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Relationship between the Methane Production and the CNCPS Carbohydrate Fractions of Rations with Various Concentrate/roughage Ratios Evaluated Using *In vitro* Incubation Technique

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ABSTRACT: The objective of the trial was to study the relationship between the methane (CH_4) production and the Cornell Net Carbohydrate and Protein System (CNCPS) carbohydrate fractions of feeds for cattle and the suitability of CNCPS carbohydrate fractions as the dietary variables in modeling the CH_4 production in rumen fermentation. Forty-five rations for cattle with the concentrate/roughage ratios of 10:90, 20:80, 30:70, 40:60, and 50:50 were formulated as feed samples. The Menke and Steingass's gas test was used for the measurement of CH_4 production. The feed samples were incubated for 48 h and the CH_4 production was analyzed using gas chromatography. Statistical analysis indicated that the CH_4 production (mL) was closely correlated with the CNCPS carbohydrate fractions (g), i.e. CA (sugars); CB_1 (starch and pectin); CB_2 (available cell wall) in a multiple linear pattern: CH_4 = (89.16±14.93) CA+ (124.10±13.90) CB_1+(30.58±11.72) CB_2+(3.28±7.19), R^2 = 0.81, p<0.0001, n = 45. Validation of the model using 10 rations indicated that the CH_4 production of the rations for cattle could accurately be predicted based on the CNCPS carbohydrate fractions. The trial indicated that the CNCPS carbohydrate fractions CA, CB_1 and CB_2 were suitable dietary variables for predicting the CH_4 production in rumen fermentation *in vitro*. (**Key Words:** Methane Production, CNCPS, Cattle, *In vitro* Incubation)

INTRODUCTION

To accurately predict the CH_4 production in rumen fermentation is important for identifying the strategies for mitigating CH_4 production from ruminants. Many studies indicated that the nutrient composition of feeds is closely correlated with the CH_4 production in rumen fermentation. Since there is a correlation between the feed composition and the CH_4 production, the feed composition is used to predict the CH_4 production. The dietary variables including dry matter intake (DMI) (Kriss, 1930; Axelsson, 1949; Mills et al., 2003; Ellis et al., 2007), energy digestibility (Blaxter and Clapperton, 1965), digestible carbohydrates (dCHO) (Bratzler and Forbes, 1940), intakes of non-structural carbohydrate (NSC), hemicellulose and cellulose (Moe and Tyrell, 1979) and digestible crude protein (CP), ether extract (EE), crude fibre and N-free extract of the diets (Jentsch et al., 2007) etc. were used in CH_4 predicting models. The use of different dietary variables in different models affected the accuracy of CH_4 predicting models. Screening the dietary parameters which are easily determined and closely correlated with the CH_4 production would be helpful for accurately modeling and predicting the CH_4 production from ruminants.

The Cornell Net Carbohydrate and Protein System (CNCPS) divided the carbohydrates and the nitrogenous compounds of feeds into detailed fractions based on the fermentative characteristics in the rumen (Sniffen et al., 1992). Many trials indicated that the CNCPS fractions closely correlated with some indices of rumen fermentation, including the duodenal flow of microbial N (Offner and Sauvant, 2004) and the *in situ* undegraded dietary protein (UDP) for ruminants (Shannak et al., 2000) etc. Since CH_4 is one of the important products of microbial fermentation of carbohydrates in the rumen, it could be speculated that the CH_4 production in rumen fermentation could be closely correlated with the CNCPS carbohydrate fractions.

The objectives of the present trial were to study the relationship between the rumen CH_4 production from feeds for cattle and the CNCPS carbohydrate fractions, and the suitability of the CNCPS carbohydrate fractions as dietary

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variables in modeling CH_4 production in rumen fermentation.

MATERIALS AND METHODS

Animals and feeding management

Two castrated Simmental bulls, aged 1.5 yrs, with average liveweight of 372±6 kg and fitted with permanent rumen fistulas made of polyethylene (Beijing *Jinniuweiye* Science and Technology Co., Ltd., Beijing, China), were used as the donors of rumen fluid. The daily ration for the cattle included 6.0 kg Chinese wildrye and 2.0 kg concentrate mixture. The concentrate mixture was composed of 58% corn, 20% soybean meal, 18% wheat bran, 2% calcium hydrogen phosphate, 1% sodium chloride, and 1% trace element mixture. The cattle were fed twice daily at 07:00 h and 17:00 h, in two equal meals, and had free access to fresh drinking water. The management of the cattle was according to The Administration Regulations on Laboratory Animals (The Administrative Department of Beijing Municipal Science and Technology, 2002).

