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Original Article

Optimum proportion of sweet corn by-product silage (SCW) and rice straw in total mixed ration using *in vitro* gas production

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ABSTRACT

An *in vitro* gas technique was used to study the effects of different proportions of sweet corn by-product silage (SCW) and rice straw (RS) on *in vitro* fermentation. The dietary treatments were ratios of SCW and RS all on a on a dry matter (DM) basis: T1 = SCW: RS at 60:40; T2 = SCW: RS at 50:50; and T3 = SCW:RS at 40:60. The ration of concentrate and roughage was 60:40 on a DM basis. The DM, crude protein (CP), ether extract (EE), acid detergent fiber (ADF) and neutral detergent fiber (NDF) of SCW were 22.56, 7.11, 1.89, 41.34 and 78.45%, respectively. The results showed that cumulative gas production at 48 h and 72 h after incubation with the ratio of SCW to RS at 60:40 on a DM basis was significantly ($p < 0.05$) higher than the results from the ratio of SCW to RS at either 50:50 or 40:60 on a DM basis. The proportion of SCW and RS among treatments had no effect on true digestibility parameters. However, the *in vitro* organic matter digestibility parameters in the treatment group with SCW:RS at 60:40 on a DM basis were higher ($p < 0.05$) than in the other two treatments. The total volatile fatty acid in the treatment group with SCW:RS at 50:50 and 40:60 on a DM basis were higher ($p < 0.05$) than in the treatment group with SCW:RS at 60:40 on a DM basis. Acetic acid (C_2), propionic acid (C_3) butyric acid (C_4) and the proportion of C_2:C_3 were not different ($p < 0.05$) among treatments. The levels of NH_3–N in all groups were not significant ($p < 0.05$) among treatments. It was concluded that the optimum level of SCW:RS was 60:40 on a DM basis.

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Introduction

The *in vitro* gas production technique has proved to be potentially useful for feed evaluation (Menke et al., 1998) as it can be used to measure the rate and extent of nutrient degradation (Cone et al., 1997). In addition, the *in vitro* gas production technique is less expensive (Getachew et al., 2004), provides easy determination (Khazaal et al., 1993) and is suitable for use in developing countries (Blummel and Becker, 1997). Rice straw (RS) is the main crop residue generated by farming in Thailand and is usually stored for use as ruminant feed. However, RS has low nutritive value with a low level of protein (2–5% on a dry matter (DM) basis), a high fiber and lignin content (neutral detergent fiber (NDF) > 50%) and low DM digestibility (below 65%) (Wanapat et al., 1985).

Sruamsiri et al. (2007) analyzed the chemical composition of agro-industrial by-products. Their results showed that all by-products were not a good roughage source and should not be used as the main roughage for ruminants because of their low contents of crude protein (CP) and DM. However, baby corn husk had the highest CP content (9.88%) but the lowest NDF and acid detergent fiber (ADF) contents (54.44% and 22.38%, respectively). Moreover, supplementation with RS increased the DM content of the silage but decreased the CP content. The utilization of less traditional feeds such as by-products combined with roughage sources may provide farmers with a variety of feeding options. By-product feeds are produced using a number of food processing industries, and such resources may impact traditional ruminant feeding practices by reducing the amount of concentrates fed to ruminants, providing feeding options when there is a scarcity of feed and reducing the feed cost (Nkosi, 2009). The objective of this study was to evaluate the proportion of sweet corn waste silage (SCW) and RS as roughage sources on *in vitro* gas production.

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Materials and method

Diets and management

This study was conducted using an *in vitro* gas technique at different levels of SCW and RS. The method used for the gas production was described by Menke and Steingass (1988). The ration concentrate (14.38% CP) and roughage ratio was 60:40%. A completely randomized design (CRD) with five replicates per treatment was used. The treatments used different ratios of SCW to RS and concentrate: Treatment 1: 60% concentrate + 16% RS and 24% SCW (on a DM basis); Treatment 2: 60% concentrate + 20% rice straw and 20% SCW (on a DM basis); and Treatment 3: 60% concentrate + 24% rice straw and 16% SCW (on a DM basis) (Table 1).

