Journal of Animal and Feed Sciences, 16, Suppl. 2, 2007, 272–277

In vitro gas production technique to evaluate associative effects among lucerne hay, rice straw and maize silage*

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ABSTRACT

In vitro gas technique was used to evaluate the associative effects between lucerne hay and rice straw (Trial 1) and between their mixture and maize silage (Trial 2) in terms of gas production and microbial protein. Significant positive associative effect existed in gas production and microbial protein when lucerne hay and rice straw were mixed. The optimal ratio was 20:80 at which lucerne hay and rice straw had the highest associative effect. Markedly negative associative effects were found when lucerne hay/rice straw (20/80) mixture was mixed with maize silage. The associative effects based on gas production and microbial protein behaved inconsistent.

KEY WORDS: associative effect, gas production, lucerne hay, rice straw, maize silage

INTRODUCTION

The amount of nutrients that ruminant can extract from one feed can be modified by the type and quantity of other feeds consumed simultaneously. This phenomenon is commonly referred to as associative effect. Prediction of associative effect is an important aspect of efficient utilization of locally available feed resources. The effects of concentrates on the intake, digestion and utilization of relatively high quality forages have been thoroughly investigated (Doyle et al., 2005). However, there is limited information on the interactions that may occur when two or three forages are offered together.

* Supported by the National Science Funds for Distinguished Young Scholar, Grant No. 30325033 and Trans-Century Training Program Foundation for the Talents by the MOE, China
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Since high correlation existed between digestibility measured *in vivo* and predicted from an *in vitro* rumen gas production technique in combination with chemical composition, a considerable amount of researchers has used *in vitro* gas techniques to study associative effects of various types of feedstuffs, and examine influences on rumen fermentation (Liu et al., 2002; Getachew et al., 2003). The objectives of this study were to investigate the associative effects between lucerne hay (LH) and rice straw (RS) (Trial 1) and between their mixture and maize silage (MS) (Trial 2) using *in vitro* gas production (GP) technique.

MATERIAL AND METHODS

Feedstuffs and experimental design

The feedstuffs (LH, RS and MS) were obtained from the experimental farm of Zhejiang University, Huajiachi Campus (China), and the CP of them were 17.1, 5.7 and 11.9% DM, respectively.

In trial 1, RS was incubated together with LH at proportions of 0, 20, 40, 60, 80 and 100% as a basis of dry matter and the optimal ratio was achieved when they had the highest associative effect. Trial 2 was conducted to investigate the interactions between MS and the mixture of LH and RS with highest associative effect. The levels of MS were 0, 25, 50, 75 and 100%.

In vitro gas production

The semi-automated *in vitro* GP technique was employed as described by Mauricio et al. (1999). About 750 mg substrates were accurately weighed into serum bottles (approximate 180 ml). Rumen fluid was collected from three rumen-fistulated sheep before morning feeding. After being strained through a double-layer of cheesecloth under CO_2 in a water-bath at 39°C, 10 ml rumen inoculum was added into each flask and mixed with 90 ml pre-injected buffer medium (Theodorou et al., 1994). The incubations were conducted in two consecutive runs, each involving quadruples of samples. Two runs were incubated for 24 and 96 h for sample collection and GP study, respectively. The GP parameters were estimated using the equation: $GP = a + b\,(1\text{-}e^{-kt})$ (Ørskov and McDonald, 1979), where a, b and k were constants and GP was the gas production from the substrate at time t.

Measurement of in vitro fermentation parameters

The pH was measured using a portable pH meter (Model PB-20, Sartorius) after 24 h incubation and concentration of volatile fatty acids (VFA) was determined by gas chromatography (GC-2010, Shimadzu), as described elsewhere (Hu et al., 2005). Concentrations of microbial protein (MP) were estimated from the ratio of purines to N of isolated bacteria (Makkar and Becker, 1979). Yeast RNA was used as a standard.

Statistical analysis

Data were analysed using the general linear model (GLM) procedure of SAS (1996). The differences among means for treatments were tested using Duncan's new multiple range test.

RESULTS

The mean gas volumes at 24 h increased with increasing ratio of LH in mixtures (Table 1). Inclusion of 20% LH increased 24 h GP and GP rate by 81 and 116% (P<0.05), respectively, while no significant differences existed in potential GP. Total VFA concentration increased with increasing levels of LH, while no significant differences existed in MP among different LH inclusion levels.