Feed samples

Air-dried feeds for cattle, milled to pass a screen with the pore size of 1 mm, were used as the materials for formulating rations. Forty-five rations for cattle with the concentrate/roughage ratios of 10:90, 20:80, 30:70, 40:60, and 50:50 were formulated as the feed samples, of which 9 rations were formulated for each concentrate/roughage ratio. The components of the rations were shown in Table 1.

In vitro incubation

The Menke and Steingass's (1988) gas test was used for the measurement of CH_4 production of feed samples. Glass syringes with a calibrated volume of 100 mL were used as the incubation vessels.

Two hundred mL of rumen fluid was taken from each cattle through the rumen fistulas 2 h after morning feeding. The rumen fluid from the two cattle was well mixed and immediately strained through four layers of gauze into pre-warmed bottle (39°C). Three hundred mL of rumen fluid and 600 mL buffer were mixed and continuously gassed with carbon dioxide. Each syringe contained 0.2000 g feed sample and the syringes were pre-warmed at 39°C. Four syringes were used for each ration as replicates and three syringes without feed samples were used as the blanks for each batch of samples. Each syringe was filled with 30 mL rumen fluid-buffer mixture. The air in the syringes was transpired and the heads of the syringes were sealed. The syringes were kept in a water bath at 39°C for incubation. The total gas production of feed samples was recorded and the pH of incubation residue was immediately determined after incubated for 48 h. A 5 mL gas sample was taken through a syringe needle connector fitted between the sampling syringe and the incubation syringe for the analysis of gas composition.

Determinations and analysis

The dry matter (DM), EE and ash of the feed samples were determined according to AOAC (1990) using the methods of no. 934.01, 920.39, and 924.05, respectively. The CP of feed samples was analyzed using the Kjeldahl method. The neutral detergent fibre (NDF) was analyzed using the method of Van Soest et al. (1991). The neutral detergent insoluble CP (NDICP) was analyzed by determination of the CP in the NDF residues. The acid detergent lignin was analyzed using the method of Goering and Van Soest (1970). The starch content of feed samples was determined using spectrophotometry (UV-9100, Beijing *Ruili* Analytical Instruments, China) after converting starch to glucose using an enzyme kit containing thermostable α-amylase and amyloglucosidase (Megazyme International Ireland Ltd., Wicklow, Ireland; Method 996.11, AOAC, 1990).

The concentrations of CH_4 and CO_2 in the gas samples were analyzed using gas chromatography (TP-2060T, Beijing *Beifen Tianpu* Instrument Technology Co., Ltd., Beijing, China). The conditions for the analysis were as following: TCD detector, TDX-01 column, size 1 m×2 mm ×3 mm, column temperature 70°C, detector temperature 100°C. The carrying gas was argon, with the flowing rate of 30 mL/min. The standard gas used was composed of 26.796% CH_4, 65.300% CO_2, 0.605% O_2, 7.100% N_2 and 0.199% H_2 (v/v).

Calculation and statistical analysis

The CNCPS carbohydrate fractions of the rations for modeling were calculated according to Sniffen et al. (1992) and listed in Table 2.

$$CA = NSC - Starch$$

$$CB_1 = Starch$$

$$CB_2 = NDF - NDICP - CC$$

$$CC = Lignin \times 2.4$$

$$NSC = CHO - CB_2 - CC$$

$$CHO = 100 - CP - Ash - EE$$

Where CA refers to sugars; CB_1, starch and pectin; CB_2, available cell wall; CC, unavailable cell wall; NSC, non-structural carbohydrate; CHO, carbohydrate; CP, crude protein; NDICP, neutral detergent insoluble crude protein.

Table 1. The components of the rations for modeling (%, air dry basis)

