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Effects of Forage Type and Dietary Concentrate to Forage Ratio on Methane Emissions and Rumen Fermentation Characteristics of Dairy Cows in China

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Abstract

This study was conducted to evaluate the effects of dietary forage type and concentrate-to-forage ratio (CTFR) on CH_4 emissions from dairy cows in China using the SF_6 tracer method. Rumen fluid concentrations of NH_4-N and VFA, rumen fluid pH, and daily milk yield were measured as well. The dietary regimens included: corn stalks (dry corn stalks after corn harvest) as the forage source at CTFR of 40:60 (ration A), corn silage as the forage source at CTFR of 40:60 (ration B), and corn silage as the forage source at CTFR of 60:40 (ration C). Twelve dairy cows (body weight = 525 Â±12 kg, mean Â±SE) were divided into three groups of four animals each, balanced in age and body weight, and randomly allocated to the dietary treatments. The study was run for 25 days, with the first 15 days used for adjustment and the subsequent 10 days used for response measurement. The dietary treatments ranked ration A > ration B > ration C for CH_4emissions quantity (L d^{-1} $head^{-1}$), rumen fluid pH, acetic acid concentration, and ratio of acetic acid and propionic acid concentration. For example, CH_4 emissions (mean Â±SE) for rations A, B, and C were 353 Â±13.4, 283 Â±7.48, and 263 Â±9.04 L d^{-1} $head^{-1}$, respectively, or 335 Â±12.75, 270 Â±7.12, and 250 Â±8.6 L d^{-1} AU^{-1} (AU = 500 kg live weight). On the other hand, the treatments ranked ration A < ration B < ration C for concentrations of NH_3-N, propionic acid, and butyric acid in the rumen fluid. This study indicates that CH_4 emissions intensity from dairy cows (g kg^{-1} milk output) can be significantly reduced by using corn silage as a forage source and by increasing the content of concentrates in the ration. Compared with rations B and C, ration A increased CH_4 emissions by 25% and 34% ($p < 0.01$), respectively. A full assessment of the effectiveness of such an improved feeding management, through life cycle analysis, in reducing carbon footprint would be warranted in future studies.

Keywords

concentrate-to-forage ratio, dairy cows, enteric fermentation, methane emissions, SF6 tracer method

Disciplines

Agriculture | Bioresource and Agricultural Engineering

Comments

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EFFECTS OF FORAGE TYPE AND DIETARY CONCENTRATE TO FORAGE RATIO ON METHANE EMISSIONS AND RUMEN FERMENTATION CHARACTERISTICS OF DAIRY COWS IN CHINA

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ABSTRACT. *This study was conducted to evaluate the effects of dietary forage type and concentrate-to-forage ratio (CTFR) on CH_4 emissions from dairy cows in China using the SF_6 tracer method. Rumen fluid concentrations of NH_4-N and VFA, rumen fluid pH, and daily milk yield were measured as well. The dietary regimens included: corn stalks (dry corn stalks after corn harvest) as the forage source at CTFR of 40:60 (ration A), corn silage as the forage source at CTFR of 40:60 (ration B), and corn silage as the forage source at CTFR of 60:40 (ration C). Twelve dairy cows (body weight = 525 ±12 kg, mean ±SE) were divided into three groups of four animals each, balanced in age and body weight, and randomly allocated to the dietary treatments. The study was run for 25 days, with the first 15 days used for adjustment and the subsequent 10 days used for response measurement. The dietary treatments ranked ration A > ration B > ration C for CH_4 emissions quantity (L d^{-1} $head^{-1}$), rumen fluid pH, acetic acid concentration, and ratio of acetic acid and propionic acid concentration. For example, CH_4 emissions (mean ±SE) for rations A, B, and C were 353 ±13.4, 283 ±7.48, and 263 ±9.04 L d^{-1} $head^{-1}$, respectively, or 335 ±12.75, 270 ±7.12, and 250 ±8.6 L d^{-1} AU^{-1} (AU = 500 kg live weight). On the other hand, the treatments ranked ration A < ration B < ration C for concentrations of NH_3-N, propionic acid, and butyric acid in the rumen fluid. This study indicates that CH_4 emissions intensity from dairy cows (g kg^{-1} milk output) can be significantly reduced by using corn silage as a forage source and by increasing the content of concentrates in the ration. Compared with rations B and C, ration A increased CH_4 emissions by 25% and 34% ($p < 0.01$), respectively. A full assessment of the effectiveness of such an improved feeding management, through life cycle analysis, in reducing carbon footprint would be warranted in future studies.*

Keywords. *Concentrate-to-forage ratio, Dairy cows, Enteric fermentation, Methane emissions, SF_6 tracer method.*

Globally, annual methane (CH_4) emissions from ruminant livestock amount to 65 to 85 Tg (10^{12} g), representing 32% of the total non-CO_2 emissions from agriculture in 2005 (IPCC, 2007). The CH_4 emissions from ruminant animals account for 97% of the CH_4 emissions from livestock in the world, and CH_4 emissions from cattle (excluding buffalo) account for about 75% (Johnson et al., 1991). CH_4 emissions from ruminant animals in China were estimated to be 10.18 Tg (China, 2004).

