Journal of Animal and Feed Sciences, 16, Suppl. 2, 2007, 139–144

Effect of nitrogen-energy balance on the associative effects of feedstuffs *in vitro*[*]

W.J. Chen, P.F. Sun, X.D. Zhang and J.X. Liu[1]

College of Animal Sciences, Institute of Dairy Science, Ministry of Education
Key Laboratory of Molecular Animal Nutrition, Zhejiang University
Hangzhou 310029, P.R. China

ABSTRACT

The effect of nitrogen-energy balance on associative effects of feedstuffs was investigated using *in vitro* gas test. Ammoniated rice straw diet was supplemented with 40% of mixture of rapeseed meal and maize in which their ratio was 25, 50, 75 and 100%, respectively, so that different ratio of rumen degradable nitrogen to digested organic matter were obtained. The associative effect was defined as the difference between the observed digestibility and the predicted value from individual feed fermented alone. The associative effect values ranged from 4 to 9 %, varied with ratio of rapeseed meal to maize. The greatest response was pronounced when the ratio was 75%, at which the microbial protein synthesis was also maximum. It is inferred that the associative effect is influenced by the nitrogen-energy balance.

KEY WORDS: associative effects, gas production, fermentation parameters, nitrogen-energy balance

INTRODUCTION

Rice straw is low in quality and its intake is usually limited by the high level of fibre. One of the most effective methods to improve the nutritive value of rice straw is treatment with ammonia derived from anhydrous ammonia or urea. When cattle are fed only ammonium bicarbonate treated rice straw (ABRS), the growth rate is low, but there are dramatic increases in performance when sources of bypass nutrients, such as oilseed cakes, are supplied (Zhang et al., 1994), suggesting an associative effect (AE) between basal straw and supplementary protein. However,

[*] 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
[1] Corresponding author: e-mail: liujx@zju.edu.cn

the responses to supplemental rumen degradable protein may be dependent on the level of supplemental non-fibre carbohydrates. Schroeder et al. (2006) suggested that nitrogen (N) retention was increased by energy supplementation in growing steers. The balance of N and energy may have a profound effect on AE of feedstuffs.

The AE of feedstuffs in ruminants has been realized for a long time. *In vitro* gas production (GP) technique has been recently used to study the AE (Liu et al., 2002). However, measurement of GP only could lead to misleading results, because GP only accounts for substrate that is used for volatile fatty acids (VFAs) and does not consider substrate utilized for microbial growth (Getachew et al., 2004). Therefore, it is reasonable to evaluate the AE between feedstuffs based on both GP and other fermentation parameters, such as microbial protein (MP), methane production and carboxymethyl cellulase (CMCase). The present study was undertaken to investigate the AE of different combinations of rapeseed meal (RSM) and maize on ABRS using the *in vitro* GP technique.

MATERIAL AND METHODS

Samples preparation and experimental design

The rice straw used was from the late season rice (Variety *V. Yiu64*) cultivated in Zhejiang Province. Following the harvest, rice straw was air-dried and manually chopped to 3-5 cm lengths, and then treated with NH_4HCO_3 solution in polyethylene bags. Dosage of NH_4HCO_3 was 90 g/kg dry matter (DM), with initial moisture content of the treated straw at about 500 g/kg. After rice straw was thoroughly mixed with NH_4HCO_3 solution, the materials were left in a thermostated container at 35°C for 10 d.

Basal ABRS diet was supplemented with mixture of RSM and maize at a level of 40%. Ratio of RSM to maize in the mixture was at 25, 50, 75 and 100%, respectively, so that different ratio of rumen degradable N (RDN) to digested organic matter (DOM) was obtained.

Chemical analysis and in sacco degradability

All feeds were analysed for DM, N and ether extract (EE). *In sacco* degradability was determined using nylon bag technique (Ørskov and McDonald, 1979). About 4 g substrate was accurately weighed into nylon bags (7.5 × 15 cm) and incubated for 24 h *in sacco* in the rumen of three fistulated sheep. The OM and crude protein (CP) were determined after oven drying at 65°C to constant weight. The RDN/DOM was estimated from *in sacco* OM and CP degradability of each components.