Ration no.	Corn	Soybean meal	Wheat bran	Cottonseed meal	Rapeseed meal	DDGS	Wheat middlings	Rice straw	Corn stover	Corn silage	Wheat straw	Millet straw	Chinese wildrye	Concentrate/ roughage ratio
1	28.5	11.5	10.0	-	-	-	-	50	-	-	-	-	-	50:50
2	22.8	9.2	8.0	-	-	-	-	60	-	-	-	-	-	40:60
3	17.1	6.9	6.0	-	-	-	-	70	-	-	-	-	-	30:70
4	11.4	4.6	4.0	-	-	-	-	80	-	-	-	-	-	20:80
5	28.5	11.5	10.0	-	-	-	-	-	-	25	-	25	-	50:50
6	22.8	9.2	8.0	-	-	-	-	-	-	39	-	21	-	40:60
7	17.1	6.9	6.0	-	-	-	-	-	-	56	-	14	-	30:70
8	5.7	2.3	2.0	-	-	-	-	-	-	45	-	45	-	10:90
9	27.5	-	9.5	13.0	-	-	-	-	50	-	-	-	-	50:50
10	22.0	-	7.6	10.4	-	-	-	-	60	-	-	-	-	40:60
11	11.0	-	3.8	5.2	-	-	-	-	80	-	-	-	-	20:80
12	5.5	-	1.9	2.6	-	-	-	-	90	-	-	-	-	10:90
13	26.5	7.5	9.5	-	6.5	-	-	-	-	50	-	-	-	50:50
14	15.9	4.5	5.7	-	3.9	-	-	-	-	70	-	-	-	30:70
15	10.6	3.0	3.8	-	2.6	-	-	-	-	80	-	-	-	20:80
16	5.3	1.5	1.9	-	1.3	-	-	-	-	90	-	-	-	10:90
17	18.8	6.0	6.0	4.0	-	2.4	2.8	-	-	-	-	-	60	40:60
18	14.1	4.5	4.5	3.0	-	1.8	2.1	-	-	-	-	-	70	30:70
19	9.4	3.0	3.0	2.0	-	1.2	1.4	-	-	-	-	-	80	20:80
20	4.7	1.5	1.5	1.0	-	0.6	0.7	-	-	-	-	-	90	10:90
21	23.5	7.5	7.5	5.0	-	3.0	3.5	-	25	-	25	-	-	50:50
22	18.8	6.0	6.0	4.0	-	2.4	2.8	-	39	-	21	-	-	40:60
23	14.1	4.5	4.5	3.0	-	1.8	2.1	-	56	-	14	-	-	30:70
24	9.4	3.0	3.0	2.0	-	1.2	1.4	-	60	-	20	-	-	20:80
25	26.0	-	9.0	7.5	7.5	-	-	-	-	-	-	50	-	50:50
26	20.8	-	7.2	6.0	6.0	-	-	-	-	-	-	60	-	40:60
27	15.6	-	5.4	4.5	4.5	-	-	-	-	-	-	70	-	30:70
28	5.2	-	1.8	1.5	1.5	-	-	-	-	-	-	90	-	10:90
29	26.0	-	9.0	7.5	7.5	-	-	-	-	-	25	25	-	50:50
30	20.8	-	7.2	6.0	6.0	-	-	-	-	-	39	21	-	40:60
31	10.4	-	3.6	3.0	3.0	-	-	-	-	-	60	20	-	20:80
32	5.2	-	1.8	1.5	1.5	-	-	-	-	-	45	45	-	10:90
33	25.0	7.5	8.5	5.0	4.0	-	-	-	-	-	50	-	-	50:50
34	15.0	4.5	5.1	3.0	2.4	-	-	-	-	-	70	-	-	30:70
35	10.0	3.0	3.4	2.0	1.6	-	-	-	-	-	80	-	-	20:80
36	5.0	1.5	1.7	1.0	0.8	-	-	-	-	-	90	-	-	10:90
37	20.0	6.0	6.8	4.0	3.2	-	-	-	-	20	30	-	10	40:60
38	15.0	4.5	5.1	3.0	2.4	-	-	-	-	35	21	-	14	30:70
39	10.0	3.0	3.4	2.0	1.6	-	-	-	-	52	14	-	14	20:80
40	5.0	1.5	1.7	1.0	0.8	-	-	-	-	30	30	-	30	10:90
41	27.0	-	8.0	-	15.0	-	-	-	25	-	25	-	-	50:50
42	21.6	-	6.4	-	12.0	-	-	-	39	-	21	-	-	40:60
43	16.2	-	4.8	-	9.0	-	-	-	56	-	14	-	-	30:70
44	10.8	-	3.2	-	6.0	-	-	-	60	-	20	-	-	20:80
45	5.4	-	1.6	-	3.0	-	-	-	45	-	45	-	-	10:90

DDGS refers to dried distiller's grains with solubles.

The unit for all the CNCPS fractions is % DM.

The CH_4, CO_2 or total gas production of feed samples (mL) was calculated as following:

$$Y_{sample} = Y_{total} - Y_{blank}$$

Where, Y_{sample} refers to the CH_4 production of feed sample in 48 h; Y_{total}, the CH_4, CO_2 or total gas production of incubation in 48 h; Y_{blank}, the CH_4, CO_2 or total gas production of the blank in 48 h. The CH_4, CO_2 and total gas production and the pH for modeling were listed in Table 3.

