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# In vitro gas production measurements to evaluate interactions between untreated and chemically treated rice straws, grass hay, and mulberry leaves<sup>1</sup>

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**ABSTRACT:** In vitro gas production was measured to investigate associative effects of untreated and chemically treated rice straw (RS) and of RS and grass hay or mulberry leaves (ML). The RS was treated with NaOH, urea, or (NH<sub>4</sub>)HCO<sub>3</sub>. Cumulative gas production was recorded at 2, 4, 6, 8, 12, 16, 24, 36, 48, 60, 72, and 96 h of incubation, and the Gompertz function was used to describe the kinetics of gas production. Treatment with NaOH, urea, and NH<sub>4</sub>HCO<sub>3</sub> increased ( $P < 0.05$ ) gas production at 48 h by 55, 52, and 37% and the maximum rate of gas production of RS from 0.64 to 1.51, 1.27, and 1.13 mL/h, respectively. The inclusion of treated straws, hay, and ML in a mixture with RS at increasing proportions (25, 50, 75, 100%) elevated cumulative gas production and its rate. Maximum gas production was not different between the RS and its mixtures with the treated straws, but inclusion of hay into RS or NH<sub>4</sub>HCO<sub>3</sub>-treated RS increased the maxi-

mum gas production. Associative effects were defined as the difference between the observed gas production for the mixtures and the sum of the individual component feeds. The NaOH-treated RS, included at higher proportions, had positive associative effects at all times of incubation < 96 h; effects were negative or absent at 96 h. No effects were observed with mixtures of RS with urea-treated RS. The NH<sub>4</sub>HCO<sub>3</sub>-treated RS mixture tended to have negative associative effects at all three levels. Positive associative effects ( $P < 0.05$ ) were observed for both RS and NH<sub>4</sub>HCO<sub>3</sub>-treated RS at almost all inclusion levels of hay or ML after 12 to 96 h of incubation. The response was more pronounced with ML than with hay. Associative effects generally declined with duration of incubation. We conclude that positive associative effects on in vitro gas production occurred more consistently when RS was incubated in mixtures with hay or ML than when incubated in mixtures with chemically treated RS.

Key Words: Chemical Treatment, Fermentation, Gases, In Vitro, Rice Straw, Rumen

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## Introduction

Performance of ruminants given cereal straw diets is low due to low intake and digestibility, which is caused by low nitrogen and high fiber concentration, and the presence of antinutritional factors such as silica, tannins, and lignin (Nicholson, 1984). There is growing interest in the use of feeds with a high content

of rapidly degradable fiber as supplements to ruminants consuming poor-quality forage diets. Supplementation of barley straw with feeds providing digestible fiber improved the degradation of straw (Silva and Ørskov, 1988). Manyuchi et al. (1996) reported that supplementation of poor-quality natural pasture (veld) hay with napier and peanut hays increased the intake of veld hay. In vitro gas production measurements confirmed positive associative effects between both supplements and veld hay (Wood and Manyuchi, 1997). Associative effects occur when the observed value for a mixture of feeds is not equal to the sum of the individual component feeds (Blaxter, 1969). When Chinese Gelbvieh cattle were offered diets with ammoniated wheat straw, corn stover, or their mixtures (50:50, wt/wt), BW gain was higher for cattle on the mixed diet than for those on ammoniated straw or corn stover as sole forage source (Li et al., 1998). In situ ruminal DM disappearance confirmed positive associative effects between forage mixtures.

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**Table 1.** Composition of rice straws, hay, and mulberry leaves used for in vitro trials

Feedstuff	DM, g/kg	OM, g/kg DM	N, g/kg DM	NDF, g/kg DM	ADF, g/kg DM
Rice straw (RS)	938	906	9.8	750	491
NaOH-RS	219	845	9.6	654	484
Urea-RS	532	907	22.4	709	501
(NH <sub>4</sub> )HCO <sub>3</sub> -RS	538	892	18.7	724	495
Ryegrass hay	905	918	14.7	549	290
Mulberry leaves	912	833	35.5	301	234

The in vitro gas production technique has been used as a measure of ruminal degradation of feeds (Menke and Steingass, 1988; Getachew et al., 1998) and as an indicator of digestible DMI and growth rate of cattle fed cereal straws (Blümmel and Ørskov, 1993; Williams et al., 1996). This technique also has potential to investigate associative effects between feeds (Prasad et al., 1994; Wood and Manyuchi, 1997; Liu et al., 2000). The present study was undertaken to test associative effects between untreated and chemically treated rice straw (**RS**) and between RS and hay or mulberry leaves (**ML**) using the gas production technique.

