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原　著

稲わらとタンパク質補助飼料のルーメン内消化動態に対する相乗効果の評価

Aye Sandar CHO・上田宏一郎*・近藤　誠司
北海道大学大学院農学研究院
〒060-8589　札幌市北区北９条西９丁目
*連絡著者（Corresponding author）: ueko@anim.agr.hokudai.ac.jp

Evaluation of associative effects on ruminal digestion kinetics between rice straw and protein supplements

Aye Sandar CHO, Koichiro UEDA and Seiji KONDO
Laboratory of Animal Production System, Graduate School of Agriculture, Hokkaido University
Kita-ku Kita-9 Nishi-9, Sapporo, 060-8589, Japan

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要　約

インビトロガスプロダクション(GP)法を用いて、稲わらとタンパク質源補助飼料のルーメン内消化に対する相乗効果を評価した。稲わら(RS)を、大豆粕(SB)、ヒマワリ粕（SF)、ラッカセイ粕（GN）およびゴマ粕（SS）と70：30の比で混合して培養した。累積GPを培養96時間まで測定し、ガス生成曲線を指数回帰モデルにあてはめてGP動態パラメータを推定した。培養後残渣から乾物消化率（DMD）および有機物消化率（OMD）を算出した。有効有機物消化率（OMED）をGP生成速度およびルーメン通過速度２％ h^{-1}から推定した。タンパク質補助飼料をRSに混合した場合に、GP生成速度はSBとの混合で最も速くなった。RS＋SBもしくはRS＋GNは他の補助飼料の混合と比べてDMD, OMDおよびOMEDは高い値を示した。タンパク質補助飼料とRSとの混合培養は、その種類に関わらずGP生成速度とOMEDに正の相乗効果をもたらした。しかし、OMEDへの正の相乗効果の程度はいずれのタンパク質飼料についても１～３％程度と小さかった。

ABSTRACT

In vitro gas production (GP) was measured to investigate associative effects on ruminal digestion between rice straw and protein source supplements. Rice straw (RS) was incubated with protein supplements, soybean meal (SB), sunflower meal (SF), groundnut meal (GN) and sesame meal (SS) at a ratio of 70:30. Cumulative GP volume was recorded up to 96 h incubation and the simple exponential equation was fitted to the GP production curve to obtain the kinetics of GP. After incubation, the residues were used to measure dry matter and organic matter digestibility (DMD and OMD). Organic matter effective degradability (OMED) was determined using the rate of GP, OMD and 2% $h^{-1}$of ruminal passage rate. When supplements were mixed with RS, the rate of GP was highest for SB mixture. The mixture of RS plus SB or GN showed higher DMD, OMD and OMED than other mixtures. A significant positive associative effects on the rate of GP and OMED were observed when RS was mixed with protein supplements regardless their source. However, the positive associative effect on OMED were only 1 to 3% points for all protein sources.

INTRODUCTION

The productivity of ruminant animals in many tropical countries is restricted by their feeds which consist of low nitrogen and high fiber contents. Thus crop residues and agricultural by-products are major source of feedstuff in these regions (TIN NGWE, 2003; KABI *et al.* 2005). An

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improvement in ruminant productivity requires a means for upgrading poor roughages such as rice straw through increasing their digestibility.

In Myanmar, intensive smallholder dairy farmers often feed ruminants on rice straw supplemented with various types of protein sources. It is well known that supplementing low quality straw-based diets with protein sources elevates ruminal ammonia nitrogen concentration to improve the slow rate of straw fiber fermentation in the rumen (FIKE *et al.* 1995). TIN NGWE (1990) have revealed that feeding protein supplements from several sources increased the ruminal digestibility compared with the sole rice straw produced in Myanmar. This improvement can be considered as "associative effect" between feeds so that the ruminal digestion of one feed in the diet affected that of other feed. It is necessary to quantify the associative effect between rice straw and varied sources of protein for an efficient use of rice straw. A better understanding of associative effects between rice straw and supplements could help to improve current feeding systems and thus contribute to the development of a more suitable animal production in Myanmar as well as some other Southeast Asia counties.

Although the positive benefits of protein supplementation for the ruminal fermentation of straw fiber appear to be well established, there is a limited amount of quantitative information regarding on associative effects. The quantification of associative effects between feeds requires to be tested both singly and in one or more generally binary combinations (VAN SOEST, 1994). In spite of an importance of *in vivo* approach, it is difficult to compare the associative effects due to a wide variety of supplements. An alternative *in vitro* approach especially gas production method could be suitable for a quantitative evaluation of the associative effects between feeds on the ruminal digestion kinetics and the digestibility.