The amount of CH_4 emissions from ruminants is mainly affected by ration quality (e.g., forage type or its digestibility, and concentrate-to-forage ratio), intake level, ambient temperature, and the outflow rate of chyme (Lovett et al., 2003; McCaughey et al., 1997; Christopherson, 1976; Kennedy and Milligan, 1978). Considerable efforts have been devoted to improving feed utilization and controlling ruminant CH_4 emissions (Hironaka et al., 1996; Hensen et al., 2006).

Recently, a series of studies concerning CH_4 emissions from ruminants were conducted at the Chinese Academy of Agricultural Sciences. These include nutritional effects on CH_4 emissions from beef cattle, evaluation of IPCC (Intergovernmental Panel on Climate Change) methods (tiers 1 to 2) for assessing CH_4 emissions, and development of China-specific CH_4 emission factors for dairy cattle (Peng, 2002; Fan, 2004; Fan et al., 2006; You, 2007; Dong et al., 2008). However, the effect of ration manipulation on CH_4 emissions from dairy cows has not been addressed. This study attempted to fill this knowledge gap, improve the greenhouse gas (GHG) emissions inventories for animal production, and provide management guidance that may reduce CH_4 emissions.

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Mention of company or product names is for presentation clarity and does not imply endorsement of the company or products by the authors or their affiliations nor exclusion of other suitable product.

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Table 1. Ingredients of concentrate.

Ingredient	Proportion (%)
Corn	50.0
Cottonseed cake	9.5
Rapeseed cake	7.0
DDGS	6.0
Germ cake	5.0
Yeast powder	3.5
Fruit dreg	3.5
Bran	3.0
Puffed urea	2.5
$CaCO_3$	2.0
Soybean cake	1.5
$Ca_3(PO_4)_2$	1.0
Urea	1.0
Premix	1.0
NaCl	1.0
$NaHCO_3$	1.0
Zeolite powder	1.0
MgO	0.5

MATERIALS AND METHODS

EXPERIMENTAL DESIGN

Twelve lactating Holstein cows (approx. 130 days in milk) of average 3.5 years age were selected from the research dairy farm of the Inner Mongolian Agriculture University and subdivided into three groups of four animals each according to balanced age and body weight. The animal groups were then randomly allocated three different rations (rations A, B, and C) featuring different forage types and concentrate-to-forage ratios (CTFR). Table 1 contains the concentrate ingredients and proportions on dry matter (DM) basis. On DM basis, rations A and B had 40:60 CTFR, whereas ration C had 60:40 CTFR. The forage ingredient for ration A was corn stalk, while the corresponding forage component for rations B and C was corn silage. The experiment lasted 25 days, which included a 15-day adjustment period followed by a 10-day measurement period. The first five days of the measurement period were used to take breath samples and to estimate CH_4 emissions, and this period also was used for measurement of milk yield. The remaining five days of the measurement period were used for sampling of rumen fluid for determination of rumen fermentation characteristics.

FEEDS, FEEDING, AND ANIMALS MANAGEMENT

The feed was sampled continuously for five days during the gas sampling period. The concentrate, corn silage, and corn stalk were sampled when the five-day feed samples were collected. All three types of samples (concentrate, corn silage, and corn stalk) taken over five days were well mixed and resampled to provide three replicate samples, so a total of nine samples were sent to the laboratory for analysis. The samples were frozen, lyophilized, and ground to pass a 1 mm screen. The DM content was determined by drying the samples at 95°C for 17 h, followed by hot weighing. The Kjeldahl digestion method was used for CP analysis (N × 6.25). Gross energy was determined using a bomb calorimeter (model 1261, Parr Instruments Co., Moline, Ill.). The concentrations of NDF and ADF were determined by the filter bag technique (Ankom Technology Corp., Fairport, N.Y.). Ca and P were determined by inductively coupled plasma emission spectrometry (Optima 5300 DV, Perkin Elmer, Waltham, Mass.) after dry-ashing and extraction of the respective minerals.