The regression relationship between the CH_4, CO_2 and total gas production (mL) and the CNCPS carbohydrate fractions (g) was analyzed using the following equation:

$$y = b_1(\text{CA})+b_2(\text{CB}_1)+b_3(\text{CB}_2)+a$$

Where, y refers to the CH_4, CO_2 or total gas production;

Table 2. The CP and CNCPS carbohydrate fractions of the rations for modeling (% DM)

Ration no.	CP	Carbohydrates	Carbohydrate fractions				NSC
			CA	CB_1	CB_2	CC	
1	12.79	77.34	10.03	19.56	39.97	7.75	29.60
2	11.22	77.95	9.45	15.94	43.96	8.57	25.40
3	9.64	78.56	8.87	12.33	47.95	9.40	21.20
4	8.07	79.17	8.29	8.71	51.93	10.23	17.00
5	12.81	80.68	14.41	19.15	38.13	8.96	33.56
6	11.20	82.03	13.99	15.47	43.02	9.51	29.46
7	9.58	83.40	13.33	11.81	48.34	9.90	25.14
8	6.54	85.78	15.59	4.35	52.61	13.24	19.93
9	15.04	76.89	12.74	18.38	36.27	9.48	31.12
10	13.96	77.40	13.26	14.82	39.78	9.51	28.08
11	11.80	78.41	14.28	7.71	46.82	9.58	21.99
12	10.72	78.91	14.80	4.16	50.34	9.62	18.95
13	13.14	79.92	12.01	17.92	40.90	9.06	29.93
14	9.79	82.93	11.98	11.06	49.86	10.02	23.04
15	8.11	84.44	11.96	7.63	54.34	10.49	19.59
16	6.44	85.94	11.94	4.21	58.82	10.97	16.15
17	12.59	80.35	14.40	13.92	37.91	14.10	28.32
18	10.98	81.69	14.57	10.53	41.20	15.38	25.10
19	9.37	83.03	14.74	7.14	44.48	16.66	21.88
20	7.75	84.37	14.92	3.74	47.77	17.94	18.66
21	14.81	77.33	11.78	17.39	35.99	12.15	29.17
22	13.73	77.81	12.39	14.03	39.56	11.81	26.41
23	12.79	78.11	13.30	10.67	43.14	10.98	23.97
24	11.25	79.17	12.88	7.29	46.69	12.29	20.17
25	13.24	79.54	15.02	17.36	33.35	13.78	32.38
26	11.63	80.97	15.98	13.99	36.51	14.46	29.97
27	10.01	82.39	16.95	10.62	39.67	15.13	27.57
28	6.79	85.24	18.88	3.88	46.00	16.48	22.76
29	13.21	79.19	11.34	17.32	34.48	16.02	28.66
30	11.58	80.42	10.24	13.94	38.28	17.94	24.18
31	8.33	82.97	9.07	7.17	45.55	21.17	16.24
32	6.74	84.61	12.25	3.82	48.03	20.50	16.07
33	14.19	78.02	8.92	16.66	35.59	16.83	25.59
34	10.54	80.92	7.40	10.15	42.82	20.53	17.55
35	8.71	82.37	6.63	6.89	46.44	22.38	13.53
36	6.89	83.81	5.87	3.64	50.06	24.24	9.51
37	12.41	79.95	10.52	13.48	40.87	15.06	24.00
38	10.58	81.75	11.18	10.29	45.82	14.45	21.46
39	8.71	83.57	11.57	7.10	51.08	13.81	18.67
40	7.12	84.60	10.91	3.75	52.16	17.78	14.66
41	13.95	77.32	9.85	17.78	35.44	14.22	27.63
42	13.04	77.80	10.84	14.34	39.12	13.47	25.18
43	12.27	78.10	12.14	10.91	42.81	12.23	23.04
44	10.90	79.16	12.11	7.45	46.47	13.12	19.55
45	8.67	81.34	10.14	3.95	50.10	17.14	14.09

a refers to a constant; b_1, b_2, and b_3 refer to coefficients.

Ten rations with the same range of concentrate/roughage ratio as that of the 45 rations for modeling with two rations for each ratio were formulated to validate the relationships between the CH_4, CO_2 and total gas production and the CNCPS carbohydrate fractions. The components, the nutrient composition and the CH_4, CO_2 and total gas production of the rations for validation were listed

Table 3. The CH_4, CO_2 and total gas production and pH of the rations for modeling

