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Use of *in vitro* gas production to evaluate associative effects on gas production of rice straw supplemented with lucerne*

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ABSTRACT

A semi-automated *in vitro* gas production (GP) system was used to investigate the associative effects on GP of rice straw (RS) supplemented with 0, 20, 40, 60, 80 and 100% lucerne hay (LH). Rate of GP increased with increasing levels of LH ($P<0.05$). Potential GP values were also affected by LH levels in the substrates, but only some significant effects were observed ($P<0.05$). The associative effects were maximal at 24 h incubation and then declined with duration of incubation at all inclusion levels of LH. Supplementation with 40-60% of LH to RS could produce the optimal associative effects on GP.

KEY WORDS: *in vitro* gas production, rice straw, lucerne hay, associative effects

INTRODUCTION

Rice straw (RS) is one of the major cereal straws for ruminants in China. Lack of dietary degradable protein may limit ruminal digestion and performance of ruminants fed low protein straw diets. One of the efficient ways to improve straw utilization is to increase microbial activity by providing critical nutrients. Positive associative effects of lucerne hay (LH) and chemically treated crop residues have been reported. However, associative action of LH with untreated roughages is conflicting. The *in vitro* gas production (GP) technique has the potential to

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investigate associative effects between feeds (Wood and Manyuchi, 1997; Liu et al., 2002). Therefore, the objective of the current study was to investigate the associative effects between RS and LH using the semi-automated *in vitro* GP system (Reading Pressure Technique, RPT) (Mauricio et al., 1999).

MATERIAL AND METHODS

Substrates and design

Samples of RS and LH were obtained from the Experimental Farms of Zhejiang University. All the samples were ground to pass a 1 mm sieve, and analysed for crude protein (CP), dry matter (DM) and neutral detergent fibre (NDF) according to AOAC (1990) and Van Soest et al. (1991). Contents (g/kg DM) of CP and NDF were 51 and 696 for RS; 212 and 437 for LH, respectively. RS was incubated alone or with LH supplemented at the graded levels of the total substrate in RPT. Substrate treatments consisted of the following ratios (DM basis) of RS to LH: 100:0 (LH0), 80:20 (LH20), 60:40 (LH40), 40:60 (LH60), 20:80 (LH80), 0:100 (LH100).

In vitro gas production test

Samples (1000±10 mg) of the air-dried RS or LH and mixtures were accurately weighed in triplicate into 160 ml capacity fermentation bottles and immersed in 100 ml buffered rumen fluid (ratio of rumen fluid to buffer 1:9, v/v) and incubated at 39ºC for 144 h in RPT system. Rumen fluid was obtained pre-feeding (07.00 h) from three fistulated Hu sheep, fed on a hay-based diet (70% Chinese wild rye plus 30% concentrate mixture). Two runs of incubation were carried out. Negative controls (rumen fluid plus buffer alone) were also included in triplicate. Head-space gas produced by substrate fermentation was measured at 3, 6, 9, 12, 24, 48, 72, 96, 120 and 144 h post-incubation. Pressure values, corrected by the amount of substrate OM incubated and for gas released from negative controls, were used to generate volume estimates.

Gas production kinetics

The kinetics of GP were estimated using the model $p = a+b\ (1-e^{-ct})$ described by Ørskov and McDonald (1979), where: ‘a’ is the intercept, which ideally reflects the fermentation of the soluble fraction, ‘b’ the fermentation of the insoluble fraction, (a+b) the potential extent of GP, ‘c’ the rate of GP (% h^{-1}), and ‘p’ the gas produced at time t.

Statistical analysis

The results of parameters of GP were analysed by one-way analysis of variance using the GLM procedure of SAS (1996). The calculated and actually measured GP values of the mixtures were compared by T-test. Unless otherwise stated, significance was declared at P<0.05.

RESULTS

Gas production (GP) during the fermentation of the inoculum and the kinetics are presented in Table 1. Gas produced after 144 h incubation ranged between 166.5 and 197.5 ml/g OM substrate. The GP increased with the proportion of LH in the substrate at 12, 48 and 144 h, but the difference was not significant (P>0.05) between LH60, LH80 and LH100 at each time after 24 h incubation.

Table 1. Cumulative gas produced at different times of incubation from the mixture of rice straw and lucerne hay, and parameters of gas production

	Rice straw : lucerne hay						SEM
	100:0	80:20	60:40	40:60	20:80	0:100	
Cumulative gas, ml/1g OM substrate							
12	48.7^{h}	60.9^{g}	71.7^{f}	79.0^{e}	91.4^{d}	96.7^{d}	2.14
24	89.7^{g}	104.8^{f}	115.8^{e}	127.0^{d}	125.6^{d}	131.8^{d}	2.96
48	120.2^{f}	136.6^{e}	144.1^{e}	156.4^{d}	156.6^{d}	162.4^{d}	3.77
96	153.4^{g}	168.3^{f}	172.6^{ef}	185.0^{d}	182.2^{de}	186.6^{d}	4.89
144	166.5^{f}	178.2^{ef}	186.9^{de}	193.1^{de}	193.3^{de}	197.5^{d}	5.53
Kinetics of gas production							
*a,*ml/1gOM	1.77^{ef}	2.03^{ef}	1.48^{f}	2.17^{e}	6.67^{d}	6.55^{d}	0.21
b,ml/1gOM	173.7^{e}	199.5^{d}	200.0^{d}	212.3^{d}	179.2^{e}	182.0^{e}	5.21
*a+b,*ml/1gOM	175.4^{f}	201.5^{de}	201.4^{de}	214.5^{d}	185.9^{ef}	188.6^{ef}	5.64
c,%/h	0.029^{i}	0.031^{h}	0.039^{g}	0.041^{f}	0.048^{e}	0.051^{d}	0.0003

