STABILIZATION WITH SULFURIC ACID OF THE CRUDE PROTEIN IN UREA-TREATED RICE STRAW

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Summary

The effect of neutralization of urea-treated rice straw with sulfuric acid was investigated. Long-cut (15-20 cm) and short-cut (2-3 cm) rice straw were treated with 6% urea for 21 days, and the treated straw was mixed with an acid-molasses solution to neutralize free ammonia and kept airtightly in a plastic bag for 24 hours. The neutralized and non-neutralized straw were dried and subjected to chemical analysis and *in vitro* dry matter (DM) digestibility determination. The *in vitro* DM digestibility as well as crude protein (CP) content were remarkably improved by neutralization. Short-cutting of the straw before treatment gave a better result than the long-cut samples. Neutralization with sulfuric acid also affected the chemical composition and increased sulfur content of samples. The CP thus fixed by neutralization was proven to be kept stable for 3 months, and *in vitro* DM digestibility was not affected by the storing period.

(**Key Words**: Urea-treated Rice Straw, Neutralization, Protein Content)

Introduction

The dry matter (DM) digestibility and crude protein (CP) content of rice straw can be improved by urea/ammonia treatment (Jayasuriya and Perera, 1982; Wanapat et al., 1982). Such urea-treated rice straw contains a high amount of free ammonia which is reduced by exposure to air. Jayasuriya and Perera (1982) reported that the CP content of urea-treated rice straw declined as the length of aeration increased. To minimize CP loss, it has been recommended that urea-treated rice straw be given to livestock soon after the stack opened.

The alkalinity of urea-treated straw can be high due to the appearance of ammonia; in fact, the pH value of 6% urea-treated straw was reported to be 9.0-9.2 (Wanapat, 1985; Pradhan, 1991). Borhami et al. (1982) reported that the CP content of ammonia-treated barley straw could be stabilized by neutrailization with organic acids. Since the price of organic acids is high, it is impractical to use such acids, especially in unindustrialized countries such as Thailand. There is interest, therefore, in determining whether low priced inorganic acid such as sulfuric acid can be used. If so, ammonium sulfate, the neutralized product, could provide sulfur to the cattle, as it has been shown to have no adverse effects on the health and milk production of dairy cattle (Sobczak et al., 1962). However, ammonium sulfate is somewhat unstable when left under humid and hot tropical conditions. Thus, if neutralized straw can be stored for the whole dry season (approximately 3 months from December to March) in the same way as hay, cattle feeding practice should benefit.

The objectives of this study were to assess whether sulfuric acid could be used to fix the free ammonia in urea-treated rice straw, and to determine the extent of changes in CP content and DM digestibility of dried neutralized urea-treated straw stored for periods of different length.

Materials and Methods

The experiment consisted of 2 experiments; the first to study the neutralization of urea-treated rice straw with sulfuric acid, and the second to investigate the effect of storage time on CP content and DM digestibility of neutralized urea-treated straw.

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Experiment 1

The rice straw used in this experiment was a non glutinous rice variety straw, and collected from paddy field near Chiang Mai, Thailand. The straw was harvested in the late of December, one week after grain separation. It was hand-chopped into 15-20 cm lengths, and mixed well. The experiment consisted of four treatments of seven replicates each; 1) Untreated rice straw, 2) Urea-treated rice straw, 3) Urea-treated and neutralized rice straw, and 4) Urea-treated and neutralized rice straw which was chopped by hand into 2-3 cm lengths.

For each treatment, 3 kg of straw (2.75 kg DM) was randomly taken from the stack and put into a plastic bag (60 × 90 cm), to which was added 3 kg of water containing 0.18 kg urea (6 % of straw). After through mixing, the bag was made air-tight with a plastic rope, and stored for 21 days under cover.

The neutralization procedure consisted of taking the treated straw from the bag after 21 days and mixing it with an acid-molasses solution (40 g concentrate sulfuric acid and 100 g molasses in 500 ml water for each 1.75 kg urea-treated straw). It was returned to the plastic bag and left for another 24 hours to complete the neutralization reaction, as indicated by no odour of ammonia. All bags of urea-treated straw, both with and without neutralization, were opened and the treated straw was sun-dried. Samples of fresh and sun-dried treated straw were detected and cut into 1 - 2 cm lengths, then checked CP content as a fresh form. The dried straw samples were subjected to chemical analysis and *in vitro* DM digestibility determination. The chemical analysis were conducted by the standard method of AOAC (1975), and the *in vitro* DM digestibility was determined by the modified NDF-cellulase enzyme method of Roughan and Holland (1977) using ONOZUKA FA (Yakult Co.). The analysis of neutral detergent fiber (NDF) and acid detergent fiber (ADF) was conducted by the method of Goering and Van Soest (1970).