Ration no.	CH_4 (mL/g DM)	CO_2 (mL/g DM)	Total gas (mL/g DM)	pH
1	50±0	189±2	248±2	6.63±0.00
2	47±1	186±3	241±3	6.61±0.01
3	45±1	174±6	227±6	6.63±0.01
4	40±0	155±1	202±1	6.71±0.01
5	49±1	213±1	272±1	6.58±0.00
6	47±1	209±3	266±3	6.58±0.00
7	42±1	198±3	250±3	6.58±0.01
8	37±0	185±3	232±5	6.63±0.02
9	51±1	172±3	240±3	6.66±0.01
10	48±0	150±6	214±6	6.74±0.01
11	37±1	160±3	207±4	6.77±0.00
12	33±1	143±6	186±5	6.78±0.01
13	49±1	218±4	281±2	6.51±0.00
14	45±1	216±3	272±2	6.57±0.00
15	40±1	173±5	223±5	6.55±0.01
16	37±1	162±11	208±11	6.57±0.00
17	47±1	175±3	235±4	6.47±0.02
18	43±1	156±3	211±2	6.51±0.02
19	40±1	144±4	195±4	6.55±0.01
20	36±0	135±0	181±0	6.58±0.00
21	45±1	177±2	232±3	6.64±0.02
22	43±1	173±2	225±1	6.56±0.01
23	39±1	166±2	213±4	6.59±0.01
24	37±1	151±2	195±3	6.59±0.01
25	52±1	194±5	260±5	6.73±0.00
26	51±0	182±1	245±0	6.75±0.00
27	48±0	161±8	219±9	6.69±0.01
28	41±0	155±11	191±14	6.77±0.00
29	42±1	163±3	214±3	6.61±0.01
30	39±1	162±4	210±4	6.66±0.01
31	33±1	135±2	176±2	6.75±0.01
32	30±1	127±1	165±1	6.74±0.00
33	43±1	135±3	191±4	6.65±0.00
34	36±1	128±11	179±10	6.69±0.01
35	36±2	123±1	173±3	6.71±0.01
36	30±1	126±5	167±5	6.69±0.01
37	43±1	192±7	254±7	6.69±0.00
38	38±1	180±4	244±2	6.71±0.00
39	38±1	171±3	226±2	6.74±0.00
40	34±1	137±2	189±4	6.77±0.00
41	39±0	171±8	219±9	6.58±0.01
42	39±0	168±2	220±4	6.49±0.01
43	39±1	160±8	208±8	6.57±0.03
44	37±1	158±8	206±7	6.54±0.00
45	34±1	141±5	183±5	6.57±0.04

Values were presented as mean±standard error (SE).

in Table 4, 5 and 6, respectively.

The accuracy of the multiple regression relationships for predicting the CH_4, CO_2 and total gas production was evaluated in three ways. The observed and the predicted CH_4, CO_2 and total gas production were compared using the paired *t*-test; the relationships between the observed and the predicted CH_4, CO_2 and total gas production were analysed using the equation:

$$y = bx+a$$

Where, x refers to the observed CH_4, CO_2 or total gas production, mL/g DM; y refers to the predicted CH_4, CO_2 or total gas production, mL/g DM; The root mean square prediction error (RMSPE) between the observed and the predicted CH_4, CO_2 or total gas production was also calculated for evaluating the multiple regression relationship established in the trial. The RMSPE was calculated as:

$$\text{Mean square prediction error (MSPE)} = \sum_{i=1}^{n}(Oi - Pi)^2 / n$$

$$\text{RMSPE\%} = \text{MSPE}^{1/2}/\text{average observed value}\times 100$$

Where, $i = 1, 2, \ldots, n$; O_i refers to the observed value; P_i, the predicted value; n, the number of determinations. RMSPE%, the percentage of the prediction error/the average observed value.

The SAS Statistical Package 9.2 (SAS Institute Inc., Cary, NC, USA, 2008) was used for the statistical analysis of the trial. The prediction equations were developed using the PROC GLMSELECT Procedure. All the independent variables included in the equations were selected using the stepwise regression analysis by deleting non-significant variables (p>0.05). The PROC REG Procedure was used for the analysis of the relationship between the observed and the predicted values.

RESULTS

In vitro incubation

At the end of *in vitro* incubation for 48 h, the pH value of the incubation residue was within the range of 6.40 to 6.80, and the microscopic check indicated that the rumen microorganisms were active.

Relationships between the CH_4, CO_2, and total gas production and the CNCPS carbohydrate fractions

The CNCPS carbohydrate fractions of the mixed rations and the CH_4 production were shown in Table 2 and 3.

Significant multiple linear regression relationships were found between the CH_4, CO_2 and total gas production (mL)

Table 4. The components of the rations for validation (%, air dry basis)

Ration no.	Corn	Soybean meal	Wheat bran	Cottonseed meal	Rapeseed meal	DDGS	Wheat middlings	Rice straw	Corn stover	Corn silage	Wheat straw	Millet straw	Chinese wildrye	Concentrate/ roughage ratio
1	5.7	2.3	2.0	-	-	-	-	90	-	-	-	-	-	10:90
2	11.4	4.6	4.0	-	-	-	-	-	-	60	-	20	-	20:80
3	16.5	-	5.7	7.8	-	-	-	-	70	-	-	-	-	30:70
4	21.2	6.0	7.6	-	5.2	-	-	-	-	60	-	-	-	40:60
5	23.5	7.5	7.5	5.0	-	3.0	3.5	-	-	-	-	-	50	50:50
6	4.7	1.5	1.5	1.0	-	0.6	0.7	-	45	-	45	-	-	10:90
7	10.4	-	3.6	3.0	3.0	-	-	-	-	-	-	80	-	20:80
8	15.6	-	5.4	4.5	4.5	-	-	-	-	-	56	14	-	30:70
9	20.0	6.0	6.8	4.0	3.2	-	-	-	-	-	60	-	-	40:60
10	25.0	7.5	8.5	5.0	4.0	-	-	-	-	30	10	-	10	50:50

DDGS refers to dried distiller's grains with solubles.