## Materials and Methods

### Feedstuffs

Rice straw (*Oryza sativa* var. Zhenongda 402) was obtained from the experimental farm of the Zhejiang University, Huajiachi Campus, China. Rice straw was manually chopped to 5-cm length and treated with 40 g NaOH dissolved in 1 kg H<sub>2</sub>O/kg of straw at 70°C for 30 min in an experimental reactor (Amandus Kahl, Reinbek, Germany) to obtain NaOH-treated rice straw (**NaOH-RS**). Urea and (NH<sub>4</sub>)HCO<sub>3</sub> were used as sources of NH<sub>3</sub> to treat RS. The concentrations of urea and (NH<sub>4</sub>)HCO<sub>3</sub> were 50 and 100 g/kg RS, respectively. The amount of water added was 700 mL/kg RS. The Urea-RS and (NH<sub>4</sub>)HCO<sub>3</sub>-RS were prepared in polyethylene bags at room temperature for 21 d. After completion of the treatment, the three treated RS were dried at < 65°C, ground, and used for chemical analyses and in vitro incubations. Second-cut perennial ryegrass (*Lolium perenne* var. Fennema) hay was obtained from the "Schädtebek" experimental farm of the Federal Dairy Research Center, Kiel, Germany. Mulberry leaves (**ML**) (*Morus alba* var. Tuantouheyebai) were harvested in late August 1998 at the experimental mulberry garden, Zhejiang University, Huajiachi Campus, China (Yao et al., 2000). All feed samples were successively ground in mills with 3- and 1-mm sieves prior to chemical analyses and in vitro gas production measurements. The chemical composition of the feeds used for in vitro gas production measurements is presented in Table 1.

### Experimental Design

In two trials, in vitro gas production was measured from mixtures consisting of untreated and chemically treated RS and of hay or ML in different proportions with RS or (NH<sub>4</sub>)HCO<sub>3</sub>-RS. In Trial 1, RS was incubated together with NaOH-RS, Urea-RS, and (NH<sub>4</sub>)HCO<sub>3</sub>-RS at proportions of 0, 25, 50, 75, and 100%, respectively. In Trial 2, RS and (NH<sub>4</sub>)HCO<sub>3</sub>-RS were used as mixtures with hay or ML at levels of 0, 25, 50, 75, and 100%, respectively.

### Chemical Analyses

All feeds were analyzed for DM, N, ash, NDF, and ADF. The DM was determined by oven-drying at 105°C for 16 h, and ash content was determined by incinerating samples at 550°C for 4 h. Total N was determined using the Kjeldahl procedure with Cu<sup>2+</sup> as a catalyst according to the official German procedure (Bassler, 1993). The NDF and ADF were determined according to Van Soest et al. (1991).

### Measurement of Gas Production

In vitro gas production was determined according to Menke and Steingass (1988). Rumen fluids were collected from two ruminally fistulated German Red Pied steers fed a mixed diet of perennial ryegrass hay and mixed concentrates (2:1, wt/wt). Ingredient and chemical composition of the concentrates are reported in Table 2. Rumen fluid was strained through two layers of gauze into a prewarmed, insulated bottle. All laboratory handling of rumen fluid was carried out under a continuous flow of CO<sub>2</sub>.

Samples (200 ± 10 mg) of the air-dry feedstuffs and the respective mixtures were accurately weighed into 100-mL glass syringes fitted with plungers. In vitro incubations were conducted in two consecutive runs, each involving triplicates of 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), except that the concentration of NaHCO<sub>3</sub> was reduced to 33 g/L and that of (NH<sub>4</sub>)HCO<sub>3</sub> increased to 6 g/L to prevent a shortage in N during prolonged incubation times. Three blanks containing 30 mL of medium only were included in each assay. Triplicates of a standard hay and a stan-