The cows were housed individually in pens and fed manually. The animals in each dietary regimen were fed fixed amounts daily, consisting of 5.33 ±0.05, 4.83 ±0.26, and 7.63 ±0.29 kg head^{-1} d^{-1} of concentrate and 8.10 ±0.07, 27.75 ±0.07, and 18.58 ±0.28 kg head^{-1} d^{-1} of forage for rations A, B, and C, respectively, The *ad-libitum* feed intake was determined by pre-experiment in the adjustment period. During the experiment period, feed was supplied according to the feed intake of the pre-experiment. The concentrate was delivered twice daily at 7:00 h (about 40% of daily amount) and again at 19:00 h (about 60% of daily amount), whereas the forage was delivered three times daily (at 7:30, 13:30, and 19:30 h) in equal amounts. The cows had free access to drinking water, and they were milked twice a day (6:30 h and 18:30 h). Daily milk production was equal to the total milk weight of the two milking periods, and five-day milk production was recorded continually for this study.

SF_6 TRACER TECHNIQUE AND GAS SAMPLING FOR ESTIMATION OF CH_4 EMISSIONS

The SF_6 tracer technique (Johnson et al., 1994) was used to estimate CH_4 emissions from individual animals, and its application at our institute was described in detail by Fan (2004).

The SF_6 permeation tubes were manufactured using copper tubing with an inside diameter (ID) of 9.5 mm. The permeation element was 1 mm thick Teflon membrane with 2 μm stainless steel frit with an outside diameter (OD) of 9.5 mm (W.R. Grace, Deerfield, Ill.). All SF_6 permeation tubes were monitored and calibrated by placing tubes in a 460 L incubator (maintained at 39°C) that was ventilated with pure nitrogen (99.999%) gas at 40 mL min^{-1} flow rate to simulate the rumen environment of the animal. After three months monitoring, the tubes with steady permeation rates were used to measure CH_4 emissions from cows.

A SF_6 permeation tube with steady release rate was placed into the rumen of each cow about 15 days before emission rate measurements began. The SF_6 release rates of each permeation tube were measured over three-month periods by weighting each tube weekly. Regression analysis was used, and strong linear relationships were observed of the SF_6 release rate with time (R^2 = 0.9891 to 0.9994). The parameters of the linear regression equations for the individual SF_6 tubes are listed in table 2.

The breath sample collection canisters were made from PVC pipe to form a U-shaped device (fig. 1). The devices were evacuated to a pre-sampling internal pressure of -95 kPa (vacuum). A 1.5 m long capillary tube (1.58 mm or 1/16 in. OD and 0.127 mm or 1/200 in. ID) served as the transfer line or flow restrictor. A copper filter (15 μm, Swagelok, Solon, Ohio) near the nose sampling inlet was connected to the capillary tubing inlet to protect the tube. A halter was used to fit the gas container and capillary tube in the right location to provide good air sampling with no

Table 2. Cow ID numbers and regression equations for SF_6 tubes.

Cow ID	Regression Equation[a]	Permeation Rate (mg d^{-1})	Linear Correlation (R^2)
Ration A			
A1	$Y = 35.413 - 0.0018X$	1.8	0.9979
A2	$Y = 38.319 - 0.002X$	2.0	0.9984
A3	$Y = 36.629 - 0.0033X$	3.2	0.9994
A4[b]	$Y = 32.773 - 0.0026X$	2.6	0.9978
	$Y = 36.788 - 0.0011X$	1.1	0.9913
Ration B			
B1[b]	$Y = 39.71 - 0.0017X$	1.7	0.9975
	$Y = 39.714 - 0.0017X$	1.7	0.9983
B2[b]	$Y = 35.716 - 0.0018X$	1.8	0.9983
	$Y = 37.547 - 0.0015X$	1.5	0.9977
B3[b]	$Y = 35.597 - 0.0016X$	1.6	0.9980
	$Y = 36.786 - 0.0013X$	1.3	0.9976
B4	$Y = 36.875 - 0.0029X$	2.9	0.9981
Ration C			
C1	$Y = 35.304 - 0.0023X$	2.3	0.9980
C2[b]	$Y = 36.787 - 0.0014X$	1.4	0.9981
	$Y = 37.211 - 0.0023X$	2.3	0.9984
C3[b]	$Y = 37.932 - 0.0018X$	1.8	0.9983
	$Y = 35.588 - 0.0016X$	1.6	0.9984
C4[b]	$Y = 32.809 - 0.0025X$	2.5	0.9980
	$Y = 32.538 - 0.0011X$	1.1	0.9891

[a] Y = tube weight (g), and X = time (d).
[b] These cows received two permeation tubes in order to have similar SF_6 permeation rates.

impact on feeding. The canisters had a remaining vacuum of -50 kPa after a 24 h breath collection period.

