.73[a,b,c]	1.90[a,b,c]	2.03[a,b,c]	2.07[a,b,c]	0.096	.017	0.001	0.005	2.65	2.25	2.47	2.44	0.147	.110	0.447	0.223
NDF excretion[‡] (kg DM/day)	0.48	0.50	0.53	0.57	0.057	.455	0.101	0.256	1.40[‡]	1.16[a,b,c]	1.22[a,b,c]	1.45[a,b,c]	0.098	.033	0.585	0.012
Energy balance[‡], MJ/day																
GEI	62.0[a,b,c]	67.3[a,b,c]	72.4[a,b,c]	74.3[a,b,c]	3.15	.023	0.002	0.007	131.7	110.5	123.0	122.0	8.13	.121	0.575	.252
DEI	48.9	53.8	56.4	55.7	2.84	.079	0.022	0.029	94.8[a,b,c]	77.5[a,b,c]	87.5[a,b,c]	79.7[a,b,c]	5.10	.020	0.100	0.157
MEI	38.8[a,b,c]	42.2[a,b,c]	45.7[a,b,c]	43.6[a,b,c]	2.08	.037	0.023	0.018	76.9[a,b,c]	61.4[a,b,c]	70.4[a,b,c]	62.4[a,b,c]	4.67	.019	0.079	0.149
FE	13.2[a,b,c]	14.9[a,b,c]	16.0[a,b,c]	18.6[a,b,c]	1.68	.025	0.003	0.008	37.0[a,b,c]	33.0[a,b,c]	35.5[a,b,c]	42.3[a,b,c]	2.70	.039	0.099	0.012
UE	6.4	7.5	6.9	7.2	0.47	.133	0.242	0.294	9.8	8.3	9.0	10.0	1.04	.469	0.775	0.300
CH_4-E	3.7[a,b,c]	4.0[a,b,c]	3.9[a,b,c]	4.8[a,b,c]	0.28	.008	0.007	0.012	8.0	7.8	8.2	7.3	0.73	.647	0.411	0.614
HP	33.2	35.1	36.1	37.2	2.99	.588	0.154	0.369	64.0	52.8	60.1	56.6	4.60	.147	0.372	0.403
RE	5.6	7.1	9.6	6.4	4.67	.845	0.736	0.726	12.9	8.5	10.2	5.9	2.88	.149	0.049	0.154
Energy utilization efficiency[‡], MJ/MJ																
DEI:GEI	0.786	0.800	0.781	0.751	0.0220	.198	0.094	0.093	0.721[a,b,c]	0.702[a,b,c]	0.712[a,b,c]	0.654[a,b,c]	0.0192	.033	0.005	0.019
MEI:GEI	0.622	0.628	0.632	0.588	0.0262	.360	0.169	0.099	0.585[a,b,c]	0.555[a,b,c]	0.572[a,b,c]	0.513[a,b,c]	0.0218	.044	0.013	0.051
MEI:DEI	0.791	0.785	0.809	0.782	0.0110	.141	0.985	0.557	0.811	0.791	0.804	0.781	0.0136	.199	0.115	0.308
FE:GEI	0.214[a,b,c]	0.200[a,b,c]	0.219[a,b,c]	0.249[a,b,c]	0.0140	.044	0.032	0.016	0.279[a,b,c]	0.298[a,b,c]	0.288[a,b,c]	0.346[a,b,c]	0.0179	.033	0.015	0.027
UE:GEI	0.104	0.112	0.096	0.098	0.0086	.275	0.241	0.457	0.075	0.076	0.074	0.082	0.0139	.879	0.577	0.780
CH_4-E:GEI	0.060[a,b,c]	0.060[a,b,c]	0.053[a,b,c]	0.065[a,b,c]	0.0031	.046	0.616	0.148	0.061[a,b,c]	0.070[a,b,c]	0.066[a,b,c]	0.060[a,b,c]	0.0033	.023	0.574	0.017
HP:GEI	0.540	0.520	0.505	0.502	0.0495	.855	0.379	0.668	0.487	0.478	0.488	0.462	0.0147	.320	0.192	0.332
RE:GEI	0.082	0.108	0.127	0.087	0.0628	.878	0.861	0.731	0.099	0.078	0.084	0.051	0.0266	.371	0.084	0.276

[‡]AH, alfalfa hay; CVH, common vetch hay; NDF, neutral detergent fiber; GEI, gross energy intake; DEI, digestible energy intake; MEI, metabolizable energy intake; FE, feces energy; UE, urinary energy; CH_4-E, methane energy; HP, heat production; RE, retained energy.

