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Use of *In vitro* Gas Production Technique to Investigate Interactions between Rice Straw, Wheat Straw, Maize Stover and Alfalfa or Clover

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ABSTRACT : Measurement of gas produced during *in vitro* fermentation was used to investigate the fermentation characteristics and interactions of rice straw, wheat straw or maize stover mixed with alfalfa or clover at proportions of 100:0, 75:25, 50:50, 25:75 and 0:100, respectively. Cumulative gas production was recorded at 2, 4, 8, 12, 16, 24 and 48 h of incubation, and the Gompertz function was used to describe the kinetics of gas production. *In vitro* dry matter and organic matter disappearances (IVDMD and IVOMD) were determined after 48 h incubation. The rate of gas production of clover was higher ($p<0.05$) than that of rice straw, wheat straw, maize stover and alfalfa when straws and hays were incubated separately. Increasing the proportion of alfalfa in the straw-alfalfa mixtures increased ($p<0.05$) the rates, but not the maximum volume of gas production. However, both rate and the maximum volume of gas production were increased ($p<0.01$) as the proportions of clover increased in the straw-clover mixtures. The cumulative gas production at 48 h, IVDMD and IVOMD showed no consistent interaction effects between different mixtures of cereal straws and hays. The extent of interactive effects was affected by the types of cereal straw, legume hay and their proportions in the mixture. The appropriate combination for the mixture of rice straw or maize stover with leguminous hays was 75:25 and 25:75, respectively. The better combination occurred at a proportion of 50:50 for the mixture of wheat straw and alfalfa. We conclude that the suitable proportion of low-quality straw and high quality legume hay combination should be considered in the ration formulation system of ruminants according to the extent of positive interactive effects. (**Key Words :** Interactive Effect, Straw, Legume Hay, *In vitro* Gas Production)

INTRODUCTION

Agricultural by-products such as cereal straws are carbohydrate-rich residues that represent a large potential source of dietary energy for ruminants. The performance of ruminants on cereal straw diets is usually low due to the low intake and digestibility caused by low nitrogen and high fiber content and the presence of anti-nutritional factors such as silica, tannins, and lignin in the straw (Nicholson, 1984). However, the poor-quality cereal straws, such as air-dried rice straw, wheat straw and maize stover, are usually supplemented to ruminants in the winter season in less developed countries because of the shortage of high quality hays. Thus, efficient utilization of cereal straws is one of the challenges to animal nutrition researchers.

Feedstuffs are usually assessed individually for their nutritive values, not as the mixture of the whole diet which animals generally consume. The nutritive values of the feed in diet are assumed to be additive. For ruminant feeds, such evaluation can be misleading as one forage can influence the others in terms of digestion (Moss et al., 1992). Therefore, interactions between feeds may be particularly important for the ration formulation system of ruminants. There is a growing interest in the use of supplements to ruminants consuming poor-quality forages which are high in fiber and low in nitrogen. Silva and Ørskov (1988) found that supplementation of digestible cellulose and/or hemicellulose improved rumen conditions and thereby increased the rate and extent of degradation of straw. Manyuchi et al. (1996) observed that supplementation of Napier hay and groundnut hay increased the intake of veldt hay for sheep. Liu et al. (2002) found that the supplementation of treated or untreated rice straws with mulberry leaves and ryegrass hay increased the *in vitro* gas production of rice straw. Positive associative effects were also reported with supplementing rapidly degradable fiber to poor-quality straws or grasses (Wood and Manyuchi,

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1997; Liu et al., 2002).

The production of gas has been used as a measure of the *in vitro* degradation of feeds by rumen micro-organisms (Theodouou et al., 1994; Xi et al., 2007). This technique has also been used to investigate interactions between feeds (Prasad et al., 1994; Wood and Manyuchi, 1997; Liu et al., 2002), where interactions have been defined as the differences between the gas production from substrate mixtures and that predicted from the gas production of the substrates when fermented alone. The objective of the present study was to determine the interactions between rice straw, wheat straw or maize stover and alfalfa or clover using the *in vitro* gas production technique.