Table 5. The CP and CNCPS carbohydrate fractions of the rations for validating the model (% DM)

Ration no.	CP	Carbohydrates	Carbohydrate fractions				NSC
			CA	CB_1	CB_2	CC	
1	6.50	79.78	7.71	5.09	55.92	11.05	12.80
2	8.02	84.66	13.71	8.10	51.82	11.02	21.81
3	12.88	77.90	13.77	11.27	43.30	9.55	25.04
4	11.46	81.42	11.99	14.49	45.38	9.54	26.48
5	14.20	79.02	14.22	17.32	34.63	12.82	31.54
6	8.84	81.34	10.52	3.87	50.21	16.73	14.39
7	8.40	83.82	17.91	7.25	42.84	15.81	25.16
8	9.95	81.60	8.70	10.54	42.21	20.14	19.24
9	12.36	79.47	8.16	13.41	39.20	18.68	21.57
10	14.20	78.73	11.96	16.78	38.21	11.75	28.74

and the CNCPS carbohydrate fractions CA, CB_1 and CB_2 (g).

$$CH_4 = (89.16\pm14.93)\ CA+(124.10\pm13.90)\ CB_1+(30.58\pm11.72)\ CB_2+(3.28\pm7.19)$$
$R^2 = 0.81$, n = 45, $p<0.0001$ (Equation I)

$$CO_2 = (435.58\pm69.51)\ CA+(707.76\pm64.69)\ CB_1+(410.42\pm54.58)\ CB_2-(145.17\pm33.49)$$
$R^2 = 0.78$, n = 45, $p<0.0001$ (Equation II)

$$\text{Total gas} = (486.04\pm78.87)\ CA+(845.98\pm73.41)\ CB_1+(439.20\pm61.94)\ CB_2-(126.92\pm38.01)$$
$R^2 = 0.80$, n = 45, $p<0.0001$ (Equation III)

Validation of the equations between the CH_4, CO_2 and total gas production and the CNCPS carbohydrate fractions

Paired *t*-test showed that no difference was found

Table 6. The CH_4, CO_2 and total gas production and pH of the rations for validating the models

Ration no.	CH_4 (mL/g DM)		CO_2 (mL/g DM)		Total gas (mL/g DM)		pH
	Observed	Predicted	Observed	Predicted	Observed	Predicted	
1	35±1	34	142±3	154	183±3	199	6.69±0.01
2	42±1	42	185±3	184	237±2	236	6.62±0.01
3	40±0	43	167± 0	172	216±0	225	6.76±0.01
4	46±1	46	224±5	196	282±4	253	6.50±0.01
5	50±0	48	177±2	181	242±2	241	6.46±0.01
6	30±0	33	151±7	134	187±8	177	6.65±0.01
7	41±1	41	176±21	160	214±6	210	6.78±0.01
8	36±0	37	150±3	140	195±4	190	6.71±0.01
9	39±1	39	142±11	146	196±10	198	6.71±0.01
10	47±1	47	200±2	182	261±2	241	6.62±0.01

Values were presented as mean±standard error (SE).

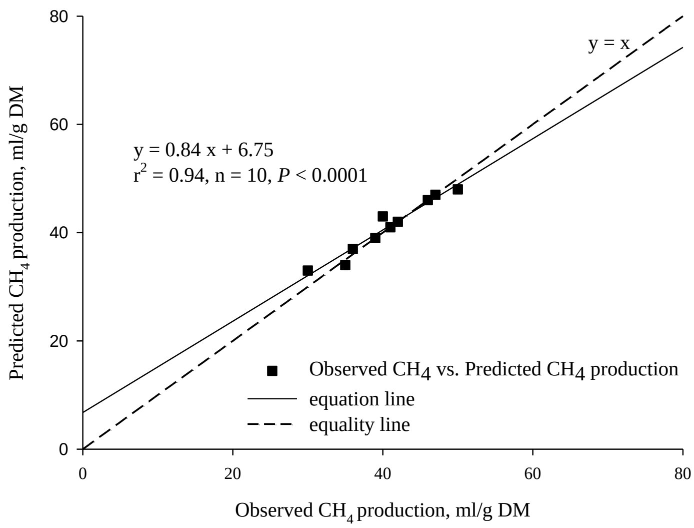

Figure 1. Relationship between the observed vs the predicted CH_4 production.