[†]Exp 1, AH-0, 60% oat hay and 0% alfalfa hay; AH-8, 52% oat hay and 8% alfalfa hay; AH-16, 44% oat hay and 16% alfalfa hay; AH-24, 36% oat hay and 24% alfalfa hay. Exp 2, CVH-0, 60% oat hay and 0% common vetch hay; CVH-10, 50% oat hay and 10% common vetch hay; CVH-20, 40% oat hay and 20% common vetch hay; CVH-30, 30% oat hay and 30% common vetch hay.

[§]Feeding level, metabolizable energy (ME) intake divided by the ME requirement for maintenance from the AFRC (1993).

[¶]*SEM*, standard error of the mean, L, linear, Q, quadratic.

[a,b,c]Means within rows with a different superscript letter differ ($p < .05$).

TABLE 2 Effects of diet on ruminal fermentation parameters in Simmental crossbred cattle (four replicates per diet) for experiments 1 (Exp 1) and 2 (Exp 2)

	Exp[‡,†] 1						Polynomial contrast[§]		Exp[‡,†] 2						Polynomial contrast[§]	
Item	AH-0	AH-8	AH-16	AH-24	*SEM*[§]	*p* Value	*L*	Q	CVH-0	CVH-10	CVH-20	CVH-30	*SEM*[§]	*p* Value	*L*	Q
Total VFA, mmol/L	80.2[b]	93.5[a]	91.6[ab]	98.3[a]	3.91	.004	0.002	0.006	95.9	96.9	93.5	92.4	2.83	.252	0.267	0.516
pH	6.70	6.76	6.52	6.44	0.131	.794	0.006	0.017	6.31	6.35	6.26	6.29	0.275	.895	0.774	0.946
Molar proportions (mol/100 mol)																
Acetate	65.0	65.1	64.1	65.0	3.21	.988	0.960	0.959	69.4	71.2	69.2	72.2	4.16	.854	0.503	0.774
Propionate	20.5[a]	18.1[b]	18.5[b]	17.9[b]	0.84	.011	0.009	0.023	16.9	15.9	15.3	16.7	2.38	.985	0.803	0.589
Butyrate	11.4	13.6	11.7	12.9	2.03	.682	0.599	0.790	10.8[ab]	9.6[ab]	11.6[a]	8.1[b]	1.02	.040	0.091	0.079
Iso-butyrate	0.9	1.0	1.8	1.2	0.9	.707	0.161	0.370	1.3	1.1	1.3	1.1	0.31	.619	0.735	0.941
Valerate	1.0	0.9	1.1	1.4	0.37	.597	0.074	0.161	0.7	0.7	0.8	0.7	0.129	.707	0.866	0.852
Iso-valerate	1.3[b]	1.3[b]	2.7[a]	1.6[ab]	0.31	.034	0.438	0.538	0.8[b]	1.4[ab]	1.7[a]	1.3[ab]	0.191	.011	0.263	0.131
Acetate:propionate ratio	3.2	3.6	3.5	3.6	0.29	.280	0.021	0.073	4.3	4.5	4.5	4.4	0.25	.592	0.758	0.831

[‡]AH, alfalfa hay; CVH, common vetch hay.

[†]Exp 1, AH-0, 60% oat hay and 0% alfalfa hay; AH-8, 52% oat hay and 8% alfalfa hay; AH-16, 44% oat hay and 16% alfalfa hay; AH-24, 36% oat hay and 24% alfalfa hay. Exp 2, CVH-0, 60% oat hay and 0% common vetch hay; CVH-10, 50% oat hay and 10% common vetch hay; CVH-20, 40% oat hay and 20% common vetch hay; CVH-30, 30% oat hay and 30% common vetch hay.

[§]*SEM*, standard error of the mean, L, linear, Q, quadratic.

[a,b,c]Means within rows with a different superscript letter differ ($p < .05$).

The similar CH_4:DMI (Figure 1f) and CH_4:GEI (Table 1) between the CVH-0 and CVH-30 groups indicated CVH-30 could be an alternative option for smallholders to save the feeding cost on soybean meal that was the most expensive of the concentrate in the CVH-0 group.

In ruminal fermentation, acetate fermentation usually accelerates CH_4 emission while propionate fermentation, which would compete with methane for available hydrogen, reduces CH_4 emissions (Pen et al., 2006). In Exp 2, there were no differences in the proportions of acetate and propionate; however, proportion of butyrate in the CVH-30 group was significantly lower than that in the CVH-20 group, which may account for the relative lower CH_4-E:GEI in the CVH-30 group than the CVH-20 group (Table 1), even if the ruminal butyrate only accounted 7%–9% of CH_4 production (Mackie & Bryant, 1981). This may be due to some other anti-nutrition factors of seeds in CVH (harvested at the podding stage), such as tannins, phenolics, trypsin inhibitors, and β-cyano-L-alanine (Huang et al., 2017); the concentration of these may reach a dietary threshold and subsequently have a negative effect on the activity of some enzymes on butyrate fermentation in the rumen. In addition, the different type and origin of saponins from alfalfa and common vetch might have differential effects on rumen fermentation (Pen et al., 2006). Therefore, further research should elucidate the effects of legumes containing saponins on the rumen microbiome and microbial synthesis.