**Table 2.** Ingredient and chemical composition of the mixed concentrates

Item	%
<b>Ingredient</b>	
Wheat	20
Corn gluten feed	19
Rapeseed meal	15
Palm kernel expeller	15
Rye bran	12
Molassed sugar beet pulp	10
Wheat feed meal	4
Molasses	3
CaCO <sub>3</sub>	1.25
Mineral and vitamin mix	0.75
<b>Chemical composition (% of DM unless stated)</b>	
DM, %	89.2
Ash	7.6
Crude fat	3.6
CP	18.4
NDF	37.1
ADF	15.1
NFC <sup>a</sup>	33.3

<sup>a</sup>NFC = nonfiber carbohydrates (100 - [CP + NDF + crude fat + ash]).

standard concentrate that can be obtained from the Institute of Animal Nutrition, Hohenheim University, 70593 Stuttgart, Germany, were included. Incubations were repeated when gas volumes of these two feedstuffs deviated by more than 10% from the reference values provided by the supplier of the standards. The syringes were placed in a rotor inside the incubator (39°C) with about one rotation per min. The gas production was recorded after 2, 4, 6, 8, 12, 16, 24, 36, 48, 60, 72, and 96 h of incubation.

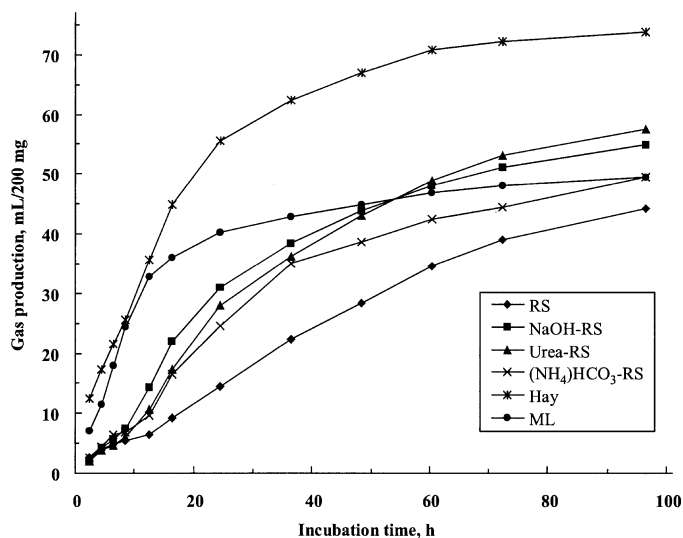
### Mathematical and Statistical Analyses

The effects of treatments and time of incubation on gas production were analyzed by the mixed model procedure of SAS (SAS Inst. Inc., Cary, NC) according to the model  $GP_{ijk} = M + Treat_i + Time_j + (Treat \times Time)_{ij} + e_{ijk}$ , where  $GP_{ijk}$  is the observed gas volume,  $M$  overall mean,  $Treat_i$  the effect of treatment  $i$ ,  $Time_j$  the effect of incubation time  $j$ ,  $(Treat \times Time)_{ij}$  the effect of interaction between treatment  $i$  and incubation time  $j$ , and  $e_{ij}$  the residual error of the measurement using a heterogeneous autoregressive covariance structure for repeated measurements over time within one syringe.

To describe the dynamics of gas production over time the following Gompertz function (Schofield et al., 1994) was chosen:

$$GP = A \exp \left\{ - \exp \left[ 1 + \frac{be}{A}(LAG - t) \right] \right\}$$

where  $GP$  is cumulative gas production (mL),  $A$  the theoretical maximum of gas production,  $b$  the maximum rate of gas production (mL/h) that occurs at the point of inflection of the curve,  $LAG$  the lag time (h),



**Figure 1.** Cumulative gas production of individual feedstuffs at different times of incubation. RS = rice straw; NaOH-RS = NaOH-treated RS; Urea-RS = urea-treated RS; (NH<sub>4</sub>)HCO<sub>3</sub>-RS = (NH<sub>4</sub>)HCO<sub>3</sub>-treated RS; Hay = ryegrass hay; ML = mulberry leaves.

which is defined as the time-axis intercept of a tangent line at the point of inflection, and  $t$  time (h).