OM - organic matter, a - the soluble and rapidly fermentable fraction, *b* - the insoluble but slowly fermentable fraction, *c* - the rate of gas production;

^{d,e,f,g,h,i} means within the same row with different superscripts differ significantly (P<0.05)

Rate of GP (*c*) increased linearly (P<0.05) as LH inclusion in the substrates increased, indicating that nonstructural carbohydrates and ruminally degradable protein of LH were readily available to the microbial population. However, the potential GP was similar for LH20 and LH40, suggesting that supplementation of 20% LH could improve nutrient utilization similar to that of 40% LH. Potential GP (a+b) values were also affected by levels in the substrates, and reached to a maximum (P<0.05) at LH60, but the differences among LH20, LH40 and LH60 were not significant (P>0.05), nor among the LH0, LH80 and LH100 (P>0.05).

Table 2. Difference[1] (%) between the gas production (GP) observed for the different mixtures of rice straw (RS) and lucerne hay (LH) and that predicted from RS and LH fermented separately

RS:LH	3	6	12	24	48	72	96	120	144
0.8:0.2	0.29	0.30	4.54	6.82	6.19	6.10	5.14	4.40	3.20
0.6:0.4	2.20	2.47	5.57[a]	8.70[a]	5.06	4.56	3.56	3.55	4.18
0.4:0.6	1.28	1.96	1.93	10.51[A]	7.46[a]	7.87[a]	6.74	5.49	4.30
0.2:0.8	1.70	5.22[a]	6.08[a]	1.84	1.69	1.62	1.27	1.20	1.05

[1] difference (%) = [(observed GP- predicted GP)/ predicted GP]×100

[a] significant at P<0.05; [A] significant at P<0.01

Table 2 presents data on the percentage difference between the observed GP of the mixtures of RS and LH and the calculated values from GP of RS and LH fermented individually. The RS-LH combinations had positive associative effects on GP at all inclusion levels and incubation times, and the magnitude increased before 24 h incubation and declined thereafter. However, only some significant effects were observed. Numerically, associative effect at LH60 showed the maximum (P<0.01) at 24 h.

DISCUSSION

One of the biggest challenges when feeding RS to ruminants is to increase their digestibility. Chemical treatments, while successful, present several practical problems for smallholder agriculture. Supplementation of high quality feeds such as legume forages to a poor-quality basal diet is more practicable. It has been demonstrated that the kinetics of GP are highly correlated with digestibility and feed intake when compared to data using the nylon bag technique (Khazaal et al., 1993). The RS used in this study was low in CP and high in fibre content. Lack of highly digestible carbohydrates caused an initial inhibition of GP and lowest value of fraction a from LH0 (Table 1).

When RS was incubated with LH, the rate of GP increased with the level of LH. However, the maximum gas volume did not differ between different mixtures (LH20, LH40 and LH60) except for LH80. Although the overall extent of digestion of LH20, LH40 and LH60 were similar, the rate of fermentation could be ranked as LH60>LH40>LH20. This is consistent with the findings of Liu et al. (2002), who incubated RS-based diets supplemented with 0, 25, 50, 75 and 100% treated straws, hay or mulberry leaves, and observed that the maximum gas volume and lag time did not differ between different mixtures. The rate of GP increased with the proportion of supplements. Manyuchi et al. (1992) also observed that the rate of *in vitro* GP of untreated barley straw was increased with the inclusion level of ammonia-treated straw. Cumulative GP at each sampling time was affected by levels of LH in the substrate. This finding suggest that the inclusion of LH as a substrate results in an increased rate and extent of fermentation of the substrates. CP content of the substrate increased with the level of LH, but it appeared to have little effect on final GP (Table 1). The fermentation medium contained nitrogen and minerals essential for microbial growth, so nitrogen and mineral deficiencies were unlikely to have occurred.

Positive associative effects were observed at all inclusion levels of LH (Table 2), the effects being most pronounced in LH60. This is consistent with the findings of Wood and Manyuchi (1997), who 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 GP and DM disappearance. Similar interactions were observed for mulberry leaves or hay supplementation to RS, although statistical significance was not always achieved for supplementation levels (Liu et al., 2002). Similar to the LH in our study, mulberry leaves 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.

CONCLUSIONS

Positive associative effects on *in vitro* gas production occurred when rice straw was supplemented with lucerne hay. Desired associative effects could be produced at the 40-60% of lucerne hay.

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