MATERIALS AND METHODS

Feedstuffs

Rice straw (*Oryza sativa* var. D68) was collected from the experimental farm of the Institute of Subtropical Agriculture, Changsha, China. Alfalfa (*Medicago sativa* var.Sijiwang), clover (*Trifolium* var.Haifa), wheat straw (*Triticum aestivum* var.Taishan 9818) and maize stover (*Zea mays* var.Keyu No.2) were obtained from the Yucheng Ecological Experimental Station, Chinese Academy of Sciences, Shandong, China. These feedstuffs were sampled with three replicates at the harvest period. All feed samples were oven dried at 65°C for 48 h, and subsequently ground in mills with 1-mm sieves prior to chemical analyses and *in vitro* gas production measurements.

Experimental design

In the present trial, the simplex lattice mixture design was used to investigate the interactive effect of rice straw, wheat straw or maize stover mixed with alfalfa or clover. *In vitro* gas production was measured from mixtures consisting of alfalfa or clover with rice straw, wheat straw or maize stover at proportions of 0, 25, 50, 75 and 100%.

Chemical analyses

All feeds were analyzed for dry matter (DM), crude protein (CP), ash, neutral detergent fiber (NDF), and acid detergent fiber (ADF). The DM was determined by oven-drying at 105°C for 24 h, and ash content was determined by incinerating samples at 550°C for 5 h. Crude protein was determined using the Kjeldahl procedure with Cu^{2+} as a catalyst (AOAC, 1990). The NDF and ADF were determined according to Goering and Van Soest (1970).

Measurement of gas production

In vitro gas production was determined as described by Menke and Steingass (1988). Rumen fluid was collected before feeding in the morning from three ruminally fistulated Liuyang black wether goats (a local breed, 20±1.0 kg) fed a mixed diet of maize stover and concentrates (1:1, w/w) at the experimental farm of the Institute of Subtropical Agriculture. Rumen fluid was strained through four layers of gauze into a pre-warmed, insulated bottle. All laboratory handling of rumen fluid was carried out under a continuous flow of CO_2.

Samples (200±1 mg) of the oven-dry feedstuffs and the respective mixtures were accurately weighed into 100-ml glass syringes fitted with plungers. *In vitro* incubations were conducted in one run involving quintuplicate samples. Syringes were filled with 30 ml of medium consisting of 10 ml of rumen fluid and 20 ml of buffer solution as described by Menke and Steingass (1988). Three blanks containing 30 ml of medium only were included in each assay. The syringes were placed in a rotor inside an incubator (39°C) with about one rotation per min. The gas production was recorded after 2, 4, 8, 12, 16, 24 and 48 h of incubation. At the end of the fermentation period, the fermented residues were filtered into pre-weighed filter crucibles (porosity P160; British Standard grade 1), dried for 24 h at 105°C and weighed and *in vitro* dry matter disappearance (IVDMD) was calculated. The *in vitro* organic matter disappearance (IVOMD) was obtained by incinerating the dried residues at 550°C for 5 h.

Calculation and statistical analysis

Hemicellulose (HC) was estimated as the difference of NDF and ADF, and neutral detergent soluble (NDS) was calculated by the equation (1):

$$NDS = 1{,}000\text{-}NDF \qquad (1)$$

Gas data were analyzed as described by Schofield et al. (1994) to obtain the dynamics of gas production over time. The following Gompertz function was fitted to the cumulative gas production data:

$$GP = A\exp(-\exp(1+\frac{be}{A}(LAG-t))) \qquad (2)$$

Where GP is cumulative gas production (ml), A is the theoretical maximum of gas production, b is the maximum rate of gas production (ml/h) that occurs at the point of inflection of the curve, LAG is the lag time (h), which is defined as the time-axis intercept of a tangent line at the point of inflection, t is the incubation time (h) and e is the Euler constant. The parameters A, b and LAG were estimated by nonlinear regression analysis with weighted least squares means using the PROC NLIN procedure (SAS, 1996).