The opening and closing times of each air sampling container were recorded during the sampling period to ensure that the gas sample in the sampling container represented exhaled air from the cow over the 24 h period. A total of five samples were taken for each cow. Immediately prior to sampling, the gas collection container was evacuated to -95 to -99 kPa. After the sampling period was completed, the container was taken off the cow, and the container vacuum was measured again to make sure it was still under negative pressure after the 24 h gas sample period. N_2 gas was then added slowly until the pressure in the container increased to approximately 120 kPa for transportation and gas analysis. Two gas samples from each container were taken in parallel for gas concentration analysis by GC.

Rumen Fluid Sample Collection

Rumen fluid was collected by stomach tubing (Guan et al., 2006; Zijderveld et al., 2011) from two randomly selected cows in each treatment. A total of six rumen fluid samples were collected per day for each cow. Rumen fluid samples were taken 1 h before the morning feeding and five times after feeding at 2 h intervals (2, 4, 6, 8, and 10 h). Approximately 200 mL of fluid was taken in each sample, with the first 100 mL of fluid discarded to minimize saliva contamination. During the five-day rumen sampling period, a total of 30 rumen fluid samples were collected for each cow. The rumen samples were filtered through two layers of gauze before determination of pH with an EL20 pH meter (Mettler-Toledo, Shanghai, China). The samples were subsequently frozen for further determination of ammonia nitrogen (NH_3-N) and volatile fatty acid (VFA) concentrations.

Analytical Methods

The CH_4 and SF_6 concentrations were determined by gas chromatography (model GC-14B, Shimadzu Co., Japan) with an electron capture detector (ECD) for SF_6 concen-

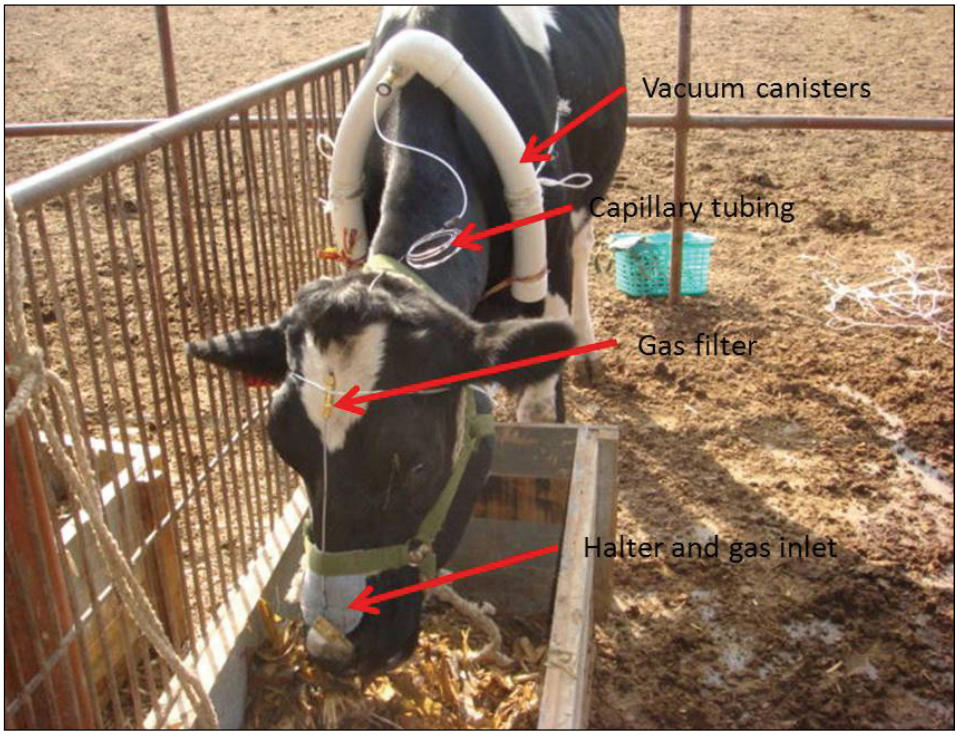

Figure 1. Gas sampling container and tubing installed on a cow.

tration and flame ionization detector (FID) for CH_4 concentration. The chromatographic conditions used were: inlet temperature of 80°C, column temperature of 100°C, detector temperature of 200°C, airflow rate of 400 mL min^{-1}, hydrogen flow rate of 60 mL min^{-1}, nitrogen flow rate of 50 mL min^{-1}, and sample volume of 1 mL. The standard SF_6 calibration gas concentration was 10.4×10^{-12} v v^{-1}. The standard CH_4 calibration concentration gas was 24.9×10^{-6} v v^{-1} (National Standard Material Center, Beijing, China).