The parameters  $A$ ,  $b$ , and  $LAG$  were estimated by nonlinear regression analysis with weighted least squares means (PROC NLIN; SAS Inst. Inc.), where the least squares means for treatment  $\times$  time from the above mixed model analysis were used as time series measurements and the standard error of least squares means as weights. Parameters were considered to be significantly different between treatments when the 95% confidence intervals of treatments did not overlap.

The cumulative volumes of gas produced after 12, 24, 48, and 96 h were used for detecting possible associative effects of feedstuffs. These effects were assumed to be statistically significant ( $P < 0.05$ ), when the calculated gas production for the mixture according to their proportions lay outside the 95% confidence interval of the measured gas production.

## Results

### Chemical Composition and Gas Production Characteristics for Individual Feeds

The gas production curves of untreated and treated straws and of hay and ML are given in Figure 1, and the parameters of the Gompertz function are presented in Table 3. The maximum gas volume ( $A$ ) was highest for hay ( $P < 0.05$ ) and greater for the treated straws ( $P < 0.05$ ) than for RS or ML. The maximum rate of gas production ( $b$ ) was highest for ML ( $P < 0.05$ ), followed by hay ( $P < 0.05$ ) and the treated straws ( $P < 0.05$ ), and lowest for untreated RS ( $P < 0.05$ ). The lag time showed no difference among straws, but the

**Table 3.** Mean values (n = 6) of cumulative gas produced at different times of incubation for individual feedstuffs and parameters of gas production estimated with the Gompertz function (Trials 1 and 2)

Feedstuff	Cumulative gas (mL) produced at				Parameters of Gompertz function <sup>b</sup>		
	12 h	24 h	48 h	96 h	A, mL	b, ml/h	LAG, h
Rice straw (RS)	6.5	14.1	28.3	44.2	48.96 <sup>cd</sup>	0.638 <sup>f</sup>	2.3 <sup>b</sup>
NaOH-RS	14.2	31.0	43.9	54.8	49.61 <sup>cd</sup>	1.506 <sup>d</sup>	2.9 <sup>b</sup>
Urea-RS	10.7	27.9	43.1	57.5	53.83 <sup>c</sup>	1.267 <sup>de</sup>	3.8 <sup>b</sup>
(NH <sub>4</sub> )HCO <sub>3</sub> -RS	9.7	24.5	38.7	49.5	47.92 <sup>d</sup>	1.130 <sup>e</sup>	2.5 <sup>b</sup>
Ryegrass hay	35.6	55.5	66.9	73.7	70.48 <sup>d</sup>	2.305 <sup>c</sup>	-3.3 <sup>c</sup>
Mulberry leaves	32.8	40.2	44.8	49.4	45.17 <sup>d</sup>	2.739 <sup>b</sup>	-0.4 <sup>c</sup>

<sup>a</sup>For all equations the R<sup>2</sup> was > 0.99.

<sup>b,c,d,e,f</sup>Means within the same column with different superscripts differ ( $P < 0.05$ ).

values of all straws were higher than those of hay or ML ( $P < 0.05$ ).

#### Associative Effects on Gas Production of Untreated and Treated Rice Straws

The mean gas production for the mixtures of untreated and treated straws at selected times of incubation and parameters of gas production estimated with the Gompertz function are given in Table 4. The gas volumes at all times of incubation increased with increasing proportions of treated straws in the mixtures. The rate of gas production ( $b$ ) also increased with the

proportions of the treated straws. However, there were no differences in maximum volume ( $A$ ) and lag time ( $LAG$ ) between RS and its mixtures with treated straws.

Table 5 presents data on the percentage difference between the observed gas production from the mixtures of the untreated and treated straws and the calculated values from gas production of straws fermented individually. Positive values indicate positive associative effects of feeds in the mixture. The NaOH-RS, when included at higher proportions, showed positive associative effects at all times of incubation < 96 h, whereas no or negative effects were observed at 96

**Table 4.** Mean values (n = 6) of cumulative gas produced at different times of incubation when untreated rice straw (RS) was mixed with straw treated with NaOH, urea, or (NH<sub>4</sub>)HCO<sub>3</sub>, and parameters of gas production estimated with the Gompertz function (Trial 1)