4.2 | Energy metabolism and FCE

Forage composition affects energy metabolism and energy utilization efficiency (Win et al., 2015). In Exp 1, GEI increased quadratically from the AH-0 group to the AH-24 group (Table 1), however, DEI had a decrease in the AH-24 group compared to the AH-16 group under a quadratic tendency from the AH-0 to the AH-24 groups (Table 1). This might be due to the relative higher FE output and FE:GEI in the AH-24 group than that in the AH-16 group (Table 1), which was likely attributed to the more NDF output that is the primary source of FE (Hales et al., 2014). Eventually, it led to an approximate quadratic trend for DEI:GEI from the AH-0 group to the AH-24 group ($p = .093$, Table 1). In Exp 2, although no differences were found in the GEI, the differences in FE output, FE:GEI, and DEI:GEI among these four groups were likely a result of quadratic trend of nutrient digestibility (table 3 of Du et al., 2019) and NDF excretion (Table 1). The lower averaged DEI:GEI in Exp 2 compared to Exp 1 (0.697 vs. 0.780 respectively) might be due to the higher feeding level (Table 1) that increased the rate of passage and decreased the digestibility (Chaokaur et al., 2015), which was also confirmed in our study (table 3 of Du et al., 2019). No differences in UE and UE:GEI were found among the four diet groups in each experiment, indicating that UE was not affected by increasing legume proportion. This is because UE loss was derived primarily from urinary N concentration (Hales et al., 2014), the differences of which were not significant in our study (14.8, 13.3, 14.9, and 13.2 g/L for AH-0, AH-8, AH-16, and AH-24 in Exp 1, respectively; 16.2, 17.4, 15.9, and 15.7 g/L for CVH-0, CVH-10, CVH-20, and CVH-30 in Exp 2, respectively). Furthermore, the sum of UE and CH_4-E only occupied around 14–17% for both experiments, which was relatively stable. There were no differences in RE and RE:GEI among the diet groups in each Exp (Table 1). Nkrumah et al. (2006) reported that part of the variation in energy retention efficiency was associated with MEI:GEI above maintenance levels. In the present study, the change in RE and RE:GEI was consistent with MEI and MEI:GEI, respectively, regardless of Exp 1 or 2 (Table 1), which confirmed the previous finding (Nkrumah et al., 2006).

The FCE (kg DMI/kg BWG) has a major impact on the cost of beef production, which varies both within and across breeds and ages (Garg et al., 2013). In our study, although there were no differences in the FCE among the four diets in each experiment, a higher FCE was observed in Exp 2 than in Exp 1 (Figure 1d and h). This may be due to the difference in digestibility of AH and CVH; however, the relatively lower OM digestibility in Exp 2 compared with Exp 1 (table 3 of Du et al., 2019) was inconsistent with the results reported by Karabulut et al. (2007), who showed that the OM digestibility of CVH was significantly higher than that of AH. This could be attributed to the higher DMI (table 3 of Du et al., 2019) and feeding level in Exp 2 compared with Exp 1, which increased the rate of passage through the rumen and then decreased digestibility, resulting in a higher FCE. A previous study found an average FCE of 8.21 with a low energy diet, and 7.66 with a high energy diet in finishing Simmental steers (Mandell et al., 1998). The FCE values found in our study (average 3.31 in Exp 1 and 5.19 in Exp 2) were lower than those reported by Mandell and may be attributed to the higher proportion of concentrate (average 40% in our experimental diets vs. maximum value of 5.3% in Mandell et al. [1998]), which is the main source for BWG.

5 | CONCLUSION

The results of this study suggest that a 16% AH diet with an approximately 50:50 forage-to-concentrate ratio significantly reduced CH_4 energy to gross energy intake ratio (CH_4-E:GEI) and CH_4 emissions (g/kg DMI) compared with a 24% AH diet, and no significant differences between 16% AH diet and 0 or 8% AH diets in Exp 1. A 30% CVH diet resulted in significantly lower CH_4-E:GEI and CH_4 emissions (g/kg DMI) than a 10% CVH diet, with no significant difference observed between 30% CVH diet and 0 or 20% CVH diets in Exp 2. Additionally, leguminous forage proportion did not affect the feed conversion efficiency. Our results suggest that strategic feed compositions containing alfalfa (16%) and common vetch (30%) are optimal, respectively, compared with 0, 8, or 24% AH, and 0, 10, or 20% CVH, which leads to lower CH_4 emission per unit DMI while maintaining feed conversion efficiency in crossbred Simmental beef cattle in dryland environment.

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CONFLICT OF INTEREST

There is no conflict of interest.

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