The CH_4 emissions rate was calculated using equation 1 with the measured SF_6 and CH_4 concentrations and the known release rate of SF_6:

$$ER_{CH4} = \frac{ER_{SF6}}{6.518} \times \frac{C_{CH4}}{C_{SF6}} \times 1000 \quad (1)$$

where

ER_{CH4} = CH_4 emissions rate of the animal (L d^{-1})

ER_{SF6} = SF_6 release rate (mg d^{-1})

6.518 = density of SF_6 (kg m^{-3})

C_{CH4} = concentration of CH_4 gas in the collected samples (10^{-6} v v^{-1})

C_{SF6} = concentration of SF_6 gas in the collected gas samples (10^{-12} v v^{-1}).

The NH_3-N concentration was determined with a spectrophotometer at a wavelength of 700 nm (Feng and Gao, 1993). The VFA concentration was determined with the common method (Ding et al., 2006) involving gas chromatography (model GC-7A, Shimadzu, Kyoto, Japan).

The percentages of milk protein, milk fat, dry matter, and lactose contents were measured according to Chinese national standards GB/T 5413.1-1997, GB/T 5413.3-1997, GB/T 5413.8-1997, and GB/T 5413.5-2003 using a visible spectrophotometer and Kjeldahl apparatus as the main instruments. Milk yield was converted to the standard level of 4% fat content.

Statistical Analysis

The collected data were collated in an Excel 2003 spreadsheet. Significant differences in the response variables among the treatment groups were compared with GLM, followed by Duncan's multiple mean comparisons (SAS ver. 8.2, SAS Institute, Inc., Cary, N.C.). The results are presented as means ± standard error (SE). A probability value of 5% or lower was considered significant.

Results and Discussion

Dairy Cow Feed Intake and Properties

Table 3 lists the compositions for the concentrate, corn silage, and corn stalk on DM basis, The crude protein (CP) concentration of the concentrate was 2.8 times the CP concentration of corn silage and 3.9 times the CP concentration of corn stalk, while the acid detergent fiber (ADF) and neutral detergent fiber (NDF) contents of the concentrate were only 23.6% of corn silage ADF, 19.4% of corn stalk ADF, 41.6% of corn silage NDF, and 36.5% of corn stalk NDF. Table 4 lists the compositions of the rations on DM basis. Rations A, B, and C had similar gross energy contents, while the NDF of rations A and B was, respectively, 1.3 and 1.2 times that of ration C, and the ADF of rations A and B was, respectively, 1.5 and 1.3 times that of ration C.

Table 3. Composition of the raw materials on dry matter (DM) basis.

Composition[a]	Concentrate	Corn Silage	Corn Stalk
Dry matter (%)	89.94	22.07	86.13
Gross energy (MJ kg^{-1} DM)	15.31	17.36	17.65
Crude protein (% DM)	18.83	6.72	4.79
NDF (% DM)	28.68	68.88	78.65
ADF (% DM)	10.58	44.83	54.40
Ca (% DM)	1.92	0.78	0.43
P (% DM)	0.48	0.11	0.10

[a] NDF = neutral detergent fiber, and ADF = acid detergent fiber.

Table 4. Composition of the experimental rations.

Composition	Ration A[a]	Ration B[b]	Ration C[c]
Dry matter (%)	87.65	49.22	62.79
Gross energy (MJ kg^{-1})	16.71	16.54	16.13
Crude protein (% DM)	10.41	11.56	13.99
NDF (% DM)	58.66	52.80	44.76
ADF (% DM)	36.87	31.13	24.28
Ca (% DM)	1.03	1.24	1.46
P (% DM)	0.25	0.26	0.33

[a] Forage was corn stalk and CTFR was 40:60.

[b] Forage was corn silage and CTFR was 40:60.

[c] Forage was corn silage and CTFR was 60:40.

Methane Emissions

The CH_4 emissions from dairy cows with different diets are listed in table 5. The CH_4 emissions (mean ±SE) were 353 ±13.44, 283 ±7.48, and 263 ±9.04 L d^{-1} for rations A, B, and C, respectively. The CH_4 emissions accounted for 7.13%, 6.50%, and 5.91% of the gross energy (GE) intake for ration A, B, and C, respectively. Ration A resulted in significantly higher CH_4 emissions than rations B or C (20% and 25%, respectively; $p < 0.01$). No difference in CH_4 emissions between rations B and C was detected ($p = 0.32$). This outcome presumably resulted from the reduced fiber in corn silage as compared to corn stalks, leading to reduced rumen acetic acid but increased propionic acid, which in turn led to reduced CH_4 formation due to the highly negative correlation between propionic acid and CH_4 (Han et al., 1997). The current study showed results similar to the lower CH_4 emissions from beef cattle fed corn silage than from cattle fed dry corn stalks (Han et al., 1997; Fan et al., 2006).