In vitro gas production

In vitro GP test was performed according to the method of Menke et al. (1979). About 200 mg DM of samples (ABRS, RSM, maize and their mixtures) were introduced into a glass syringe (Model Fortuna, Häberle Labortechnik, Germany) together with 20 ml of artificial liquor and 10 ml of rumen fluid that was collected from three rumen-fistulated Huzhou sheep. Then the syringes were immediately placed in a water bath at 39°C. The *in vitro* digestibility of OM was estimated from the *in vitro* GP, CP and EE (Menke et al., 1979).

Measurement of in vitro fermentation parameters

The fermentation parameters were determined after the termination of 24 h incubation. The pH of rumen fluid was determined immediately after removal using a pH meter (Model PB-20, Sartorius). Concentrations of methane and VFAs were determined by gas chromatography (GC-2010, Shimadzu). Ammonia-N concentration was determined by a spectrometer (Model 721) using colorimetry with the NH_4Cl solution as a standard (Feng and Zhang, 1993). Concentrations of the microbial protein were estimated from the ratio of purines to N of isolated bacteria (Makkar and Becker, 1999). Yeast RNA was used as a standard. The CMCase activities were measured according to Wood and Mahalingeshwara (1998).

Statistical analysis

Data for AE were analysed using the GLM procedure of SAS (1996). The differences among means for the treatments were tested using Duncan's new multiple range test. The interrelations between AE values and RDN/DOM were analysed using regression procedure.

RESULTS

Chemical composition and degradability of OM and CP after 24 h incubation are shown in Table 1.

Results for *in vitro* fermentation parameters are summarized in Table 2. With the increasing levels of RSM, RDN/DOM value increased from 20.0 to 33.3 g/kg, while the GP decreased at high RSM levels. The same tendency existed in estimated OM digestibility. The AE value was defined as the difference between the observed *in vitro* OM digestibility and the calculated values from RSM, maize and ABRS fermented individually. Positive AE was observed in all the mixtures (Table 2). The values of AE increased with the increasing values of RDN/DOM up to 28.7

g/kg, and then decreased. The pH values were not significantly affected by ratio of RSM to maize, with all values within the normal range. Ammonia-N concentration increased with increased levels of RSM, while the opposite tendency was observed in the concentrations of VFAs. Methane production and CMCase activity increased with the increasing values of RDN/DOM up to 24.2 g/kg, and then decreased. The MP yield showed the same tendency as AE value.

Table 1. Chemical composition, *in sacco* degradability and *in vitro* gas production of ammonium bicarbonate treated rice straw (ABRS), rapeseed meal (RSM) and maize

	ABRS	RSM	Maize
Chemical composition			
dry matter, %	93.4	90.9	91.4
organic matter, % of DM	88.0	91.7	98.5
crude protein, % of DM	8.9	40.8	9.5
ether extract, % of DM	1.2	3.8	4.3
In sacco degradability at 24 h, %			
organic matter	32.7	65.7	76.8
crude protein	60.0	38.9	48.1
In vitro gas production at 24 h, ml			
gas production	16.4	33.1	58.5

A high quadratic relationship existed between the AE (y, %) and the RDN/DOM value (x, g/kg): $y = -0.0929x^2 + 5.0572x - 60.572$ ($R^2=0.8802$). It is estimated that optimal AE occurred when the RND/DOM was at 27.2 g/kg.