The results were subjected to the GLM procedure (SAS, 1996) according to the following statistical model:

Table 1. Chemical composition (g/kg DM, n = 3) of experimental feedstuffs

	DM	OM	CP	NDF	ADF	HC	Ash	NDS
Rice straw	859	863	69	606	383	224	137	395
Wheat straw	898	927	29	795	503	292	73	205
Maize stover	893	932	56	786	489	297	67	214
Alfalfa	906	777	274	350	176	175	223	650
Clover	853	881	177	349	320	29	119	651

DM = Dry matter, OM = Organic matter, CP = Crude protein, NDF = Neutral detergent fiber.
ADF = Acid detergent fiber, HC = Hemicellulose, NDS = Neutral detergent soluble.

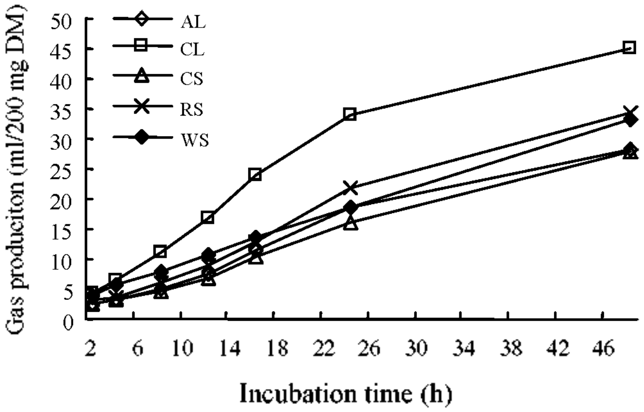

Figure 1. Cumulative gas production of individual feedstuffs at different hours of incubation. MS = Maize stover, RS = Rice straw, WS = Wheat straw, AL = Alfalfa, CL = Clover.

$$Y_{ij} = \mu + T_i + e_{ij}$$

Where Y_{ij} is dependent variable, μ is overall mean, T_i is proportion, and e_{ij} is residual error. Differences among means were tested using Duncan's multiple range tests. Parameters were considered to be significantly different between treatments when the 95% confidence intervals of treatments did not overlap. The pooled standard errors of the means of the gas production, IVDMD and IVOMD (calculated by the weight difference before and after incubation) for substrate mixtures were calculated. The cumulative volumes of gas produced after 24 and 48 h incubation, IVDMD and IVOMD were used to detect the interactive effects of feedstuffs. The percentage difference was calculated between the observed values of gas production, IVDMD and IVOMD from the mixtures of straws and leguminous hay and the calculated values of gas production, IVDMD and IVOMD from straws and leguminous hay fermented individually. Positive values indicated positive interactive effects of feeds in the mixture. These effects were assumed to be statistically significant ($p<0.05$) when the calculated gas production or IVDMD or IVOMD for the mixture according to their proportions lay outside the 95% confidence interval of the measured value. Orthogonal polynomial contrast was used to examine their responses (linear, quadratic and cubic) to increasing the level of legume hay incorporation.

RESULTS

Chemical composition

The chemical composition of straws and hays used in the experiment is given in Table 1. Both alfalfa and clover had numerically higher crude protein and NDS contents but lower NDF contents than rice straw, wheat straw and maize stover. The numerically lowest HC content was observed in clover. Alfalfa had the highest ash content, followed by rice straw and clover, and the lowest was in wheat straw and maize stover.

Gas production, IVDMD and IVOMD for individual feed

The gas production curves of rice straw, wheat straw, maize stover, alfalfa and clover are given in Figure 1. The gas produced after 48 h incubation of clover, rice straw, wheat straw, alfalfa and maize stover was 225.0, 171.5, 167.0, 141.5 and 140.0 ml/g DM, respectively.