Source and proportion of treated rice straw, %	Cumulative gas (mL) produced at				Parameters of Gompertz function <sup>a</sup>		
	12 h	24 h	48 h	96 h	A, mL	b, mL/h	LAG, h
<b>NaOH-RS</b>							
0	6.5	14.1	28.3	44.2	49.0 (1.4)	0.638 <sup>e</sup> (0.01)	2.3 (0.5)
25	8.2	18.1	32.0	45.4	46.6 (1.2)	0.762 <sup>de</sup> (0.02)	1.8 (0.4)
50	10.9	23.9	37.7	52.2	49.7 (2.1)	1.001 <sup>cd</sup> (0.06)	1.8 (0.7)
75	13.6	28.8	42.0	55.1	50.3 (2.0)	1.244 <sup>bc</sup> (0.09)	1.5 (0.7)
100	14.2	31.0	43.9	54.8	49.6 (1.8)	1.506 <sup>b</sup> (0.11)	2.9 (0.7)
SEM	0.62	1.22	1.15	0.88			
<b>Urea-RS</b>							
0	6.5	14.1	28.3	44.2	49.0 (1.4)	0.638 <sup>e</sup> (0.01)	2.3 (0.5)
25	7.6	18.0	32.4	48.2	51.2 (1.4)	0.742 <sup>d</sup> (0.02)	1.8 (0.5)
50	8.4	20.7	35.2	50.6	50.5 (1.6)	0.885 <sup>c</sup> (0.03)	2.8 (0.5)
75	9.8	23.9	38.7	54.0	52.6 (1.9)	0.991 <sup>bc</sup> (0.04)	2.1 (0.6)
100	10.7	27.9	43.1	57.5	53.8 (2.1)	1.267 <sup>b</sup> (0.08)	3.8 (0.7)
SEM	0.34	0.90	0.99	0.87			
<b>(NH<sub>4</sub>)HCO<sub>3</sub>-RS</b>							
0	6.5	14.1	28.3	44.2	49.0 (1.4)	0.638 <sup>e</sup> (0.01)	2.3 (0.5)
25	6.4	16.0	30.2	45.8	48.6 (1.6)	0.700 <sup>d</sup> (0.02)	2.8 (0.5)
50	6.8	18.0	31.9	46.5	47.2 (1.6)	0.788 <sup>c</sup> (0.03)	3.5 (0.6)
75	8.2	21.7	35.5	48.7	47.7 (1.7)	0.912 <sup>c</sup> (0.04)	2.6 (0.6)
100	9.7	24.5	38.7	49.5	47.9 (1.3)	1.130 <sup>b</sup> (0.04)	2.5 (0.5)
SEM	0.37	0.75	0.76	0.68			

<sup>a</sup>A = theoretical maximum of gas production;  $b$  = maximum rate of gas production;  $LAG$  = lag time; for all equations the R<sup>2</sup> was > 0.99. Values are estimates with their SE in parentheses.

<sup>b,c,d,e</sup>Means within the same combination of feeds with different superscripts differ ( $P < 0.05$ ).

**Table 5.** Difference (%)<sup>a</sup> between the gas production observed for the different mixtures of untreated and treated rice straws (RS) and that predicted from untreated and treated rice straws fermented separately (Trial 1)

Treated straw and proportion, %	Incubation period, h			
	12	24	48	96
<b>NaOH-RS</b>				
25	-1.0	-1.4	0.0	-4.6 <sup>b</sup>
50	6.9	6.0	4.7	4.4 <sup>b</sup>
75	11.6 <sup>b</sup>	7.5	5.3	5.1 <sup>b</sup>
<b>Urea-RS</b>				
25	3.4	2.4	1.8	0.0
50	-1.0	-1.3	-1.0	-1.4
75	2.5	-2.4	-1.7	-0.8
<b>(NH<sub>4</sub>)HCO<sub>3</sub>-RS</b>				
25	-8.5	-3.2	-1.4	-2.8
50	-11.8 <sup>b</sup>	-5.2	-3.8	-5.2 <sup>b</sup>
75	-2.3	1.5	-0.7	-4.5 <sup>b</sup>

<sup>a</sup>Difference (%) = [(Observed gas production – predicted gas production)/predicted gas production] × 100.  
<sup>b</sup>Significant differences (*P* < 0.05).

