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Mob: +92 300 3008585, Fax: +92 41 8815544
E-mail: editorpjn@gmail.com

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Effect of Sulfur Supplementation on *in vitro* Fermentability and Degradability of Ammoniated Rice Straw

Mardiati Zain[1], N. Jamarun[1] and Nurhaita[2]
[1]Department of Animal Nutrition, Faculty of Animal Science, Andalas University, Kampus Limau Manis, Padang 25163, Indonesia
[2]Department of Animal Nutrition and Feed Technology, Faculty of Agriculture, Bengkulu University, Bali Bengkulu Stree, Indonesia

Abstract: *In vitro* studies were conducted to determine effect of supplementation various amount of sulfur (Ammonium sulphate) on fermentability and degradability of ammoniated rice straw. The *in vitro* experiment was carried out following the first stage of Tilley and Terry method. The treatments consisting of four levels of sulfur, were A = ammoniated Rice Straw (RS) (control), B = A + 0.15% Sulfur (S) supplement, C = A + 0.3% Sulfur supplement and D = A + 0.45% sulfur supplement on dry matter. Completely randomized design was used as the experimental design and differences among treatment means were examined using Duncan multiple range test. Variables measured were Ammonia (NH_3) and Volatile Fatty Acid (VFA) concentrations, as fermentability indicators, as well as degradability indicators including degradability of Dry Matter (DM), Organic Matter (OM), Neutral Detergent Fibre (NDF), Acid Detergent Fibre (ADF) and cellulose. The results indicated that fermentability and degradability of diets consisting ammoniated RS with Sulfur supplementation were significantly higher than the control diet ($p<0.05$). It is concluded that sulfur supplementation is important to improve fermentability and degradability of rations containing ammoniated RS. The best level of sulfur supplementation is obtained at 0.3% on dry matter.

Key words: Ammoniated rice straw, sulphur, fermentability and degradability

INTRODUCTION

The possibility of increasing fiber digestibility of high fiber containing material such as Rice Straw (RS) by ruminants to obtain high protein products is of particular interest in developing countries. In these countries such as Indonesia high fibrous feedstuffs constitute the main or only dietary component for animals since feeds of higher energy and protein values (cereals, legumes, etc.) are reserved for human needs. The utilization of rice straw is hampered by deficiency of nutrients, both for the animal and the rumen microbes. However, ruminants are still capable of using feedstuffs with low nutrient values and its rumen microbes are able to produce protein from simple nitrogen (N) sources such as urea, biuret, etc and Sulfur supplement. Therefore, nutritive value of some straws and other byproduct feeds can be improved simply by adding urea and Sulfur.

In recent years, sulfur content of forage diets has been recognized as a significant factor that affect the size of the rumen microbial population (Akin *et al*., 1983). Deficiency of dietary Sulfur could limit microbial growth in the rumen and hence limited their contribution to plant tissue digestion (Karsli and Russell, 2001). Sulfur was required to support *de novo* synthesis of Sulfur amino acids and thus microbial synthesis (Slyter *et al*., 1996). Bacteria can use carbohydrates as carbon skeletons for protein synthesis in combination with ammonia (Bach *et al*., 2005) and also utilize sulfur (organic or inorganic sulfur) to synthesize sulfur-containing amino acids (Kandylis, 1984) to produce microbial protein. Sulfur was also important for fiber fraction degradation in the rumen as it stimulated specifically growth of cellulolytic bacteria (Bal and Ozturk, 2006).

Sulfur supplementation is important for rumen fermentation and microbial protein synthesis. As Although there is little information explaining how the rumen bacteria and fermentation are influenced by Sulfur supplementation, it has been shown that the supplementation of poor quality fiber diets with Sulfur improved ruminal fiber degradation as well as apparent organic matter digestibility. Therefore, a study was conducted to examine effects of S addition on *in vitro* fermentability and degradability of ammoniated RS.

MATERIAL AND METHODS

The experimental was designed to study the effects of level sulfur on the *in vitro* degradability when rice straw was the substrat. The rice straw was previously treated with 4% urea on dry matter. Ammonium sulphate was used an Sulfur source and added in diet with level 0.15, 0.3 and 0.4% on dry matter respectively in rations B, C and D.

In vitro fermentability and degradability of nutrient were determined following the first stage of Tilley and Terry procedure (1969). Ruminal fluid was obtained from cannula steer. Fermentation tube consisted of 10 ml of

Corresponding Author: Mardiati Zain, Department of Animal Nutrition, Faculty of Animal Science, Andalas University, Kampus Limau Manis, Padang 25163, Indonesia

ruminal fluid and 40 ml of McDougall buffer solution. Samples were incubated in duplicate in 100 ml polyethylene tubes in 39°C in a shaker water bath for 48h. Two fermentation tubes that did not contain diets were also incubated and used as blanks. Fermentation was terminated at 48 h by injecting the tubes with 1 ml of $HgCl_2$. Tubes were then centrifuged at 2000 x g for 15 min and the supernatant was removed. Tubes with residue were dried at 60°C for 48 h and weighed and the data were used for degradability determination. These residues were also analyzed for its DM, OM and N by standard procedures (AOAC, 1990), the NDF, ADF and cellulose of residues were determined by Goering and Van Soest (1970) procedures. Supernatants were used to determined NH_3 concentration (microdifusion Conway method) and total VFA concentration (steams distillation, Strorry and Miller (1965).

A completely randomized design was used as experimental design consisting of four treatments. Variables measured were fermentability indicators (ammonia and total VFA concentration) and degradability of Dry Matter (DM), Organic Matter (OM) and fibrous fractions (NDF, ADF and cellulose). Data were analyzed by ANOVA using the GLM procedure. Differences between the control treatment and S supplementation treatment were analyzed by Duncan Multiple Range Test (DMRT) (Steel and Torrie, 1981).