The objective of this study was, therefore, to evaluate associative effects on ruminal digestion between rice straw and different protein supplements (soybean meal, sunflower meal, groundnut meal and sesame meal) produced in Myanmar, using *in vitro* gas production method. The hypothesis tested in this study was whether associative effects on ruminal fermentation would be varied with protein supplements source due to their ruminal degradation rate.

Materials and Methods

Feedstuffs and chemical analysis

Rice (*Oryza sativa* L.) straw was obtained from the experimental farm of Agriculture University, Yezin, Myanmar. Soybean (*Glycine max* L.) meal (SB); sunflower (*Helianthus annuus* L.) meal (SF); groundnut (*Arachis hypogaea* L.) meal (GN) and sesame (*Sesamum indicum* L.) meal (SS) were obtained from a local market in the central region of Myanmar. All feed samples were ground by a cutting mill to pass through 1-mm screen prior to the chemical analyses and the *in vitro* gas production measurements. All feeds were analyzed for dry matter (DM), crude protein (CP), crude ash, organic matter (OM), neutral detergent fibre (NDF), acid detergent fibre (ADF), acid detergent lignin (ADL), ether extract (EE), and non-fibrous carbohydrate (NFC). The content of DM, crude ash, OM, CP and EE were according to the methods by AOAC (1990). The content of NDF, ADF and ADL were measured according to the methods described by GOERING and VAN SOEST (1975). The content of NFC was calculated by subtracting CP, NDF and EE from OM (VAN SOEST *et al.* 1991).

The protein fractions (A, B1, B2, B3 and C) of feedstuffs according the Cornell net carbohydrate and protein system were analysed by SNIFFEN *et al.* (1992) and LICTIRA *et al.* (1996). Fraction A is non-protein nitrogen which is trichloroacetic acid soluble. B is true protein, and C is unavailable true protein or bound protein. Fraction B is further divided into three fractions (B1, B2, and B3) that have different rates of ruminal degradation. Fractions A and B1 are soluble in borate phosphate buffer and are rapidly degraded in the rumen. Fraction B2 is fermented in the rumen at lower rates than buffer-soluble fractions, and some fraction B2 escapes to the lower gut. Fraction B3 is more slowly degraded in the rumen than Fractions B1 and B2 because of its association with the cell wall. Fraction C is the acid detergent insoluble protein (ADICP), and is highly resistant to breakdown by microbial and mammalian enzymes, and it is unavailable for the animal. Fraction B3 is the difference between neutral detergent insoluble protein (NDICP) and ADICP. Fraction B2 can be calculated with the following equation; $B2 = CP - A - B1 - B3 - C$.

Gas production measurements

Rumen digesta was taken from six ruminally fistulated Holstein dry cows (777.4 ± 18.4 kg of BW) fed on corn silage and alfalfa hay twice a day with an allowance at a maintenance level. The management and the rumen fistulation procedures for experimental cows were approved by the Animal Care and Welfare Committee of Hokkaido University, Japan. Rumen fluid inoculum was collected after the morning meal and squeezed through four layers of cotton gauze. The fluid was transferred to the glass bottle and immediately transported to the laboratory. The culture medium and rumen inoculum procedures used the method of THERODOROU *et al.* (1994).

In vitro gas production was measured from incubation of single substrates of RS and supplement (SB, SF, GN and SS) separately, and their mixtures consisting of 30% supplements with 70% RS. The strip anaerobic methods were used in all steps during the rumen fluid transfer and the incubation period. The individual feed samples and/or the respective mixtures (app. 1g DM) were transferred into 100 ml serum bottles. The bottles were filled with 80 ml medium and pre-warmed at 39℃, and then added 4 ml of reducing agent and 5 ml of rumen fluid inoculum under continuous flow of CO_2. Each sample was incubated in duplicate and two blanks containing rumen fluid and culture medium without sample in each cow was run.

The gas production was measured by reading and recording the amount of gas volume after incubation using 50 ml syringe connected to the incubation bottle with a 23 gauge, 1.25 inch needle. Gas production (GP) was recorded from 1 to 96 h after incubation periods. The incubation terminated after 96 h of incubation. At the end of fermentation period, the residue in the bottles were filtered with pre-weighed filter paper, weighed after drying 4 h at 105℃ and then dry matter digestibility (DMD) was calculated. Organic matter residue was measured by incineration (600℃ for 2 h) of filter paper and filtrate and organic matter digestibility (OMD) was calculated.