**Table 6.** Mean values (n = 6) of cumulative gas produced at different times of incubation of rice straw (RS) and (NH<sub>4</sub>)HCO<sub>3</sub>-treated RS, and of mixtures with ryegrass hay (Hay) or mulberry leaves (ML), and parameters of gas production estimated with the Gompertz function (Trial 2)

Item	Cumulative gas (mL) produced at				Parameters of Gompertz function <sup>a</sup>		
	12 h	24 h	48 h	96 h	A, mL	b, mL/h	LAG, h
<b>RS:hay</b>							
100:0	6.5	14.1	28.3	44.2	49.0 <sup>e</sup> (1.4)	0.638 <sup>f</sup> (0.01)	2.3 <sup>b</sup> (0.5)
75:25	11.6	22.3	34.9	47.4	46.0 <sup>e</sup> (1.4)	0.819 <sup>e</sup> (0.03)	-1.7 <sup>c</sup> (0.7)
50:50	21.9	36.0	49.1	56.6	55.4 <sup>d</sup> (1.1)	1.326 <sup>d</sup> (0.06)	-4.0 <sup>c</sup> (0.6)
25:75	28.5	45.5	57.6	65.6	62.3 <sup>c</sup> (1.1)	1.791 <sup>c</sup> (0.09)	-3.5 <sup>c</sup> (0.6)
0:100	35.6	55.5	66.9	73.7	70.5 <sup>b</sup> (1.0)	2.305 <sup>b</sup> (0.11)	-3.3 <sup>c</sup> (0.5)
SEM	2.00	2.79	2.67	2.23			
<b>RS:ML</b>							
100:0	6.5	14.1	28.3	44.2	49.0 (1.4)	0.638 <sup>e</sup> (0.01)	2.3 <sup>b</sup> (0.5)
75:25	14.2	22.7	35.4	43.7	44.2 (1.5)	0.835 <sup>de</sup> (0.05)	-3.5 <sup>c</sup> (0.9)
50:50	20.2	29.4	38.6	46.8	42.9 (1.9)	1.187 <sup>cd</sup> (0.15)	-3.6 <sup>c</sup> (1.5)
25:75	27.6	34.6	40.5	46.5	41.5 (1.3)	2.074 <sup>bc</sup> (0.29)	-1.3 <sup>c</sup> (1.0)
0:100	32.8	40.2	44.8	49.4	45.2 (1.0)	2.739 <sup>b</sup> (0.25)	-0.4 (0.6)
SEM	1.72	1.71	1.21	0.82			
<b>(NH<sub>4</sub>)HCO<sub>3</sub>-RS:hay</b>							
100:0	9.7	24.5	38.7	49.5	47.9 <sup>f</sup> (1.3)	1.130 <sup>f</sup> (0.04)	2.5 <sup>b</sup> (0.5)
75:25	20.0	33.9	47.0	54.8	53.2 <sup>e</sup> (0.8)	1.300 <sup>e</sup> (0.04)	-2.4 <sup>c</sup> (0.4)
50:50	24.0	42.1	55.1	61.6	59.8 <sup>d</sup> (0.8)	1.686 <sup>d</sup> (0.06)	-2.2 <sup>c</sup> (0.4)
25:75	31.1	50.8	63.2	68.6	66.9 <sup>c</sup> (0.8)	2.071 <sup>c</sup> (0.07)	-2.9 <sup>c</sup> (0.4)
0:100	35.6	55.5	66.9	73.7	70.5 <sup>b</sup> (1.0)	2.305 <sup>b</sup> (0.11)	-3.3 <sup>c</sup> (0.5)
SEM	1.71	2.05	1.97	2.00			
<b>(NH<sub>4</sub>)HCO<sub>3</sub>-RS:ML</b>							
100:0	9.7	24.5	38.7	49.5	47.9 (1.3)	1.130 <sup>e</sup> (0.04)	2.5 <sup>b</sup> (0.5)
75:25	16.6	29.8	40.8	44.9	44.4 (0.8)	1.274 <sup>de</sup> (0.06)	-0.7 <sup>c</sup> (0.5)
50:50	23.2	35.0	44.0	50.9	47.0 (1.4)	1.581 <sup>cd</sup> (0.15)	-1.7 <sup>c</sup> (0.9)
25:75	27.9	36.6	43.2	48.2	44.1 (1.2)	2.073 <sup>bc</sup> (0.23)	-1.2 <sup>c</sup> (0.8)
0:100	32.8	40.2	44.8	49.4	45.2 (1.0)	2.739 <sup>b</sup> (0.25)	-0.4 <sup>c</sup> (0.6)
SEM	1.54	1.04	0.63	0.88			

<sup>a</sup>A = theoretical maximum of gas production; b = maximum rate of gas production; LAG = lag time; for all equations the R<sup>2</sup> was > 0.98. Values are estimates with their SE in parentheses.  
<sup>b,c,d,e,f</sup>Means within the same combination of feeds with different superscripts differ (*P* < 0.05).