Sweet corn waste silage management

The SCW was composed of corn husk, cobs, discarded kernels and a small amount of stalk, all chopped into 2–3 cm pieces. The mixtures of all products were mixed and fermented in 2-layer plastic bags for 30 d. Each bag was made up to a weight of 30 kg at 30 d after fermentation.

Sample preparation

All feeds were dried at 65°C until constant weight for DM determination. Then, the samples were ground to pass through a 1 mm sieve. Feed samples (200 mg each) were placed in separate serum bottles. After weighing, each bottle was placed in an incubator at 39°C.

Rumen fluid collection and artificial saliva preparation

Ruminal fluid was collected from a slaughtered cow at an abattoir in Pathum Thani province, Thailand according to Chaudhry (2008). The rumen fluid was filtered through four layers of cheesecloth into plastic bottles and prewarmed in thermos flasks. Artificial saliva was prepared according to Menke and Steingass (1988), involving adding distilled water, buffer solution, macro mineral solution and resazurin solution into a flask and warming to 39°C. Then, reducing solution was added and the solution was placed into a magnetic flask and CO_2 was gently bubbled into the solution until it turned blue to pink and then clear. The rumen fluid was poured into the artificial saliva using a ratio of saliva rumen to fluid of 2:1. The whole process involved dispensing the rumen liquor (rumen fluid + artificial saliva) into serum bottles. A sample of 30 mL solution was added to each bottle using a dispenser. Then, the bottles were placed in an incubator at 39°C.

Sample collection and chemical analysis

Chemical analysis

The substrate including the roughage source (SCW and RS) and concentrate were analyzed for DM, ether extract (EE), ash and CP content according to methods of Association of Official Analytical Chemists (1990). NDF, ADF and acid detergent lignin (ADL) were calculated using the method of Goering and Van Soest (1970).

Gas production recording

During incubation, the gas production was recorded at 2 h, 4 h, 6 h, 8 h, 10 h, 12 h, 24 h, 48 h and 72 h. Cumulative gas production data were fitted to the model of Ørskov and McDonald (1979) as shown in Eq. (1):

$$Y = a + b \quad \left(1 - e^{-ct}\right) \tag{1}$$

where: Y is the volume of gas production (mL) at time, t (hr), a is the gas production from the immediate solution fraction (mL), b is the gas production from the insoluble fraction (mL), c is the gas production rate constant for the insoluble fraction (mL/hr) and t is the incubation time (hr).

Volatile fatty acid and NH_3–N measurement

At 4 h post inoculation, a set of samples was analyzed for volatile fatty acids (VFAs) and NH_3–N. A random sample (20 mL) was placed in a glass bottle and 1 M sulfuric acid (1 mL) added. Then, it was centrifuged at 16,000 × *g* for 15 min. The supernatant was sampled (15 mL) and frozen at −20°C. The samples were analyzed for NH_3–N (Bremner and Keeney, 1965), total VFA, acetic acid, propionic acid and butyrate (Samuel and Sagathewan, 1997).

In vitro true digestibility determination

At 48 h post incubation, *in vitro* true digestibility (IVTD) was determined according to Van Soest and Robertson (1985). A sample from the whole treatment was transferred quantitatively to a spoutless beaker by repeated washing with 100 mL neutral detergent solution. The content was refluxed for 60 min and filtered through pre weighed Gooch crucibles followed by rinsing with 25 mL acetone. Then, each sample was dried at 100°C for 5 h and the final weight recorded. The crucible was placed in a furnace at 600°C for 2 h. The DM of the residue was weighed and the IVTD of feed was calculated using Eq. (2):

Table 1
Chemical composition (% dry matter) of diets used in the experiment.