This study also showed that dietary CTFR has an impact on CH_4 emissions. Higher CTFR could reduce CH_4 emissions, and Han et al. (1997) and Fan et al. (2006) reported similar results. Han et al. (1997) fed cattle (500 ±50 kg body weight) at dietary CTFR of 0:100, 25:75, 50:50, or 75:25 (with Chinese wild rye as forage) and reported CH_4 emissions of 208, 201, 194, and 171 L d^{-1}, respectively. Fan et al. (2006) fed beef cattle (358 ±15 kg body weight) at dietary CTFR of 75:25, 40:60, or 0:100 (with corn stalk as forage) and reported CH_4 emissions of 174, 190, and 239 L d^{-1}, respectively.

The CH_4 emissions per kg DM intake, shown in table 5, were 30.06, 27.15, and 24.28 L, respectively, for rations A, B, and C ($p < 0.01$ between A and C; $p < 0.05$ between A and B; $p < 0.05$ between B and C). The results show that

Table 5. Effects of dietary type and concentrate-to-forage ratio on methane (CH_4) emissions of dairy cows (mean ± SE).

Variable[a]	Ration and Concentrate-to-Forage Ratio (CTFR)[b]		
	Ration A (Corn stalk as forage, CTFR = 40:60)	Ration B (Corn silage as forage, CTFR = 40:60)	Ration C (Corn silage as forage, CTFR = 60:40)
DM intake (kg d^{-1}head^{-1})	11.77 ±0.10 a	10.47 ±0.22 b	10.96 ±0.32 ab
GE intake (MJ d^{-1} head^{-1})	196.68 ±1.64 a	173.13 ±3.56 b	176.83 ±5.12 b
CH_4 emissions (L d^{-1} head^{-1})	353 ±13.44 a	283 ±7.48 b	263 ±9.04 b
CH_4 energy (MJ d^{-1} head^{-1})	14.03 ±0.54 a	11.26 ±0.30 b	10.45 ±0.36 b
CH_4 emissions (L kg^{-1} DM)	30.06 ±1.17 a	27.15 ±0.71 b	24.28 ±1.01 c
CH_4 emissions (L MJ^{-1} consumed)	1.80 ±0.07 a	1.64 ±0.04 ab	1.51 ±0.06 b
CH_4 emissions (L kg^{-1} milk)	32.88 ±1.25 a	22.54 ±0.60 b	20.27 ±0.70 b
CH_4 emissions (L AU^{-1} head^{-1})	335 ±12.75 a	270 ±7.1 b	250 ±8.60 b
CH_4 energy/GE (%)	7.13 ±0.27 a	6.50 ±0.17 b	5.91 ±0.20 b

[a] DM = dry matter , GE = gross energy, and AU = animal unit = 500 kg body weight.
[b] Within a row, means followed by different letters are significantly different ($p < 0.05$).

Table 6. Summary of methane emissions rates (ER) from dairy cattle as reported in the literature and the current study.

Country or Region	Method	Animal Stage	CH_4 Emission Rates[a]			Reference
			L $head^{-1}$ d^{-1}	L AU^{-1} d^{-1}	L kg^{-1} milk	
Canada	SF_6 tracer method	Lactating beef cows	374	380	N/A	McCaughey et al. (1997)
China	SF_6 tracer method	Dairy cattle	263 to 353	250 to 335	20.27 to 32.88	This study
China	Energy metabolism trials	Dairy cattle	441 to 784	477	13.37 to 25.36	Sun et al. (2008)
North American	N/A	Dairy cattle	523	N/A	22.7	
Western European	N/A	Dairy cattle	478	N/A	29.1	IPCC (2006)
Asia	N/A	Dairy cattle	278	N/A	61.5	
Japan	Energy metabolism trials	Dry cattle	256 to 336	224	N/A	Kume et al. (2003)
New Zealand	SF_6 tracer method	Dairy cattle	387	400	N/A	Ulyatt et al. (1997)
Switzerland	Open-circuit respiration chambers	Dairy cattle	330 to 429	319	18.07	Hindrichsen et al. (2005)
U.K.	Indirect calorimeter chambers	Dairy cattle	518	456	23.87	Yan et al. (2006)

[a] Emission rates per animal unit (AU = 500 kg live weight) and per kg milk from the literature were recalculated in this study.

use of corn silage as forage and/or a higher concentrate ratio can reduce CH_4 emissions on the basis of kg DM intake. Compared with rations B and C, ration A increased CH_4 emissions by 9.7% and 19.2% per kg DM, respectively. The CH_4 emissions observed in this study are comparable with the values reported by Sun et al. (2008), in which CH_4 emissions were 20.87 to 31.85 L kg^{-1} DM intake for CTFR of 20:80 to 70:30. Sun et al. (2008) concluded that high CTFR could reduce CH_4 emissions per unit of DM intake.