**Table 7.** Difference (%)<sup>a</sup> between the observed gas production of the mixtures of untreated and (NH<sub>4</sub>)HCO<sub>3</sub>-treated rice straws with ryegrass hay and mulberry leaves, and that predicted from feedstuffs fermented separately (Trial 2)

Basal feed, supplement, and proportion, %	Incubation period, h			
	12	24	48	96
Rice straw (RS)				
Ryegrass hay				
25	-14.4	-7.2	-4.9	-3.4
50	7.1	6.2	6.4	0.1
75	4.1	4.1	4.0	2.7
Mulberry leaves				
25	9.7 <sup>b</sup>	10.6 <sup>b</sup>	11.7 <sup>b</sup>	0.3
50	4.7	10.4 <sup>b</sup>	8.3 <sup>b</sup>	3.8 <sup>b</sup>
75	7.9	5.3	2.4	-0.1
(NH <sub>4</sub> )HCO <sub>3</sub> -RS				
Ryegrass hay				
25	28.0 <sup>b</sup>	7.0	7.5 <sup>b</sup>	8.8 <sup>b</sup>
50	10.4 <sup>b</sup>	8.7 <sup>b</sup>	9.5 <sup>b</sup>	8.2 <sup>b</sup>
75	12.1 <sup>b</sup>	11.1 <sup>b</sup>	10.9 <sup>b</sup>	8.0 <sup>b</sup>
Mulberry leaves				
25	9.8 <sup>b</sup>	5.5	5.3 <sup>b</sup>	-0.2
50	12.9 <sup>b</sup>	10.4 <sup>b</sup>	9.6 <sup>b</sup>	11.2 <sup>b</sup>
75	7.1	4.4	4.2 <sup>b</sup>	3.6

<sup>a</sup>Difference (%) = [(Observed gas production – predicted gas production)/predicted gas production] × 100.

<sup>b</sup>Significant differences ( $P < 0.05$ ).

h. No such effects were observed for the mixtures of RS with Urea-RS. The mixtures containing (NH<sub>4</sub>)HCO<sub>3</sub>-RS tended to have negative associative effects at all three inclusion levels and incubation times, and some significant effects were observed.

#### *Associative Effects on Gas Production of Straws and Hay or Mulberry Leaves*

The mean gas production of mixtures of RS and (NH<sub>4</sub>)HCO<sub>3</sub>-RS with hay or ML and the parameters of gas production are given in Table 6. The cumulative gas production and its rate (*b*) increased for RS and (NH<sub>4</sub>)HCO<sub>3</sub>-RS with increasing proportions of hay or ML, whereas an increase ( $P < 0.05$ ) in maximum gas production (*A*) was detected only for the RS mixtures with hay. The lag time was higher for both RS and (NH<sub>4</sub>)HCO<sub>3</sub>-RS than for the mixtures, with no differences between different proportions of hay or ML in the mixtures.

Table 7 presents the difference between the observed gas production of the mixtures of straws with hay or ML compared with the calculated values from straws and supplements fermented individually. Positive associative effects ( $P < 0.05$ ) on gas production were observed for both RS and (NH<sub>4</sub>)HCO<sub>3</sub>-RS at almost all proportions of hay or ML after 12 to 96 h of incubation. The response was more pronounced for ML than for hay. The magnitude of associative effects generally declined with time of incubation.

## Discussion

The in vitro gas production technique has the potential to reflect the in vivo digestibility of feeds for ruminants; the highest predictive value for the in vivo digestibility of straw was obtained after 45 to 52 h of in vitro fermentation (Prasad et al., 1994). When proportionality between gas production after 48 h of incubation and digestibility is assumed, the treatments with NaOH, urea, and (NH<sub>4</sub>)HCO<sub>3</sub> increased the digestibility of rice straw by 55, 52, and 37%, respectively (Table 3).