DISCUSSION

Positive AE in OM digestibilities were observed in all the mixtures, which ranged from 3.8 to 9.0%. *In vitro* OM digestibility is an important reflection of rumen fermentation, while microbial protein provides the majority of protein supplied to the small intestine of ruminants. In this study, the greatest AE value was obtained when RND/DOM was 28.7 g/kg. At this point, the MP synthesis was also strengthened, indicating the existence of positive associative effect in MP synthesis. Generally, transformation of RDN to MP or ammonia is regulated by the availability of energy. The balanced supply of N and energy ensure optimal MP synthesis, improve the utilization of protein and energy and result in positive AE. When the RND/DOM increased to 33.3 g/kg, the AE and MP synthesis decreased. It has been observed that when protein degradation exceeds the carbohydrate fermentation, large quantities of N would be lost as ammonia, while if the carbohydrate fermentation exceeds the protein degradation, MP production and efficiency of energy utilization would be

Table 2. Associative effect (AE) and rumen fermentation parameters when different combinations of rapeseed meal (RSM) and maize were incubated with ammonium bicarbonate treated rice straw (ABRS) at 24 h

Item	RSM: maize: ABRS				SEM
	10:30:60	20:20:60	30:10:60	40:0:60	
RDN/DOM[1]	20.0	24.2	28.7	33.3	
Gas production, ml	30.8^{a}	29.9^{ab}	28.9^{b}	24.7^{c}	0.35
OM digestibility, g/kg	518	524	530	502	3.6
AE, %	3.8*	6.5*	9.0*	4.6*	0.75
Fermentation parameters					
pH	6.69	6.71	6.72	6.73	0.024
NH_3-N, mg/l	146^{c}	180^{b}	193^{b}	212^{a}	5.2
total VFAs[2], mmol/l	31.9^{a}	31.1^{ab}	30.1^{bc}	29.5^{c}	0.32
methane, mmol	0.18^{b}	0.23^{a}	0.21^{ab}	0.12^{c}	0.012
CMCase[3], IU/g · min	48.5^{a}	49.5^{a}	38.9^{b}	32.8^{c}	1.28
Microbial protein, mg	9.6^{c}	11.4^{b}	14.5^{a}	10.7^{bc}	0.51

$^{a, b, c}$ means with different capital superscripts within the same row differ at P<0.05

* significant difference (P<0.05) between the measured and predicted *in vitro* organic matter (OM) digestibilities

[1] ratio of rumen degradable nitrogen (RDN) to digested organic matter (DOM)

[2] volatile fatty acids

[3] carboxymethyl cellulose

decreased (Russell et al., 1992). The available energy might be limited when RND/DOM reached 33.3 g/kg, and certain amount of N was lost as ammonia but not incorporated into MP, causing the decreased efficiency of RDN to MP.

Apart from GP and MP, there are still other parameters which changes may reflect and influence the AE. Methane production is the loss of energy during the transformation from digestible to metabolizable energy. Higher methane production, CMC activity and total VFAs relate to the higher GP. Theoretically, methane production, CMCase and total VFAs would decrease linearly with the increasing ratio of RSM to maize. In the present study, methane production increased when the levels of RDN/DOM was at 24.2 g/kg. This increase in methane production may be associated with more hydrogen, originated from the higher fermentation of substrate, to be provided for methane synthesis. The similar increasing tendency was found in CMCase, which reflected the microbial digestion of cellulose in solid-phase. The greatest activity of CMCase was observed when the value of RDN/DOM was 24.2 g/kg. The positive responses might be attributed to improvement in activity of ruminal microorganisms. High activity of cellulolytic bacteria would enhance the digestion of fibre and increase energy supply, hence cause the positive associative effects.

The results of this experiment can partly explain our previous results. With the ABRS, Xu and Liu (2005) observed that when supplementary RSM was raised from 0 to 100 g/day, liveweight gain increased from 20 to 63 g/d, but the further increasing levels of RSM only slightly increased the liveweight gain. Supplementation with small amount of RSM may be enough to balance the supply of N and energy, accordingly cause the positive associative effect.

CONCLUSIONS

The balance between degradable N and dietary available energy has great effect on the efficiency of microbial protein synthesis, energy utilization and associative effect. The increased MP synthesis and cellulolytic bacteria activity may be responsible for the positive associative effect.

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