Table 2. Parameters of gas production estimated with the Gompertz function, IVDMD and IVOMD (48 h) for feedstuffs incubated individually

Item	Parameters of Gompertz function			IVDMD (%)	IVOMD (%)
	A (ml)	*b* (ml/h)	*LAG* (h)		
Alfalfa	30.4^{b}	1.79^{bc}	1.97^{a}	64.5^{a}	58.1^{a}
Clover	46.6^{a}	3.21^{a}	0.75^{a}	63.1^{a}	60.8^{a}
Maize stover	32.3^{b}	1.48^{c}	1.51^{a}	27.3^{c}	27.0^{c}
Rice straw	35.7^{b}	1.81^{b}	1.53^{a}	37.2^{b}	38.1^{b}
Wheat straw	38.1^{b}	1.30^{c}	-1.28^{b}	30.3^{b}	27.4^{c}
SEM	1.39	0.089	0.364	1.79	1.57

a, b, c Means with different superscripts in the same column are significantly different ($p<0.05$).
A = the theoretical maximum of gas production of 200 mg DM basis, *b* = the maximum rate of gas production, *LAG* = the lag time, IVDMD = *in vitro* dry matter disappearance at 48 h incubation, OM = *in vitro* organic matter disappearance at 48 h incubation.

The parameters of the Gompterz function, IVDMD and IVOMD at 48 h incubation for rice straw, wheat straw, maize stover, alfalfa and clover are shown in Table 2. The maximum gas volume (*A*) was higher ($p<0.05$) for clover than for alfalfa, maize stover, rice straw, or wheat straw. The maximum rate of gas production (*b*) was highest ($p<0.05$) for clover, and greater ($p<0.05$) for rice straw than for maize stover and wheat straw. The lag time did not

Table 3. Parameters of gas production estimated with Gompertz function, the observed values of gas production (24, 48 h), IVDMD and IVOMD (48 h) when rice straw, maize stover or wheat straw was mixed with alfalfa or clover