Item	Concentrate	Sweet corn waste silage	Rice straw	Sweet corn waste silage: rice straw		
				T1	T2	T3
Dry matter	90.12	22.56	84.65	71.19	75.67	78.16
Crude protein	14.38	7.11	3.04	10.82	10.66	10.50
Ether extract	2.56	1.89	0.84	2.12	2.08	2.04
Ash	8.28	3.78	15.97	8.43	8.92	9.41
Nitrogen free extract	62.32	61.03	44.34	59.13	58.47	57.80
Acid detergent fiber	24.64	41.34	43.85	31.72	31.22	31.92
Neutral detergent fiber	35.25	78.45	65.54	50.46	49.95	33.70
Acid detergent lignin	2.13	4.05	9.08	3.70	3.90	4.10
Gross energy (Kcal/kg dry matter)	3.76	3.39	4.02	3.72	3.74	3.77

$$\text{True digestibility} = \frac{(\text{DM of feed taken for incubation} - \text{NDF residue})}{\text{DM of feed taken for incubation}} \times 100 \quad (2)$$

where DM is the dry matter (g) and NDF is the neutral detergent fiber (% of dry matter).

The *in vitro* organic matter disappearance (IVOM) was obtained by incinerating the dried residues at 600°C for 2 h.

The in vitro organic matter digestibility (IVOMD) of samples was calculated as described by Close and Menke, 1986 using Eq. (3);

$$\text{IVOMD} = (14.88 + 0.889\ \text{GP} + 0.045\ \text{CP} + 0.065\ \text{CA})/100 \quad (3)$$

where GP is the milliliters produced at 72 h, CP is the crude protein (g/kg dry matter), EE is the ether extract (g/kg dry matter) and CA is the crude ash (g/kg dry matter).

Table 2
Gas volume and values of kinetic parameter from fermentation with different ratios of roughage source.

Incubation time (hr)	Ratio of roughage source (dry basis)			Standard error of mean
	Sweet corn by-product silage:Rice straw (%)			
	60:40	50:50	40:60	
Gas volume (mL/200 mg dry matter)				
2	4.48	6.29	6.18	0.60
4	12.16	12.73	12.94	0.63
6	18.80	18.74	18.92	0.60
8	23.67	23.37	23.40	0.63
10	26.68	26.69	26.39	0.69
12	30.00	30.02	29.85	0.72
24	43.05	40.94	39.09	0.89
48	57.56[a*]	54.03[b]	52.20[b]	1.00
72	63.66[a]	61.12[b]	58.79[b]	1.16
Gas production parameter				
a (mL)	0.94[b]	2.24[a]	2.25[a]	0.31
b (mL)	62.53[a]	58.17[b]	54.99[b]	1.09
a+b (mL)	63.47	60.41	57.25	1.20
C (per hour)	0.05	0.03	0.04	0.80

*Mean values in the same row with different lowercase superscripts are significantly ($p < 0.05$) different.

Statistical analysis

All data obtained from the experiment were analyzed using analysis of variance under the CRD using the procedures of the Statistical Analysis System Institute (SAS, 1996). Differences between treatment means were determined using Duncan's new multiple range test (Steel and Torrie, 1980).

Results and discussion

Chemical composition of feed

The gas production from the fermentation of the ratios of SCW and RS were measured at 2 h, 4 h, 6 h, 12 h, 24 h, 48 h and 72 h *in vitro*. The gas production characteristics are presented in Table 2 and Fig. 1. The results show that the gas volumes at 48 h and 72 h after incubation were significantly different ($p < 0.05$) among treatments. Cumulative gas production rates at 48 h and 72 h after incubation at a ratio SCW to RS of 60:40 on a DM basis (57.56 mL/200 mg DM basis and 63.66 mL/200 mg DM basis, respectively) were significantly ($p < 0.05$) higher than the treatment group with a ratio of SCW to RS ratio of 50:50 on a DM basis (54.03 mL/200 mg DM basis and 61.12 mL/200 mg DM basis, respectively) and SCW to RS at 40:60 on a DM basis (52.20 mL/200 mg on a DM basis and 58.79 mL/200 mg on a DM basis, respectively). The microbes in the rumen fluid in the treatment group with a ratio of SCW to RS of 60:40 might have used the substrate rapidly which resulted in a high cumulative gas production (Fig. 1). Gas production is directly proportional to the rate at which the substrate is degraded (Dhanoa et al., 1995). Additionally, the kinetics of gas production are dependent on the relative proportions of soluble, insoluble but degradable and undegradable particles in the feed (Getachew et al., 1998). The high level of SCW in the diet (a ratio of SCW to RS of 60:40 on a DM basis) had a high level of degradable substrate and consequently, there should be a high level of gas production. SCW has a high level of water soluble carbohydrate or NC (sugar and