Computation of data and statistical analysis

The collected cumulative GP profiles were fitted to the following simple exponential equation to evaluate kinetics of GP data:

$$GP\ (ml) = A[1-\exp(-k\ (t-LT))]$$

where A is the potential GP (ml), k is the rate of GP (%/h), LT is the lag time (h) before degradation commenced, assuming that no gas was produced for $t < LT$, t is the hour since incubation.

The predictive values of cumulative GP in each time point were obtained by summation of two single feed data with considering their proportions in the mixture. These predicted data were fitted to the model again and thus predicted GP kinetics parameters were obtained. To detect possible associative effects of the mixtures, the parameters derived from predicted values of a mixture were compared with their correspondent parameters derived from observed values of mixture.

Organic matter effective degradability (OMED) was calculated from GP parameter by using at a rumen outflow per h of 0.02 according to FRANCE *et al.* (2000):

$$ED = S0\exp(-0.02)[k/(0.02+ k)]$$

where k is the rate of GP (/h) and S_0 represents the OM disappearance after 96 h of incubation (%).

For DMD and OMD, predicted value was calculated by summing the digestibility from the single feedstuff incubation with considering their proportion. Predicted value of OMD and k were used to obtain the predicted OMED.

All data obtained from the study were statistically analysed using the GLM procedure of SAS (2004) as a randomized block design regarding cows as blocks. The significance of difference between feeds was analyzed with Tukey's multiple range tests. In order to determine the associative effects, the observed values of GP parameters and digestibility were compared with the correspondent predicted values with pared t-test.

Results and Discussion

The chemical compositions and protein fraction of feedstuffs are presented in Table 1. The chemical composition of RS used in this study was typical one, as low content of OM, CP and NFC accompanied with high content of NDF, ADF and ADL. However, when compared to the Japanese feed standard table (NARO, 2001), the CP content of RS was slightly lower. Four sources of protein supplements contain a greater amount of CP but were varied between 34.5% of DM for SF to 53.9% of DM for GN. The contents of EE of all four supplements were ranged between 6.6 to 9.4% of DM and were considerably higher than the standard values (1.2 to 2.2% of DM; NARO, 2001). This could be because the protein supplements used in this study were made with a mechanical oil extraction method in Myanmar. KRISHNAMOORTHY *et al.* (1995) reported that the EE contents of groundnut meal made with mechanical oil extraction was higher (7.3%) than that made with a solvent extraction method (0.0%). Fiber content in protein supplements was generally low but SF contained considerably greater NDF, ADF and ADL compared with other tree supplements. The content of NFC was higher for SB (25.3% of DM) than other supplements (18.4 to20.4% of DM).

The contents of CP fractions of protein supplements had a wide variation with their sources (Table 1). The CP of SB contained the lowest A and the highest B1 fraction among supplements. On the contrary, the CP of GN contained the highest A fraction among supplements. The CP of SF and SS contained the moderate A and the relatively lower B1 among supplements. The content of B2 was lower for SF and GN than SB and SS. Therefore, the degradation rate of CP with the ruminal fermentation could be ranked as the highest in GN, the intermediate in SF and SS, and the lowest in SB among supplements.

Table 1　Chemical composition and crude protein fractions of experimental feedstuffs (RS: rice straw, SB: soybean meal, SF: sunflower meal, GN: groundnut meal, SS: sesame meal)

	RS	SB	SF	GN	SS
	% of dry matter				
Organic matter	83.0	93.0	92.5	94.2	85.8
Crude protein	4.2	45.2	34.5	53.9	44.1
Ether extract	1.1	6.7	9.4	7.6	6.6
Neutral detergent fiber	70.3	17.5	31.9	13.4	18.1
Acid detergent fiber	41.2	13.7	25.3	8.9	9.5
Acid detergent lignin	5.5	1.5	9.5	1.4	2.8
Non-fiber carbohydrate	9.5	25.3	19.4	20.4	18.4
Crude protein fraction					
A	0.6	6.2	20.2	40.8	24.1
B1	0.0	22.7	4.8	5.5	5.8
B2	1.4	14.6	6.7	6.4	12.8
B3	1.1	1.3	1.3	0.0	0.7
C	1.1	0.5	1.3	1.2	0.7

A: non-protein nitrogen x 6.25, B1: rapidly degradable protein, B2: intermediately degraded protein, B3: slowly degraded protein, C: bound protein

The gas production parameters, DMD, OMD and OMED measured by an incubation with a single feedstuff are presented in Table 2. The protein supplements used in this study had generally less A, higher k and shorter LT compared with RS. The digestibility (DMD, OMD and OMED) was higher for SB, GN and SS than for RS but OMD was lower for SF than for RS. Among protein sources, A was the greatest for SB and the least for SF. However, the k was not differed among SB, SF and GN but differed between SF and SS. The lag time of protein supplements did not differ among supplements. The digestibility (DMD, OMD and OMED) of SB and GN were higher than those of SF and SS. The digestibility was similar between SB and GN, and was the least for SF among supplements.