Straw:hay (%)	Parameters of Gompertz function			GP24 (ml)	GP48 (ml)	IVDMD (%)	IVOMD (%)
	A (ml)	*b* (ml/h)	*LAG* (h)				
Rice straw:alfalfa							
0:100	30.4	1.79^{b}	1.97^{bc}	18.5^{b}	28.3^{c}	64.5^{a}	58.1^{a}
25:75	32.3	1.99^{ab}	1.97^{bc}	20.3^{ab}	30.1^{bc}	63.3^{a}	58.9^{a}
50:50	29.5	2.33^{a}	3.58^{a}	21.3^{ab}	30.3^{bc}	56.9^{b}	53.6^{ab}
75:25	31.8	2.10^{ab}	3.12^{ab}	22.2^{a}	32.7^{ab}	51.9^{b}	50.0^{b}
100:0	35.7	1.81^{b}	1.53^{c}	21.9^{ab}	34.3^{a}	37.2^{c}	38.1^{c}
SEM	2.21	0.122	0.401	0.73	0.69	1.97	1.76
Contrast		L, Q	Q	L	L, Q	L, Q	L, Q
Wheat straw:alfalfa							
0:100	30.4	1.79^{b}	1.97^{a}	18.5^{c}	28.3^{b}	64.5^{a}	58.1^{a}
25:75	35.5	2.21^{a}	0.38^{b}	24.5^{a}	33.7^{a}	56.4^{b}	49.1^{b}
50:50	35.7	2.32^{a}	1.18^{ab}	23.3^{a}	33.4^{a}	51.1^{b}	45.7^{b}
75:25	35.4	2.05^{ab}	0.95^{ab}	22.2^{a}	33.2^{a}	38.9^{c}	34.1^{c}
100:0	38.1	1.30^{c}	-1.28^{c}	19.3^{c}	33.8^{a}	30.3^{d}	27.4^{d}
SEM	2.72	0.109	0.374	0.98	0.75	1.93	1.94
Contrast		L, Q	Q	Q	L, Q	L	L
Maize stover:alfalfa							
0:100	30.4^{b}	1.79^{a}	1.97^{a}	18.5^{ab}	28.3^{b}	64.5^{a}	58.1^{a}
25:75	36.5^{a}	1.62^{ab}	-0.50^{b}	20.3^{a}	32.1^{a}	52.3^{b}	48.0^{b}
50:50	33.3^{ab}	1.69^{ab}	0.59^{ab}	20.3^{a}	31.8^{a}	42.0^{c}	38.4^{c}
75:25	31.7^{ab}	1.77^{ab}	1.63^{a}	18.2^{ab}	28.7^{b}	32.5^{d}	30.3^{d}
100:0	32.3^{ab}	1.48^{b}	1.51^{a}	16.2^{b}	28.0^{b}	27.3^{d}	27.0^{d}
SEM	1.53	0.091	0.449	0.97	0.53	2.26	2.08
Contrast	Q, C		Q, C	L, Q	L, Q, C	L	L, Q
Rice straw:clover							
0:100	46.6^{a}	3.21^{a}	0.75^{b}	34.1^{a}	45.0^{a}	63.1^{a}	60.8^{a}
25:75	46.3^{ab}	2.40^{b}	0.49^{b}	28.1^{b}	42.5^{b}	56.4^{b}	57.6^{a}
50:50	43.3^{ab}	2.37^{b}	0.72^{b}	27.3^{b}	40.3^{b}	47.2^{c}	49.1^{b}
75:25	39.8^{bc}	2.05^{c}	1.60^{a}	21.6^{c}	32.9^{c}	42.2^{cd}	44.2^{c}
100:0	35.7^{c}	1.81^{c}	1.53^{a}	21.9^{c}	34.3^{c}	37.2^{d}	38.1^{d}
SEM	1.99	0.096	0.213	1.16	0.95	1.87	1.54
Contrast	L	L, Q	L	L, Q	L	L	L
Wheat straw:clover							
0:100	46.6^{a}	3.21^{a}	0.75^{a}	34.1^{a}	45.0^{a}	63.1^{a}	60.8^{a}
25:75	45.6^{a}	3.09^{a}	-0.90^{bc}	34.9^{a}	44.3^{a}	56.1^{b}	50.7^{b}
50:50	39.3^{b}	2.40^{b}	-0.61^{b}	27.3^{b}	37.4^{b}	46.5^{c}	40.8^{c}
75:25	37.0^{b}	1.94^{c}	-1.21^{c}	24.1^{c}	34.8^{c}	40.5^{d}	34.9^{d}
100:0	38.1^{b}	1.30^{d}	-1.28^{c}	19.0^{d}	33.8^{c}	30.3^{e}	27.4^{e}
SEM	1.97	0.081	0.181	0.88	0.88	1.48	1.45
Contrast	L, Q, C	L, C	L, C	L, Q, C	L, C	L	L, Q
Maize stover:clover							
0:100	46.6^{a}	3.21^{a}	0.75	34.1^{a}	45.0^{a}	63.1^{a}	60.8^{a}
25:75	43.3^{a}	2.42^{b}	0.63	27.8^{b}	40.3^{b}	53.0^{b}	51.1^{b}
50:50	38.8^{b}	2.42^{b}	1.03	26.1^{b}	37.0^{c}	43.2^{c}	42.00^{c}
75:25	32.9^{c}	1.84^{c}	1.52	18.8^{c}	29.9^{d}	32.0^{d}	31.10^{d}
100:0	32.3^{c}	1.48^{d}	1.51	16.2^{c}	28.0^{d}	27.3^{d}	27.0^{d}
SEM	1.42	0.089	0.281	1.26	1.10	1.77	1.44
Contrast	L	L		L	L	L	L

$^{a, b, c, d, e}$ Means with different superscripts within the same combination of feeds in the same column differ ($p<0.05$).

L = Linear effect, $p<0.05$; Q = Quadratic effect, $p<0.05$; C = Cubic effect, $p<0.05$.

A = the theoretical maximum of gas production of 200 mg (DM basis), *b* = the maximum rate of gas production, *LAG* = the lag time, GP24 = gas production of 200 mg (DM basis) at 24 h, GP48 = gas production of 200 mg (DM basis) at 48 h incubation, IVDMD = *in vitro* dry matter disappearance at 48 h incubation, IVOMD = *in vitro* organic matter disappearance at 48 h incubation.