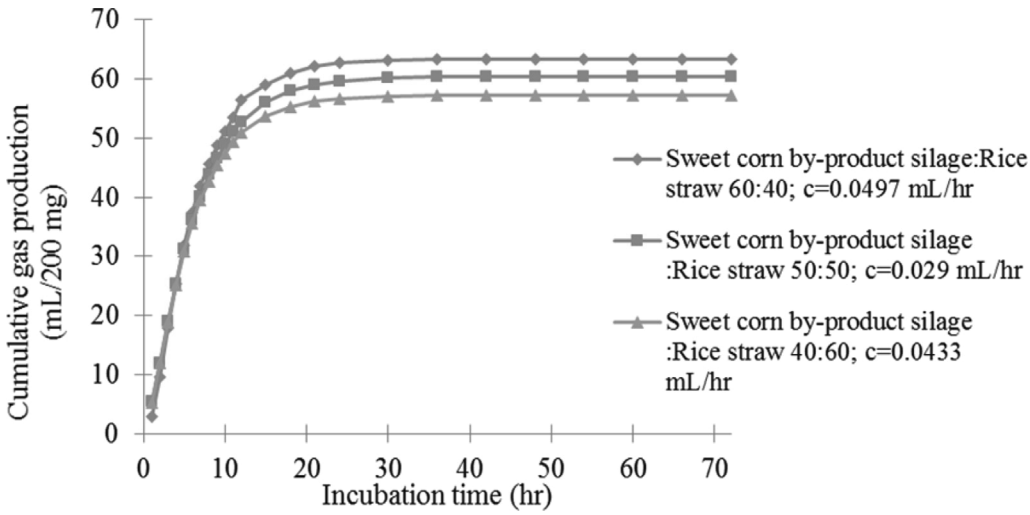

Fig. 1. Gas volume produced versus incubation time for various proportions of by-product.

Table 3
In vitro True digestibility, *in vitro* organic matter digestibility, volatile fatty acids and ammonia nitrogen in different levels of roughage source.

Item	Sweet corn by-product silage: rice straw (% dry matter basis)			Standard error of mean
	60:40	50:50	40:60	
In vitro true digestibility (%)	76.58	75.40	77.26	1.25
In vitro organic matter digestibility (%)	86.09[a*]	80.91[b]	82.55[b]	1.04
Total volatile fatty acids (VFA) (mM)	41.96[b]	45.56[a]	43.17[a]	0.65
VFA (mol/100 mol)				
Acetate (C_2)	72.98	73.33	75.56	1.45
Propionate (C_3)	15.39	15.55	16.03	1.34
Butyrate (C_4)	11.28	11.47	11.80	0.78
C2:C3	4.74	4.65	4.71	1.50
NH_3–N (mg/dL)	24.29	25.24	26.19	1.23

It was concluded that a ratio of SCW to RS of 60:40 on a DM basis had a high level of cumulative gas production at 48 h and 72 h after incubation. The IVOM in the treatment group with the ratio of SCW to RS of 60:40 on a DM basis was significantly higher than in the treatment groups with ratios of SCW to RS of 50:40 and 40:60. Furthermore, these levels had no effect on the total VFAs acetic acid, propionic acid, butyric acid (C4) and the proportion of C_2:C_3.
*Means values in the same row with different lowercase superscripts are significantly ($p < 0.05$) different.

starch) which can be used as an energy source for microbes during digestion (Aminah et al., 2004). This could promote the high level of gas production in the treatment group with a high level of SCW.