Table 1. Gas production and fermentation parameters of lucerne hay and rice straw mixtures

Items	Levels of lucerne hay, %						SEM
	0	20	40	60	80	100	
Gas production (GP) parameters							
GP at 24 h, ml/g	37^{f}	67^{e}	87^{d}	105^{c}	125^{b}	131^{a}	1.7
potential GP, ml/g	105^{d}	115^{cd}	121^{c}	142^{b}	145^{b}	159^{a}	3.7
rate of GP, $\%\cdot h^{-1}$	0.019^{d}	0.041^{c}	0.055^{b}	0.067^{b}	0.081^{a}	0.086^{a}	0.0022
Fermentation parameters							
pH	6.68^{c}	6.72^{b}	6.74^{ab}	6.73^{b}	6.78^{a}	6.74^{ab}	0.015
microbial protein, mg/ml	0.43^{c}	0.59^{ab}	0.62^{a}	0.58^{ab}	0.59^{ab}	0.52^{b}	0.028
VFA mmol/l	12.3^{f}	17.6^{e}	21.2^{d}	24.4^{c}	28.2^{b}	33.9^{a}	0.44

[a-f] means within the same line with different superscripts differ at P<0.05

Table 2. Difference (%)[1] between the values of gas production or microbial protein observed for lucerne hay and rice straw mixtures and that predicted from them fermented separately

Parameters	Levels of lucerne hay, %			
	20	40	60	80
Gas production (GP) at 24 h	20.8^{*}	16.3^{*}	12.2^{*}	11.3^{*}
Microbial protein (MP)	31.7^{*}	34.5^{*}	19.8^{*}	17.5^{*}

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

[*] significant difference (P<0.05)

The GP showed positive associative effects (P<0.05), irrespective of the inclusion level of LH, being the highest (20.8%) at 20% of LH (Table 2). Similar to GP at 24 h, significant positive associative effect existed in MP, being 31.7 and 34.5% when LH was at 20 and 40%, respectively. From above, it is indicated that optimal positive associative effect occurs in the mixture of LH and RS at 20:80.

Inclusion of 25% MS did not significantly affect 24 h GP, potential GP, GP rate and total VFA, while higher levels (>50%) of MS induced notable increase in these parameters (Table 3). The MP increased with increasing levels of MS (P<0.05), being highest for MS alone (1.31 mg/ml).

Table 3. Gas production and fermentation parameters of maize silage and lucerne hay: rice straw mixtures

Items	Levels of maize silage, %					SEM
	0	25	50	75	100	
Gas production parameters						
GP at 24 h, ml/g	67^{d}	67^{d}	75^{c}	87^{b}	105^{a}	0.9
potential GP, ml/g	115^{b}	115^{b}	114^{b}	130^{a}	132^{a}	3.2
rate of GP, $\%\cdot h^{-1}$	0.041^{d}	0.041^{d}	0.050^{c}	0.056^{b}	0.065^{a}	0.0010
Fermentation parameters						
pH	6.74^{b}	6.78^{a}	6.72^{b}	6.74^{b}	6.72^{b}	0.010
microbial protein, mg/ml	0.61^{e}	0.65^{d}	0.82^{c}	0.85^{b}	1.31^{a}	0.024
VFA acids, mmol/l	18.1^{c}	19.7^{c}	29.4^{b}	32.9^{b}	38.3^{a}	1.36

$^{a-e}$ means within the same line with different superscripts differ at P<0.05

Table 4. Difference (%)[1] between the values of gas production or microbial protein for maize silage and lucerne hay: rice straw (20:80) mixtures and that predicted from them separately

Parameters	Levels of maize silage, %		
	25	50	75
Gas production (GP) at 24 h	-12.0^{*}	-13.0^{*}	-9.5^{*}
Microbial protein (MP)	-17.2^{*}	-14.6^{*}	-25.1^{*}

[1] difference (%) = [(observed GP or MP - predicted GP or MP)/ predicted GP or MP] × 100
* significant difference (P<0.05)

Contrary to the effects between LH and RS, negative associative effects predominated in the interaction between MS and LH:RS mixture (20:80) (Table 4). Significant negative associative effects, averaging -11.5 and -19.0%, existed in GP at 24 h and MP (P<0.05), respectively.

DISCUSSION

Fibre quality is extremely important for the complete action of cellulolytic bacteria, responsible for high rates of microbial colonization to the substrate, and resulting in efficient energetic use of the evaluated feeds. Silva and Ørskov (1988) observed that the presence of a source of readily digestible cellulose and hemicellulose increased the numbers of ruminal fibrolytic microorganisms and, consequently, may improve digestibility of other less degradable fibre sources in mixtures. The positive associative effect in GP and microbial protein when LH was mixed with RS may be attributed to the readily digestible fibre of LH.

In this study, the associative effect in GP (20.8%) was most apparent when LH was mixed with RS at 20% (Table 2). Similar ratios (27 and 17% LH) have been reported when LH was mixed with treated wheat straw for dairy heifers (Atwell et al., 1991) and barley straw for ewes (Haddad, 2000). Since the LH is relatively scarce and expensive in less developed countries, it is cost-effective to supplement 20% to rice straw.