Table 4. Difference between gas production, IVDMD and IVOMD observed for the mixtures of straws and hays and that predicted from straws and hays incubated individually

Straw:hay (%)	Predicted value				Difference (%)			
	GP24 (ml)	GP48 (ml)	IVDMD (%)	IVOMD (%)	GP24	GP48	IVDMD	IVOMD
Rice straw:alfalfa								
25:75	19.4	29.8	59.2	54.8	4.43*	0.88	6.88*	7.41*
50:50	20.2	31.3	51.9	49.0	5.49*	-3.05	9.72*	9.32*
75:25	21.0	32.8	45.0	44.0	5.94*	-0.24	15.29*	13.63*
Wheat straw:alfalfa								
25:75	18.6	29.6	55.7	50.6	31.94*	13.97*	1.23	-2.89
50:50	18.8	31.0	47.6	44.1	23.68*	7.86*	7.44*	3.72
75:25	18.9	32.4	37.9	35.4	17.70*	2.39	2.65	-3.63
Maize stover:alfalfa								
25:75	17.9	28.2	51.3	47.2	13.65*	13.93*	1.88	1.63
50:50	17.4	28.2	44.8	42.0	16.53*	12.72*	-6.25	-8.59*
75:25	16.8	28.1	33.8	32.7	8.10*	2.30	-3.75	-7.35*
Rice straw:clover								
25:75	31.0	42.3	53.6	54.9	-9.45*	0.51	5.15	4.95
50:50	28.0	39.6	46.0	47.9	-2.46*	1.87	2.50	2.52
75:25	24.9	37.0	39.6	41.7	-13.27*	-11.13*	6.69*	6.11*
Wheat straw:clover								
25:75	30.3	42.2	57.8	52.8	15.03*	4.96	-2.86	-3.90
50:50	26.6	39.4	49.3	44.0	2.61	-5.01	-5.59	-7.36*
75:25	22.8	36.6	42.6	37.4	5.65	-5.03	-4.84	-6.71*
Maize stover:clover								
25:75	29.6	40.8	51.1	50.0	-6.22*	-1.15	3.71*	2.10*
50:50	25.1	36.5	42.5	42.2	4.07	1.34	1.74	-0.43
75:25	20.7	32.3	33.0	33.1	-9.36*	-7.56*	-3.01	-6.05*

Predicted value = (observed value of straw incubated individually×its proportion in the mixture+observed value of hay incubated individually×its proportion in the mixture).

Difference (%) = ((observed value-predicted value)/predicted value)×100. * p<0.05.

GP24 = gas production of 200 mg (DM basis) at 24 h incubation. GP48 = gas production of 200 mg (DM basis) at 48 h incubation.

IVDMD = *in vitro* dry matter disappearance at 48 h incubation. IVOMD = *in vitro* organic matter disappearance at 48 h incubation.

differ among feedstuffs except that of wheat straw which was significantly lower (p<0.05) than those of alfalfa, clover, maize stover and rice straw. *In vitro* dry matter disappearance (IVDMD) was highest (p<0.05) for alfalfa and clover, followed by rice straw and wheat straw (p<0.05), and lowest (p<0.05) for maize stover. The IVOMD values of alfalfa and clover were significantly higher (p<0.05) than rice straw which in turn was higher (p<0.05) than those of maize stover and wheat straw.

Interactive effects of straws and alfalfa or clover at different proportions on gas production, IVDMD and IVOMD

The parameters of gas production estimated with the Gompertz function, IVDMD and IVOMD for the mixtures of maize stover, wheat straw or rice straw and alfalfa or clover are given in Table 3. No differences were detected in maximum volume (*A*) when alfalfa was mixed with rice straw or wheat straw, but the gas production of maize stover and alfalfa mixture (25:75) was higher (Quadratic and cubic, p<0.05) than that of alfalfa alone. The rates of gas production for rice straw, wheat straw and alfalfa mixtures were higher (linear and quadratic, p<0.05) than those of individual feedstuffs when wheat straw or rice straw was mixed with alfalfa at the proportions of 50:50, whereas there were no differences (p>0.05) in the rates of gas production for maize stover and alfalfa mixtures at different proportions. The lag time of rice straw and alfalfa mixture was prolonged (quadratic, p<0.05) at the proportion of 50:50 when compared with individual feedstuffs. The lag time was lower (p<0.05) for wheat straw and alfalfa mixture (25:75) than for alfalfa, but higher (p<0.05) than for wheat straw. The lag time of maize stover and alfalfa mixture (25:75) was lower (quadratic and cubic, p<0.05) than that of individual feedstuffs. When straws were mixed with clover, the maximum volume and the rate of gas production were decreased with increasing proportions of straws. The lag time was higher (linear, p<0.05) for the rice straw and clover mixture (75:25) than for the other mixtures and clover alone, but lower (linear and cubic, p<0.05) for the mixtures of wheat straw and clover than for clover alone. The lag time numerically increased with increasing proportions of maize stover.