The highest A and digestibility for SB could be due to the higher content of easily digestible carbohydrate (NFC). Although A of GN was less than that of SB, the digestibility was as high as that of SB. This could be due to the least content of carbohydrate (NDF + NFC) accompanied with the high soluble CP content. CONE and VAN GELDER (1999) showed that the fermentation of a protein-rich substrate causes less GP than carbohydrate-rich fractions because of the binding of carbon dioxide with ammonia. Contrarily, the lowest A and digestibility in SF could be associated with the highest EE contents that might interfere with a microbial attachment as well as the highest lignin content. This result was agreed with KIRAN and KIRASHNAMOORTHY (2007) who reported that A and true digested OM were the lowest for SF among four protein sources compared.

Table 3 shows GP parameters, DMD, OMD and OMED of observed values from an actual mixture of RS and protein supplements. There was no difference in A and LT among four mixtures of RS and protein supplements. The k was the highest for mixture RS + SB, but did not differ among other three mixtures. The order of DMD and OMD of mixtures were similar to the result from single feedstuff incubation. However, OMED of RS + SB was significantly higher than that of RS + GN reflecting the higher k for RS + SB. Other two mixtures had lower OMED compared with the mixture RS + GN.

The predicted gas production parameters and digestibility from the measurements of RS and protein supplements incubated individually are shown in Table 4. The difference in the predicted gas production parameters and digestibility among four mixtures were similar to those of observed values derived from actual mixture (Table 3).

A comparison was done between the observed values and the correspondent predicted values (Table 4). When A,

Table 2　Gas production parameters, DMD, OMD and OMED of individual feedstuffs (RS: rice straw, SB: soybean meal, SF: sunflower meal, GN: groundnut meal, SS: sesame meal) when incubated as a sole substrate (n = 6)

	RS	SB	SF	GN	SS	SEM	*P* value
A, ml/g OM	173^{a}	184^{a}	87^{c}	126^{b}	145^{ab}	7.95	<0.01
k, % / h	2.9^{c}	9.5^{ab}	11.0^{a}	9.2^{ab}	8.5^{b}	0.51	<0.01
LT, h	8.0^{a}	2.5^{b}	3.0^{b}	2.4^{b}	2.5^{b}	0.29	<0.01
DMD, %	58.0^{c}	78.7^{a}	54.4^{c}	77.4^{a}	61.4^{b}	0.47	<0.01
OMD, %	65.9^{b}	81.1^{a}	55.3^{c}	79.5^{a}	67.8^{b}	0.47	<0.01
OMED, %	37.6^{d}	65.6^{a}	45.8^{c}	62.7^{a}	53.0^{b}	1.52	<0.01

A: potential gas production; k: rate of gas production; LT: lag time; DMD: *in vitro* dry matter digestibility; OMD: *in vitro* organic matter digestibility; OMED: organic matter effective degradability; abcd Means with the same letter in the same raw do not differ significantly (P < 0.05)

Table 3　Gas production parameters, DMD, OMD and OMED when rice straw (RS) was incubated with protein supplements (SB: soybean meal, SF: sunflower meal, GN: groundnut meal, SS: sesame meal) at a proportion of 70:30 (n = 6)

	RS+SB	RS+SF	RS+GN	RS+SS	SEM	*P* value
A, ml/g OM	199	170	180	180	4.75	0.17
k, % / h	4.5^{a}	3.8^{b}	3.9^{b}	3.8^{b}	0.09	0.03
LT, h	3.1	3.8	3.4	3.5	0.24	0.26
DMD, %	64.6^{a}	58.1^{c}	64.4^{a}	60.9^{b}	0.34	<0.01
OMD, %	70.7^{a}	63.4^{c}	70.5^{a}	66.2^{b}	0.28	<0.01
OMED, %	47.8^{a}	40.8^{c}	45.4^{b}	42.5^{c}	0.34	<0.01

A: potential gas production; k: rate of gas production; LT: lag time; DMD: dry matter digestibility; OMD: organic matter digestibility; OMED: organic matter effective degradability; abc Means with the same letter in the same row do not differ significantly (P < 0.05) k and digestibility from observed values were higher and LT was less than that from predicted values, an associative effect could be regarded as positive. When the inverse trend was observed, an associative effect could be regarded as negative. The higher values of observed A were shown for four all mixtures, although they were not statistically differed except for RS + GN. The higher values were also shown in the observed k for all mixtures with statistical differences (RS + SB, RS + GN and RS + SS) or a trend (RS + SF, P=0.08). However, there were no differences in LT between observed and predicted values for all mixtures.