between the observed and the predicted CH_4, CO_2 and total gas production based on the Equation I (p = 0.443), Equation II (p = 0.150) and Equation III (p = 0.326), respectively. Significant linear regression relationship was found between the observed and the predicted CH_4 production based on Equation I (R^2 = 0.94, p<0.0001, n = 10, Figure 1), between the observed and the predicted CO_2 production based on Equation II (R^2 = 0.77, p = 0.0008, n = 10, Figure 2) and between the observed and the predicted total gas production based on Equation III (R^2 = 0.87, p< 0.0001, n = 10, Figure 3). The RMSPE% of Equations I, II, and III was found to be 3.82%, 8.16% and 5.93%, respectively.

DISCUSSION

Measurement of gas production

The normal pH range and the active rumen microorganisms in the incubation residues at the end of incubation indicated that the simulation of rumen fermentation using the Hohenheim gas test (Menke and Steingass, 1988) was successful. Since the highest predictive value for the *in vivo* digestibility of feed was obtained after 45 to 52 h of *in vitro* fermentation (Prasad et al., 1994; Liu et al., 2002), the gas production of the rations during the 48 h *in vitro* incubation was believed to be close to that of the actual rumen fermentation.

Soe et al. (2009) reported that the *in vitro* gas production of ruminant feeds abundant in CP such as soybean meal (CP 51.3% DM) and cell mass from lysine production (CMLP) (CP 72.3% DM) was significantly lower than the theoretical value, indicating that the nitrogenous compounds of the feeds interfered with the acid-base reaction, increased the pH and reduced the indirect gas production. In this case, the *in vitro* gas test might be not a suitable technique for measuring the gas

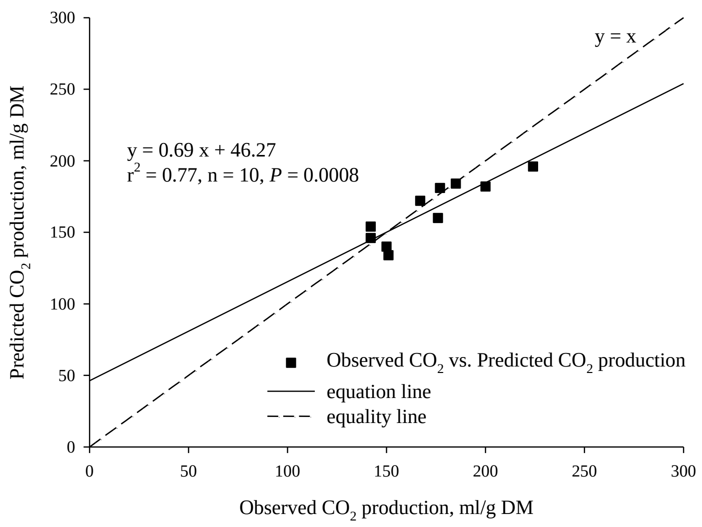

Figure 2. Relationship between the observed vs the predicted CO_2 production.

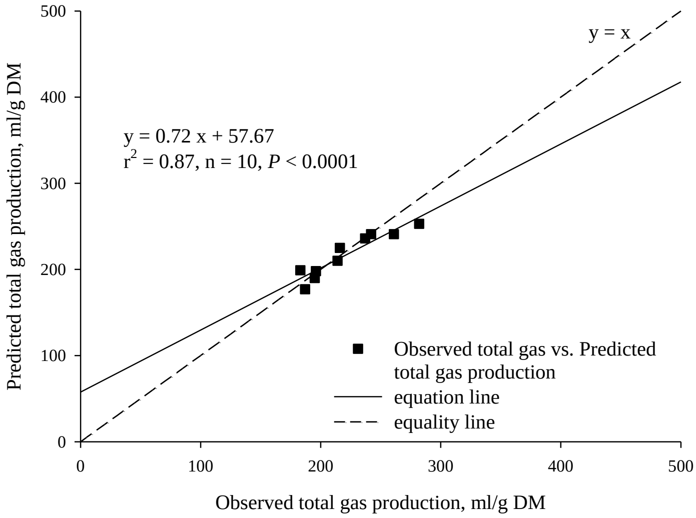

Figure 3. Relationship between the observed vs the predicted total gas production.

production. In the present trial, the CP content of the mixed rations for modeling was from 6.44 to 15.04% and that for validation was from 6.50 to 14.20%, which was in a moderate range, it could be believed that the *in vitro* gas test was suitable for the gas measurements and the results were reliable.