Sruamsiri et al. (2007) studied the DM (60.28%) and organic matter digestibility (63.68%) of sweet corn cob and husk silage with Ipil-Ipil leaves (30%) in four native beef cattle and found it was lower than in the current study and thus, as the current study had higher levels of SCW (40–60%) in the diet there was more energy for microbial action. The absolute value for *a* in Eq. (1) can be used to describe ideal fermentation of the soluble fraction. In the current study, the absolute gas production rates in the treatment group with a ratio of SCW to RS of 50:50 and 40:60 on a DM basis (2.24 mL/200 mg dry matter and 2.25 mL/200 mg dry matter, respectively) were significantly ($p < 0.05$) higher than for the treatment group with the ratio of SCW to RS of 60:40 on a DM basis (0.94 mL/200 mg dry matter). The results showed that there might be a higher soluble fraction in the treatment groups with ratios of SCW to RS of 50:50 and 40:60 on a DM basis. The high soluble fraction makes it easier for ruminal microorganisms to become attached and leads to greater gas production. The intercept value (a) for the gas production in the different treatments from soluble fractions has been reported to be in the range 0.25–1.90 (Menke et al., 1979). The gas volume at the asymptote (*b*) describes the fermentation of the insoluble fraction. The gas production from the insoluble fraction in the current study for the treatment group with a ratio of SCW to RS of 60:40 on a DM basis was significantly ($p < 0.05$) the highest which could have been due to the high insoluble fraction in the other treatment groups. The rates of gas production (*c*) in the current study were not significantly ($p > 0.05$) different among the treatment groups. High rates of gas production are influenced by the soluble carbohydrate fraction readily available to ruminal microbes. Deaville and Givens (2001) also reported that the carbohydrate fraction could affect the kinetics of gas production. The potential extent of gas production ($a + b$) was not significantly ($p > 0.05$) different among the treatment groups. Gas production can be estimated using feed degradation which it is a good parameter to predict digestibility, fermentation end products and microbial protein synthesis of microbes *in vitro* (Bergman, 1990). The effect of the proportion of SCW to RS on the true digestibility (IVDM), *in vitro* organic matter digestibility (IVOM), volatile fatty acids (VFAs) and ammonia nitrogen (NH_3–N) are presented in Table 3. The IVDM was not significantly different ($p > 0.05$) among treatments. However, The IVOM in the treatment group with the ratio of SCW to RS of 60:40 on a DM basis (86.09%) was significantly ($p < 0.05$) higher than for the other treatment groups with ratios of SCW to RS of 50:40 and 40:60 (80.91% and 82.55%, respectively). Van Soest and Robertson (1985) reported a highly significant and positive relationship between gas production and the *in vitro* apparent and true degradability. Gas production was positively correlated to IVOM. The results of the current study were higher than those reported by Tang et al. (2008) who found IVDM and IVOM in RS mixed with alfalfa at a ratio 50:50 were 56.90% and 53.60%, respectively. The TVFA in the treatment group with the ratios of SCW to RS of 50:50 and 40:60 on a DM basis were significantly ($p < 0.05$) higher than in the treatment group with the ratio of SCW to RS of 60:40 on a DM basis. This might have been due to acetic acid (C_2) production in the former two treatment groups being higher. Feeding of tropical forage to animals results in an imbalance in the digestive products (high acetate and low propionate) which causes inefficient utilization of metabolizable energy (MacRae and Lobley, 1982). The levels of acetic acid (C_2), propionic acid (C_3) and butyric acid (C_4) were not significantly ($p > 0.05$) different among treatments nor was the proportion of C_2:C_3 (4.65–4.74). The NH_3–N levels in all treatment groups were not significantly ($p > 0.05$) different among treatments (24.29–26.19 mg/dL). The optimum level of NH_3–N reported by Wanapat (1990) was 15–30 mg/dL and was considered to be suitable for microbial protein synthesis, feed digestibility and voluntary feed intake in ruminants fed on low quality roughage.

Conflict of interest

None declared.

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