RESULTS

Fermentability: Table 1 shows results of Sulfur supplementation effects on of rumen fermentability. Effects of treatments were significant ($p<0.05$) on the following variables: ammonia and total VFA concentrations.

Ammonia concentrations decreased from 56.35 mg/100 ml in control diet to 54.63 mg/100 ml, 51.60 mg/100 ml and 49.10 mg/100 ml, respectively in rations B, C and D which consisted of Sulfur supplements of 0.15, 0.30 and 0.45. Differences in ammonia concentration among treatment diets were significant ($p<0.05$). Reverse results from ammonia concentrations were obtained in total VFA concentrations. The addition of Sulfur supplement increased total VFA concentrations from 68.75 mM in diet A to 82.50 mM in diet B, 92.50 mM in diet C and 100.75 mM in diet D. Total VFA concentrations differed significantly between all rations ($p<0.05$), except that between rations C and D.

***In vitro* degradability:** Data on *in vitro* degradability of ammoniated RS are presented in the Table 2. The addition of Sulfur at different level affected all degradability variables ($p<0.05$). Control diet (A) had the lowest DM, OM, NDF, ADF and cellulose degradabilities ($p<0.05$). An increase in S supplementation increased the degradabilities of DM, OM, NDF, ADF and cellulose, and the increase in degradabilities of DM, OM and fibrous fractions followed linier patterns with the levels of Sulfur supplementation.

DISCUSSION

Fermentability: These *in vitro* results indicated that sulfur could limit maximum ruminant utilization of rice straw ammoniated. The supplement sulfur used in this study apparently reduced ammonia concentration. This is consistent with the result of Hegarty *et al.* (1991), who found that high S supplementation tended to reduce NH3 concentration (78 vs. 88 mg N/l) in the rumen fluid. This is indicated that sulphur can promote rumen bacterial growth, because almost rumen bacteria used ammonia as source nitrogen for their growth. Increasing the population of rumen microbe which subsequently increased total VFA concentration. These results had agreed with the works reported by Qi *et al.* (1992).

Comparisons among diets supplemented with Sulfur, the best results in fermentability study had been obtained at 0.3% S supplement. This level was higher than that was obtained by Harrison and McAllan (1980) who suggested that the best 0.2% level. However, the N/S diet ratio in the present study were still in the range of 3:1 up to 18.5:1 that had been recommended by Bal

Table 1: Effect of sulphur supplementation on total NH3 and VFA concentration in the rumen (mean value)

	Treatments				
Variables	A	B	C	D	SE
N-NH_3 (mg/100 ml)	56.35^a	54.63^b	51.60^c	49.10^c	0.678
Total VFA (mM)	68.75^d	82.50^c	92.50^a	100.75^a	1.874

Values within the same rows differ significantly at ($p<0.05$). A = Ammoniated RS, B = A + o.15% S , C = A + 0.3% S and D = A + 0.45% S on dry matter

Table 2: Effect of sulphur supplementation on *in vitro* degradability of ammoniated rice straw (coefficient)

	Treatments				
Variables	A	B	C	D	SE
Dry matter degradability (%)	0.48^c	0.53^b	0.54^a	0.55^a	0.524
Organic matter degradability (%)	0.50^b	0.51^b	0.54^a	0.56^a	0.575
NDF degradability (%)	0.42^c	0.44^b	0.46^a	0.47^a	0.812
ADF degradability (%)	0.40^b	0.41^b	0.44^a	0.45^a	0.552
Cellulose degradability (%)	0.42^c	0.45^b	0.47^a	0.47^a	0.880

Values within the same rows differ significantly at ($p<0.05$). A = Ammoniated RS, B = A + o.15% S , C = A + 0.3% S and D = A + 0.45% S on dry matter

and Ozturk (2006). The different response obtained was due to differences in diet used in this experiment consisting of low quality roughage.

***In vitro* degradability:** Supplementation sulphur was effective in stimulating dry matter, organic matter and fibrous fraction degradability. The increase in degradabilities of DM, OM and fibrous fraction of ammoniater rice straw vs control demonstrate that sulphur addition develops the rumen bacterial growth, resulting in a greater rate of degradability. This study has also shown that ammoniated RS were deficient in Sulfur, and it supplementation is important to improve fibre degradation of fibrous feedstuffs. The present results were in agreements with the results of Little (1986), Komizarczuk and Durand (1991) and Akin and Benner (1988). The last researchers had indicated that improvement in fibre degradation by sulfur supplementation occurred through its specific stimulation on rowth of rumen cellulolytic bacteria and anaerobic rumen fungi. Bacteria can use carbohydrates as carbon skeletons for protein synthesis in combination with ammonia (Bach *et al*., 2005) and also utilize sulfur (organic or inorganic sulfur) to synthesize sulfur-containing amino acids (Kandylis, 1984) to produce microbial protein. The present study has suggested that the best result was obtained by supplementing Sulfur at 0.3% on dry matter.

Conclusion: Supplemental sulphur have effect of fermentability and degradability of ammoniated RS. The effects occurred through reduction in ammonia concentration, increase total VFA concentration and degradabilities of DM, OM and fibrous fraction. The best level of S supplementation is obtained at 0.3% S on dry matter. Further study is required to determine effects of supplementation on *in vivo* experiment.

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Abbreviations: RS, Rice Straw; ADF, Acid Detergent Fibre; DM, Dry Matter; NDF, Neutral Detergent Fibre