Table 4 Predicted value from the data of rice straw (RS) and protein supplements (SB: soybean meal, SF: sunflower meal, GN: groundnut meal, SS: sesame meal) incubated individually and the comparison between observed value and predicted value of gas production parameters, DMD, OMD and OMED with pared t-test (n = 6)

	Predicted value				Comparison (P value)			
	RS+ SB	RS+ SF	RS+ GN	RS+ SS	RS+ SB	RS+ SF	RS+ GN	RS+ SS
A, ml/g OM	170	142	151	160	0.11	0.14	<0.01	0.14
k, % / h	4.1	3.3	3.6	3.5	0.02	0.08	<0.01	0.01
LT, h	2.9	3.7	3.4	3.4	0.76	0.89	1.00	0.90
DMD, %	64.2	56.9	63.8	59.0	0.20	0.11	0.10	<0.01
OMD, %	70.4	62.7	70.0	66.4	0.56	0.37	0.47	0.65
OMED, %	46.2	38.1	44.0	41.6	<0.01	0.02	0.01	<0.01

A: potential gas production; k: rate of gas production; LT: lag time; DMD: dry matter digestibility; OMD: organic matter digestibility; OMED: organic matter effective degradability

Although there were no difference in OMD between observed and predicted, the OMED were significantly higher for observed value four all four mixtures (Table 4). The increases of actual mixtures in OMED were 1.6, 2.7, 1.4 and 0.9% points for RS + SB, SF, GN and SS, respectively, which were brought by the increases in k. The results suggested that when using reference values for feed formulation in Myanmar ruminant production systems based on rice straw, the protein source supplements would be underestimation and the increase in 1 to 2% points of digestibility should be taken into consideration from the point of their positive associative effects

In this study, positive associative effects in mixtures of RS and protein supplements were observed in k and OMED. The protein supplements can provide adequate nitrogen to the rumen microbes which are restricted growth and activity. Although the k of SB with single feedstuff incubation was not significantly differed with GN or SF (Table 2), the k became the most rapid when SB was mixed with RS (Table 3). It could be considered that the slow CP degradation rate of SB, which was determined with CP fraction, may be well matched to the slow rate of RS fiber degradation in the rumen and led to the increased OMED. The CP degradation rate of other supplements in this study could be too fast to match to the rice straw fiber fermentation by the rumen microbes.

SAMPATH *et al.* (1995) also studied the effect of supplementation of various nitrogen sources to finger millet straw with *in vitro* GP and showed that urea which would have the most rapid CP degradation rate brought the negative associative effects on the accumulated volume of early stage GP of the straw mixture. Moreover, cottonseed meal mixture and groundnut meal supplementation made a positive associative effect on the accumulated volume of early stage GP of the straw mixture, however, the extent of increase of observed value compared to the predicted value was largely higher for the mixture of groundnut. This could attribute to the too slow CP degradation rate of cottonseed meal, whereas the groundnut could have a suitable moderate rate of CP degradation to gain the greater associative effect during the early stage of rumen digestion. These results suggest that the CP degradation rate of protein supplements is essential to match to the degradation rate of the straw to obtain a maximum rate of rice straw fermentation with a positive associative effect.

The positive associative effects on k and OMED was not so large in this experiment. This was similar to the report by SAMPATH *et al.* (1995) who found that *in vitro* DMD of finger millet straw were increased 1.1 and 1.7% points for groundnut meal and cottonseed meal supplementation, respectively. However, *in vivo* DMD and OMD of rice straw were increased from 3.0% to 7.0% points by soybean meal, groundnut meal and sesame meal supplementation (TIN NGWE, 1990), which was slightly higher than the values in this study. A possible factor attributing to the less positive associative effect on k and OMED was the high EE contents in the protein supplements used in this study. A high EE content of the diet inhibits the ruminal microbial activity. The content of EE greater than 6.0% of DM for all protein supplements could partially cancel a potential extent of positive associative effect. A methodology of *in vitro* GP incubation using nitrogen-rich medium can be an alternative reason as suggested by DRYHURST and WOOD (1997).

It was concluded that the protein degradation rate of oil cake produced in Myanmar affected the extent of positive associative effect on the rate of RS fermentation. Rice straw can be fermented more rapidly in the rumen when supplemented with SB compared with SF, GN and SS. However, the increase of OMED due to a positive associative effect was only 1 to 3% regardless protein source.

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