Efficient microbial growth requires balance and synchronization between nitrogen and energy supply (Tebot et al., 2004). When LH was mixed with RS at 20%, the high protein solubility nature of LH may complement the deficiency of RS and resulted in synchronized supply of nitrogen and energy to the ruminal microbes (Chernev et al., 2002). Additional inclusion of MS induced negative associative effect, which might result from that a certain number of fermentation end products in silages do not contribute any energy to microbial growth (Verbic et al., 1999) and hence the homeostasis being destroyed. In fact, the synchrony index (Is) of silages (mean Is=0.30) was generally lower than that of fresh forages (0.84) and hay (0.80) in his study.

Gas production is an indirect measure of substrate degradation and it is not always positively related to microbial mass production (Liu et al., 2002). In our study, the associative effects in terms of GP and MP were highest for 20 and 40% LH in trial 1 (Table 2) and lowest for 50 and 75% MS (Table 4), respectively. Such inconsistency has been demonstrated when associative effect was investigated between mulberry leaves and oilseed meal (Su, 2002). Since truly digested substrate is partitioned among VFA, gas and microbial biomass, it is necessary to incorporate different parameters to evaluate associative effect of mixtures and further information on fermentation products partitioning is needed.

IMPLICATIONS

Positive associative effect exists in *in vitro* gas production (GP) and microbial protein (MP) when lucerne hay (LH) was mixed with maize silage (MS), while the inclusion of MS in LH:RS (20:80) mixture induced markedly negative associative effects. The associative effects in terms of GP and MP behaved inconsistent, for which further study is needed.

REFERENCES

Atwell D.G., Merchen N.R., Jaster E.H., Fahey G.C., Berger Jr. L.L., Titgemeyer E.C., Bourquin L.D., 1991. Intake, digestibility, and *in situ* digestion kinetics of treated wheat straw and alfalfa mixtures fed to Holstein heifers. J. Dairy Sci. 74, 3524-3534

Chernev D.J.R, Cherney J.H., Chase I.F., 2002. Influence of lucerne, fescue silage mixtures on milk production of early-lactation Holstein cows. J. Anim. Feed Sci. 11, 555-564

Doyle P.T., Francis S.A., Stockdale C.R., 2005. Associative effects between feeds when concentrate supplements are fed to grazing dairy cows: a review of likely impacts on metabolizable energy supply. Aust. J. Agr. Res. 56, 1315-1329

Getachew G., Robinson PH, Cone J.W., 2003. Evaluation of associative effects of feeds using *in vitro* gas production. J. Anim. Sci. 81, 337 (Abstr. 170)

Haddad S.G., 2000. Associative effects of supplementing barley straw diets with alfalfa hay on rumen environment and nutrient intake and digestibility for ewes. Anim. Feed Sci. Tech. 87, 163-171

Hu W.L., Liu J.X., Ye J.A., Wu Y.M., Guo Y.Q., 2005. Effect of tea saponin on rumen fermentation *in vitro*. Anim. Feed Sci. Tech. 120, 333-339

Liu J.X., Susenbeth A., Südekum K.H., 2002. *In vitro* gas production measurements to evaluate interactions between untreated and chemically treated rice straws, grass hay, and mulberry leaves. J. Anim. Sci. 80, 517-524

Makkar H.P.S., Becker K., 1999. Purine quantification in digesta from ruminants by spectrophotometric and HPLC methods. Brit. J. Nutr. 81, 107-113

Mauricio R.M., Mould F.L., Dhanoa M.S., Emyr O., Channa K.S., Theodorou M.K., 1999. A semi-automated *in vitro* gas production technique for ruminant feedstuff evaluation. Anim. Feed Sci. Tech. 79, 321-330

Ørskov E.R., McDonald I., 1979. The estimation of protein degradability in the rumen from incubation measurements weighted according to rate of passage. J. Agr. Sci. 92, 499-503

SAS, 1996. SAS/STAT User's Guide, Version 6.12. SAS Institute Inc. Cary, NC

Silva A.T., Ørskov E.R., 1988. Fibre degradation in the rumen of animals receiving hay, untreated or ammonia-treated straw. Anim. Feed Sci. Tech. 19, 277-287

Su H.Y., 2002. Study on associative effects between mulberry leaves (*Morus alba*) and oil-seed meals in ruminants (in Chinese). M.Sc. Thesis, Zhejiang University, pp. 43-48

Tebot I., Ibarra A.L., Purtcher F., Cirio A., 2004. Influence of energy supply on microbial protein synthesis and renal urea handling in Corriedale sheep. J. Anim. Feed Sci. 13, Suppl. 1, 223-226

Theodorou M.K., Williams B.A., Dhanoa M.S., McAllan A.B., France J., 1994. A simple gas production method using a pressure transducer to determine the fermentation kinetics of ruminant feeds. Anim. Feed Sci. Tech. 48, 185-197

Verbic J., Ørskov E.R., Zgajnar J., Chen X.B., Zindarsic-Pongrac V., 1999. The effect of method of forage preservation on the protein degradability and microbial protein synthesis in the rumen. Anim. Feed Sci. Tech. 82, 195-212