Table 6 lists the CH_4 emissions from dairy cattle as reported in the literature and in the current study per animal unit per day (AU = 500 kg body weight) (Ngwabie et al., 2011) and per kg milk production. The CH_4 emissions of 250 to 335 L d^{-1} AU^{-1} in the current study were within the range of the literature values. Although the CH_4 emissions in this study tended to be lower than most of the literature values, the CH_4 emissions per unit of milk production in the current study tended to be higher than the literature values, except for the IPCC default emissions. The main reason is that the average milk production in the current study is approximately 2 times the IPCC default values for Asia (3200 vs. 1650 kg $head^{-1}$ $year^{-1}$), which would lead to lower CH_4 emissions per unit of milk production.

In addition, because the *ad-libitum* feed intake was determined by pre-experiment in the adjustment period, and no feed residue was found during the experiment period, the cows may have been slightly restricted on feed intake, and their DM intake may have been lower than their actual requirement. This could also reduce CH_4 emissions.

RUMEN FERMENTATION CHARACTERISTICS

Rumen Fluid pH

The rumen fluid pH values for the three ration groups (table 7) were between 6.06 and 6.87, which was within the normal range (5.0 to 7.5) of rumen fluid pH of dairy cows. However, there were significant differences in rumen fluid pH among the cows fed different rations ($p < 0.05$). The pH of the stalk-based ration A was significantly greater than that of the silage-based ration C ($p < 0.05$) due to the fact that the stalk-based ration contained more crude fiber, leading to less VFA production. With corn silage as forage,

Table 7. Effects of dietary concentrate-to-forage ratios on rumen characteristics of dairy cows (mean ± SE).

Response Variable	Ration and Concentrate-to-Forage Ratio (CTFR)[a]		
	Ration A (Corn stalk as forage, CTFR = 40:60)	Ration B (Corn silage as forage, CTFR = 40:60)	Ration C (Corn silage as forage, CTFR = 60:40)
Rumen fluid pH	6.64 ±0.04 a	6.53 ±0.03 a	6.21 ±0.04 b
Rumen fluid NH_3-N (mg 100 mL^{-1})	11.56 ±0.18 b	13.10 ±0.66 a	13.70 ±0.54 a
Acetic acid (mmol L^{-1})	67.29 ±3.45 a	64.88 ±3.62 a	58.25 ±2.75 a
Propionic acid (mmol L^{-1})	16.75 ±0.70 a	17.29 ±0.77 a	21.25 ±0.99 a
Butyric acid (mmol L^{-1})	5.43 ±0.23 b	6.36 ±0.41 ab	11.24 ±0.56 a
Acetic acid / propionic acid	4.23 ±0.32 a	3.90 ±0.34 a	2.58 ±0.18 b

[a] Within a row, means followed by different letters are significantly different ($p < 0.05$).

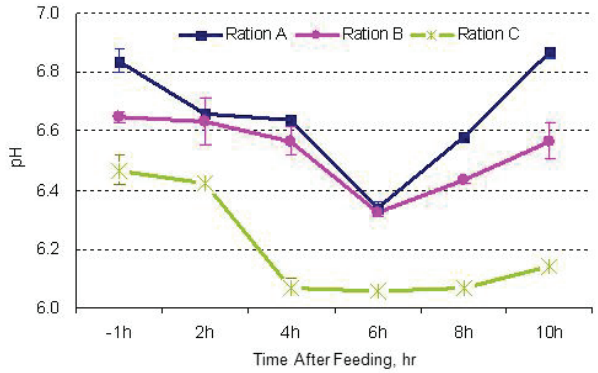

Figure 2. Effects of dietary concentrate to forage ratio on rumen fluid pH of dairy cows (vertical bars represent SD). Ration A: corn stalk as forage, CTFR = 40:60; Ration B: corn silage as forage, CTFR = 40:60; Ration C: corn silage as forage, CTFR = 60:40.

Figure 3. Effects of dietary forage to concentrate ratios on ammonia nitrogen (NH_3-N) concentration in rumen fluid of dairy cows (vertical bars represent SD). Ration A: corn stalk as forage, CTFR = 40:60; Ration B: corn silage as forage, CTFR = 40:60; Ration C: corn silage as forage, CTFR = 60:40.

the pH decreased ($p < 0.05$) with the increasing proportion of dietary concentrate. This outcome stemmed from the rapid fermentation of starch in the concentrate, which brought about more acid production, thus leading to lower pH in the rumen. This result was consistent with other studies (Kennelly et al., 1999; Bargo et al., 2002).

The dynamic profile of rumen fluid pH within 10 h after feeding is shown in figure 2. The rumen fluid pH of rations A, B, and C reached the minimum (6.34, 6.33, and 6.06, respectively) in 4 to 6 h after feeding, followed by upward trends, and the rumen pH of cows fed high-concentrate rations and silage started decreased in 2 h after feeding, which is similar to the results of Li (2007).