Relationships between the CH_4, CO_2 and total gas production and the CNCPS carbohydrate fractions

The CNCPS divides the carbohydrates of feeds for ruminants into four fractions, i.e. CA (sugar), CB_1 (starch and pectin), CB_2 (available fibre) and CC (unavailable fibre) (Sniffen et al., 1992). Fractions CA, CB_1 and CB_2 can be fermented in the rumen at fast, moderate and slow speed, respectively, whereas fraction CC is not fermentable in the rumen. The classification of the CNCPS carbohydrate fractions reflects the carbohydrate composition as well as the fermentative characteristics of the carbohydrates. Significant positive regression relationships found in the present trial between the CH_4 production (Equation I), the CO_2 production (Equation II), the total gas production (Equation III) and the CNCPS carbohydrate fractions CA, CB_1 and CB_2 indicated that the CNCPS carbohydrate fractions were suitable parameters for predicting the CH_4 production, the CO_2 production and the total gas production.

The carbohydrate fraction CC was not included in the equations because it was screened out by the stepwise regression analysis. The result was in accordance with the fermentative characteristics of fraction CC in the CNCPS (Sniffen et al., 1992). The results were also in agreement with the results of Moe and Tyrell (1979) who found that no significant regression relationship existed between the CH_4 production and the lignin intake of Holstein cows.

Ruminal fermentation of cellulose and hemicellulose mainly produces acetate and butyrate accompanied with the formation of CO_2 and H_2 which are used for CH_4 production, whereas fermentation of sugars and NSC mainly produces propionate accompanied with an uptake of H_2. It could be presumed that the fermentation of fraction CB_2 would produce more CH_4 than that of fractions A and CB_1. In Equation I, however, the coefficients of CA and CB_1, CB_2 are 89.16, 124.10, and 30.58 mL/g, respectively, indicating that fraction CB_2 produced less CH_4 than fractions CA and CB_1. The reason for the results could be that CB_2 was the available fibre with slower fermentation rate than fractions CA and CB_1.

Moe and Tyrell (1979) studied the relationship among the diet composition, intake and CH_4 production of Holstein cows and found a regression relationship between the CH_4 production and the intakes of the soluble residue, hemicellulose and cellulose (R^2 = 0.67) and the regression relationship became closer when the apparently digested soluble residue, hemicellulose and cellulose were used as the dietary variables (R^2 = 0.73). Jentsch et al. (2007) found a regression relationship between the CH_4 production and the intakes of CP, crude fat, crude fibre and N-free extract of cattle (R^2 = 0.859). The regression relationship also became closer when the digestible nutrients including CP, EE, crude fibre and N-free extract were used as the dietary variables (R^2 = 0.896). In Equation I of the present trial, the determination coefficient (R^2 = 0.81) between the CH_4 production and the CNCPS fractions CA and CB_1, CB_2 was between the determination coefficient of Moe and Tyrell (1979) and that of Jentsch et al. (2007), indicating that Equation I was reliable for predicting the CH_4 production.

Validation of the equations established in the trial

Validation of Equations I, II, and III in the present trial using 10 rations indicated that the determination coefficient (R^2) between the observed and the predicted CH_4, CO_2 or total gas production was high. The RMSPE% of Equations I, II and III was lower than that of Moe and Tyrrell (1979) (34%), Ellis et al. (2007) (14.4%) and Blaxter and Clapperton (1965) (36.5%). The high determination coefficient and the low RMSPE% indicate that Equations I, II, and III are reliable for predicting the CH_4 CO_2 or total gas production of rations with the roughage/concentrate ratios within the range of 10:90, 20:80, 30:70, 40:60, and 50:50 for cattle. The trial demonstrated that the CNCPS fractions CA and CB_1, CB_2 are suitable dietary variables for modeling CH_4 production.

It should be noted, however, that Equations I, II, and III in the present trial were developed based on the *in vitro* measurement of gas production. Since rumen is a dynamic system with the passage and digestion of digesta taking place simultaneously, and the substrate for methanogenesis shifts from hydrogen to formate after 10 h of *in vitro* batch culture (Seo et al., 2009), this trial suffers from the limitations of the 48 h *in vitro* incubation. The equations need to be validated using *in vivo* trials for predicting the CH_4 CO_2 and total gas production from rumen fermentation of cattle.

CONCLUSION

A significant multiple linear regression relationship was found between the CH_4 production and the CNCPS carbohydrate fractions CA, CB_1 and CB_2 of rations for cattle over a wide range of concentrate/roughage ratios. Evaluation results demonstrated that the *in vitro* CH_4 production of rations for cattle could be accurately predicted based on the CNCPS carbohydrate fractions CA, CB_1 and CB_2 using Equation I. To utilize the equation for predicting the CH_4 production from rumen fermentation of cattle, it is necessary to validate the equation using *in vivo* trials.

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