When RS was mixed with the treated straws, the rate of gas production increased with the proportion of treated straws, irrespective of the chemicals used for treatment (Table 4). However, the maximum gas volume and the lag time did not differ between different mixtures. Manyuchi et al. (1992) investigated the effects of supplementing NH<sub>3</sub>-treated barley straw on degradation rate and intake of untreated barley straw in sheep. The rate of in situ degradation of untreated barley straw was increased in the rumen of sheep fed supplemental ammoniated straw ( $P < 0.05$ ), and rate of in vitro gas production was also significantly higher ( $P < 0.05$ ) when rumen fluid from animals fed ammoniated straw was used to ferment the untreated straw. However, the extent of degradation or total gas production was not affected. Supplements of ammoniated straw increased intake of the untreated straw, and hence total straw intake of supplemented diets reached a level comparable to the treated straw diet.

Shi et al. (1997) observed that daily straw intake of heifers was highest (3.93 kg) when RS and  $(\text{NH}_4)\text{HCO}_3\text{-RS}$  were fed at a ratio of 50:50 (wt/wt), followed by  $(\text{NH}_4)\text{HCO}_3\text{-RS}$  (3.55 kg), and lowest with untreated RS (2.46 kg). The heifers fed the straw mixture had higher ( $P < 0.05$ ) BW gains than those on the untreated straw diet (836 vs 666 g/d) and tended to gain more BW than heifers on the  $(\text{NH}_4)\text{HCO}_3\text{-RS}$  diet (808 g/d).

In our study, cumulative gas production showed no consistent interaction effects between untreated and treated RS (Table 5). At high inclusion levels of the treated straws, NaOH-RS gave positive associative effects, whereas Urea-RS showed negative effects. Therefore, effects on straw intake and growth rate might be mainly attributed to differences in degradation rate caused by the stimulatory effect in the rumen of more rapidly fermentable energy provided by the treated straws (Silva and Ørskov, 1988; Manyuchi et al., 1992). However, the maximum rate of gas production in our study indicated only small positive or even negative associative effects when RS was mixed with treated straws, hay, or ML (Tables 4 and 6). Regarding the cumulative gas production, positive associative effects were observed when hay and ML were used as supplements (Table 7), the effects being most pronounced in ML-straw mixtures. Wood and Manyuchi (1997) observed statistically significant positive associative effects of veld hay and napier hay or peanut hay fermented in both N-rich and N-free media as assessed by gas production and DM disappearance. Different sources of rapidly fermentable fiber from the basal feed may be beneficial to the stimulation of the activity of cellulolytic bacteria.

Significant ( $P < 0.05$ ) positive associative effects on *in vitro* gas production were observed also with untreated finger millet straw at different levels of peanut cake supplementation after 12, 52, and 166 h of incubation (Sampath et al., 1995). Similar interactions were observed for cottonseed cake supplementation to untreated straw and for peanut cake supplementation to urea-treated straw, although statistical significance was not achieved for all supplementation levels. No consistent significant associative effects on gas production were observed between cottonseed cake and treated straw. Similar to the ML in our study, peanut cake and cottonseed cake contain higher amounts of rapidly fermentable carbohydrates and protein. Therefore, the effects of these supplements may be caused by improved supply of energy and(or) amino acids or peptides. However, based on these observations, the respective effects were not distinguishable (Sampath et al., 1995).

Gas production is an indirect measure of substrate degradation, particularly the carbohydrate fraction, and is a good predictor for the production of VFA, but it is not always positively related to microbial mass production. It has been demonstrated for straws that an inverse relationship existed between gas volume or

VFA and microbial biomass production *in vitro* (Blümmel et al., 1997). This may be one of the reasons why gas production measurements are not always in accordance with the results of feeding trials (Shi et al., 1997).

## Implications

This study could show that positive associative effects on *in vitro* gas production occurred more consistently when rice straw was incubated in mixtures with hay or mulberry leaves than when incubated in mixtures with chemically treated rice straw. Further work is needed to measure *in vitro* gas production with concomitant microbial mass measurements to provide more information about partitioning of fermentation products.

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