Ammonia Nitrogen Concentration in Rumen Fluid

Rumen fluid NH_3-N is the end product fermented from the feed protein and other nitrogenous compounds in rumen, which function as raw materials for rumen bacteria to synthesize microbial proteins. The NH_3-N concentration for ration A was significantly lower than that of rations B or C ($p < 0.05$) (table 7) owing to the relatively rapid degradation of corn silage compared with corn stalks. The result also showed that NH_3-N concentrations tended to increase with higher levels of dietary concentrate, which was consistent with the findings of Wang et al. (2005). The NH_3-N concentration in the rumen fluid for all three rations rose to a maximum (11.59 for ration A, 14.26 for ration B, and 14.39 for ration C, in units of mg 100 mL^{-1}) 2 h after feeding. Leng (1990) reported that NH_3-N levels increased up to 20 mg 100 mL^{-1}, which can improve bacterial growth efficiency. Rations B and C had higher NH_3-N concentrations, which would improve rumen fermentation. The NH_3-N concentrations of rations A and C decreased to a minimum (5.01 and 9.47 mg 100 mL^{-1}, respectively) 8 h after feeding, while that of ration B decreased to a minimum value of 5.51 mg 100 mL^{-1} 10 h after feeding (fig. 3).

VFA Concentration in Rumen Fluid

The ratio of acetic acid to propionic acid concentrations was significantly higher in rations A and B than in ration C ($p < 0.05$). This is consistent with the results of Miettinen and Huhtanen (1996), who reported that a higher

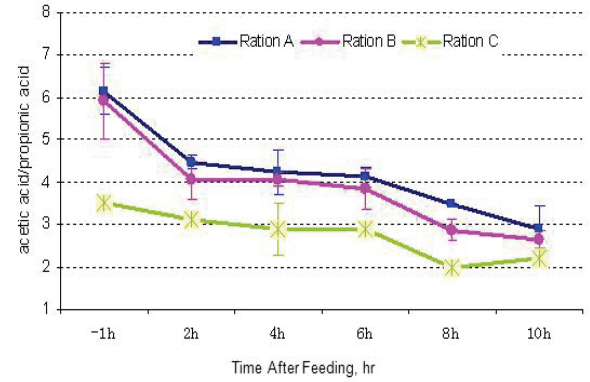

Figure 4. Change of acetic acid / propionic acid ratio in rumen fluid with time (vertical bars represent SD). Ration A: corn stalk as forage, CTFR = 40:60; Ration B: corn silage as forage, CTFR = 40:60; Ration C: corn silage as forage, CTFR = 60:40.

proportion of dietary concentrate would result in higher propionic acid in rumen and a reduced ratio of acetic acid and propionic acid. With silage-based rations, the concentrations of propionic acid and butyric acid in the rumen fluid were higher than those of stalk-based rations, and the concentrations of propionic acid and butyric acid in the rumen fluid rose with increasing proportion of concentrate. Significant differences in butyric acid concentrations were observed among the three rations ($p < 0.05$). Other studies also indicated that butyric acid concentrations increased significantly in the rumen with increasing proportions of dietary concentrate (Wang et al., 2005; Sun et al., 2008). The ratio of acetic acid and propionic acid for each ration group showed a downward trend over time after feeding (fig. 4).

SUMMARY AND CONCLUSIONS

Based on the experiments on methane (CH_4) emissions and rumen fermentation characteristics of lactating Holstein cows with three feed rations, i.e., CTFR of 40:60 with corn stalk as forage (ration A), CTFR of 40:60 with corn silage as forage (ration B), and CTFR of 60:40 with corn silage as

forage (ration C), the following conclusions were made:

The dietary regimens ranked ration A > ration B > ration C for CH_4 emissions quantity (L d^{-1} $head^{-1}$), rumen fluid pH, acetic acid concentration, and ratio of acetic acid and propionic acid. The CH_4 emissions (mean ±SE) for rations A, B, and C were 353 ±13.4, 283 ±7.48, and 263 ±9.04 L d^{-1} $head^{-1}$, respectively, or 335 ±12.75, 270 ±7.12, and 250 ±8.6 L d^{-1} AU^{-1}.

Use of corn silage as forage considerably reduced enteric CH_4 emissions of the cows as compared to corn stalk. Compared with rations B and C, the stalk-based ration A increased CH_4 emissions by 20% and 25%, respectively, on per animal basis. Ration A also increased CH_4 emissions by 9.7% and 19.2% per kg DM intake, or by 31% and 38% per kg milk production, compared with rations B and C, respectively.

The more fibrous ration increased rumen fluid pH, acetic acid content, and the ratio of acetic acid and propionic acid, but decreased NH_3-N concentration, propionic acid concentration, and butyric acid concentration.

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