The gas production at 24 h (rice straw: linear, $p<0.05$; wheat straw: quadratic, $p<0.05$; maize stover: linear and quadratic, $p<0.05$) and 48 h (rice straw: linear and quadratic, $p<0.05$; wheat straw: linear, quadratic and cubic, $p<0.05$; maize stover: linear, quadratic and cubic, $p<0.05$) increased in response to decreased alfalfa level in the mixture. For the mixtures of straws and clover, the gas production at 24 h (rice straw: linear and quadratic, $p<0.05$; wheat straw: linear and quadratic, $p<0.05$; maize stover: linear, $p<0.05$) and 48 h (rice straw: linear, $p<0.05$; wheat straw: linear and cubic, $p<0.05$; maize stover: linear, $p<0.05$) decreased as the level of clover incorporation decreased in the mixture. *In vitro* disappearance of dry matter (IVDMD) and IVOMD of straws and alfalfa or clover mixtures generally decreased with increasing proportions of straws.

Table 4 presents data on the percentage difference between the observed gas production, IVDMD, and IVOMD from the mixtures of the straw and alfalfa or clover and the calculated values from gas production, IVDMD and IVOMD of straw feedstuffs fermented individually. Positive interactive effects ($p<0.05$) on gas production were observed for almost all the mixtures of straws and alfalfa at 24 and 48 h incubation time with the exception of the mixture of rice straw and alfalfa incubated for 48 h. Positive interactions ($p<0.05$) of rice straw and alfalfa were detected in IVDMD and IVOMD, and also observed when wheat straw and alfalfa were mixed at the proportion of 50:50. However, negative effects occurred to the mixtures of maize stover and alfalfa at the proportions of 50:50 and 75:25 at 48 h incubation time. Negative interactions ($p<0.05$) were observed for all proportional mixtures of rice straw and clover at 24 h incubation time and for the mixture of 75:25 at 48 h incubation time, whereas positive interactions on IVDMD and IVOMD were noted for the mixtures of rice straw and clover. Positive effects on gas production at all three proportions were observed for the mixtures containing wheat straw and clover at 24 h incubation time, but negative interactions were observed in IVDMD and IVOMD for the mixtures of wheat straw and clover. Negative interactive effects on gas production at 24 and 48 h incubation time were observed for the mixtures of maize stover and clover except for the proportion of 50:50. When maize stover was proportionally mixed with clover, positive effects ($p<0.05$) on IVDMD and IVOMD occurred at the proportion of 25:75, whereas negative interactive effects on IVDMD and IVOMD were observed at the proportion of 75:25.

DISCUSSION

In vitro fermentation characteristics of individual feedstuffs

The values observed in this study for theoretical maximum gas production, rate of gas production and lag time of clover and alfalfa agreed with our previous findings (Tang et al., 2005), in which the maximum volume of gas production and rate of gas production were higher, and the lag time was lower for clover than alfalfa. *In vitro* gas production of wheat straw, maize stover and rice straw reported here were also consistent with our previous study (Zhou et al., 2005; Tang et al., 2006), in which maize stover had the lowest maximum volume of gas production. The differences in fermentation characteristics of different forages may partly relate to the variations in CP, NDF and ADF contents observed in this study, as well as their different configurations of cell-wall polysaccharides (Cheng et al., 1984). *In vitro* gas production appeared to conform with the IVDMD and IVOMD for individual feedstuffs except for alfalfa which had higher IVDMD and IVOMD values, although alfalfa had relatively lower gas production when compared with cereal straws. High IVDMD and/or IVOMD but low gas production of alfalfa may be due to its high crude protein content being largely degraded to ammonia which influences the equilibrium in the carbonate buffer, by binding H^+ ions and holding back the release of CO_2 (Cone et al., 1999).

In vitro fermentation characteristics of mixtures

Compared to that of mixtures of straws and alfalfa, the parameters of gas production from mixtures of straws and clover were different, probably due to differences in chemical composition (especially crude protein content) of alfalfa and clover. The maximum volume of gas production did not differ but the rate of gas production increased with increasing proportion of alfalfa in the mixture. However, the maximum volume and rate of gas production both increased when the proportion of clover increased in the mixtures. This is consistent with the findings of Liu et al. (2002) for mixtures of rice straw and mulberry leaves or ryegrasss hay. This could be explained by several factors; firstly, the feed constituents, such as fat, protein and ash, which contribute little or no gas but are degraded or dissolved *in vitro*. Cone et al. (1999) observed that the fermentation of casein, after prolonged incubation, produced only 32% gas compared with carbohydrates and it was calculated that each percentage of protein caused a reduction in gas production of 2.48 ml/g organic matter. Secondly, truly digested substrate is partitioned among VFA, gas and microbial biomass and gas measurement only accounts for substrate that is used for VFA and gas production and does not consider the amount of substrate utilized for microbial growth. Wood and Manyuchi (1997) concluded that lack of correlation between gas production and DMD could be attributed to soluble substrate not being fully degraded to gas and/or to variability in the stoichiometry of gas production.

Prasad et al. (1994) found that the highest predictive value for *in vivo* digestibility was obtained after 45-52 h *in vitro* fermentation. Wood and Manyuchi (1997) pointed out that interactive effects were generally highest between 33 and 52 h incubation when veldt hay was supplemented with Napier hay or groundnut hay. In the present study, the cumulative gas production at 48 h, IVDMD and IVOMD showed no consistent interactive effects between different mixtures of cereal straws and hays (Table 4). For instance, the statistically positive interactive effects occurred in IVDMD and IVOMD of rice straw and alfalfa mixtures, but the gas production at 48 h incubation time showed no significant interactive effects. This was inconsistent with the findings of Wood and Manyuchi (1997) who found that 52 h interactive effects of Napier hay and veldt hay mixtures on DMD were much lower than those observed in gas production. Sandoval-Castro et al. (2002) observed statistically significant positive interactive effects of *L. latisiliquum* or *L. leucocephala* and concentrate as assessed by cumulative gas production, and statistically significant negative interactive effects of *L. leucocephala* and concentrate as assessed by IVDMD and IVOMD. Blummel et al. (1997) suggested that the different nature of ingredients in the feed mixtures might cause asynchrony in the release of the nutrients, resulting in a different microbial biomass growth which in turn might change gas production and/or rate. *In vitro* fermentation is an indirect measure of substrate degradation, particularly the carbohydrate fraction, and is a good predictor of the production of VFA, but it is not always positively related to production of microbial mass and in accordance with the *in vivo* results (Wood and Manyuchi, 1997). Additional work should be carried out to estimate the extent of the interactive effect *in vivo* and the correlation between *in vitro* and *in vivo* variables when studying feed mixtures.

CONCLUSIONS

This study demonstrated that the positive interactive effects on *in vitro* gas production occurred more consistently when poor quality straws (rice straw, wheat straw and maize stover) were incubated in mixtures with alfalfa than when incubated in mixtures with clover. The extent of interactive effects was affected by types of cereal straw, legume hay and their proportions in the mixture. The appropriate combination for the mixture of rice straw or maize stover with leguminous hays was 75:25 and 25:75, respectively. The better combination occurred at a proportion of 50:50 for the mixture of wheat straw and alfalfa. In practice, the suitable proportions of low-quality straws and high quality hays should be considered in the ration formulation system of ruminants according to the extent of positive interactive effects.

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