CP was fractionated into water soluble and insoluble fractions, and they were measured by the following procedure; one gram of ground straw was mixed with 100 ml water and shaken for 1 hour. This mixture was also used for checking pH of straw using a portable pH meter. After filtering the residue was washed with an adequate amount of distilled water to obtain the water insoluble fraction. This fraction was treated with an acid detergent (AD) solution (Goering and Van Soest, 1970) to determine the AD insoluble fraction. The filtrate was mixed with 40% NaOH solution and ammonia content measured by distillation and subsequent titration with 0.1 N sulfuric acid (AOAC, 1975).

Experiment 2

In this experiment, rice straw chopped into 15-20 cm lengths was treated with 4, 6 or 8% urea, and part of the 6% urea-treated straw was neutralized with an acid-molasses solution. In addition, rice straw cut into 2-3 cm lengths was treated with 6% urea and neutralized. The urea treatment and neutralization procedures were the same as described for experiment 1. Samples of long straw were sun-dried until the DM reached about 90% to ensure sufficient drying, and baled by hand, while the short straw samples were put into a paper bag after drying. All bales and bags were kept in a room under shade with good air ventilation. At the start (0), and after 1, 2 and 3 months of storage, samples were randomly taken from the bales or bags, and CP content and *in vitro* DM digestibility determined as described for experiment 1.

Statistical analysis

The results of experiment 1 were subjected to analysis of variance for a completely randomized design, and those of experiment 2 by a two-way analysis of variance for a completely randomized design. Duncan's new multiple range test was applied for testing the differences between treatment means (Steel and Torrie, 1984).

Results and Discussion

Experiment 1

The chemical composition and *in vitro* DM digestibility of rice straw treated with or without urea and sulfuric acid are shown in table 1. The pH value of dried urea-treated straw ranged from 6.5 to 6.9, and were not significantly different from that (7.0) of the untreated rice straw. This indicates that ammonia produced from urea had evaporated during the drying process, and that no residual sulfuric acid remained. The molasses was not directly involved in the chemical reaction, but it might serve to cover the taste of the ammonium

TABLE 1. EFFECTS OF 6% UREA TREATMENT, NEUTRALIZATION AND CUTTING LENGTH OF RICE STRAW ON CHEMICAL COMPOSITION AND *IN VITRO* DRY MATTER DIGESTIBILITY

Cutting length (cm)	15-20			2-3	SEM
Urea treatment	None	Treated			
Neutralization	None		Neutralized		
pH	7.0	6.5	6.7	6.9	0.1
DM (%)	91.7	92.6	95.2	94.0	
DM digestibility (%)	46.3^{a}	50.8^{b}	55.9^{c}	58.6^{d}	1.0
CP content (DM %)					
Dried sample	3.01^{a}	7.56^{b}	12.27^{c}	14.56^{d}	0.30
Fresh sample	—	17.07^{b}	13.80^{a}	18.44^{b}	0.63
Sulfur content (%)	0.05^{a}	0.06^{a}	1.04^{b}	1.22^{c}	0.03
Composition, CP-free DM (%)					
Ash	17.71^{a}	19.72^{b}	16.94^{a}	20.40^{b}	0.56
EE	1.69^{b}	1.27^{a}	1.82^{b}	1.74^{b}	0.13
NFE	43.04^{b}	39.20^{a}	44.67^{b}	43.02^{b}	0.99
CF	36.45^{a}	40.13^{b}	36.38^{a}	34.23^{a}	0.72
NDF	66.84^{a}	77.69^{c}	68.63^{ab}	72.62^{b}	1.53
ADF	47.64	44.50	46.05	48.19	2.04

Within row, means with different superscripts differ significantly ($p < 0.05$).

sulfate.

The *in vitro* DM digestibility of untreated rice straw was 46.3%, and it was significantly increased to 50.8% by the 6% urea treatment. Neutralization with sulfuric acid increased DM digestibility a further 5% ($p < 0.05$), and even more by chopping the straw short prior to treatment. These results are in agreement with the report of Fahmy and Ørskov (1984), who found that when 60 g of sulfuric acid was added to 1 kg of ammonia-treated barley straw, DM digestibility was improved by 8 percentage units. The improvement of DM digestibility by neutralization might be due to the chemical reaction of cell wall components with sulfuric acid. The results of this experiment also suggest that chopping the straw shorter made sulfuric acid reaction with cell wall components more effective. The reaction of sufuric acid with ammonia was also more efficiently in chopped straw since CP was increased by 2.3 percentage units.

CP content was determined in both fresh and dried samples. In the untreated straw, CP content of the fresh sample seemed to be the same as that of the dried sample (3%), however, the fresh sample of the urea-treated straw contained 17.1% of CP but the dried one had only 7.6%. This means that volatile ammonia was escaped and its amount was equivalent to 9.5% CP, and only nitrogen equivalent to 4.6% CP was fixed by the urea treatment. The rate of ammonia fixation was calculated to be (7.56-3.01)/(17.07-3.01) = 32.4%. In the urea-treated and neutralized straw, CP content of the fresh sample was lower than that of the non-neutralized sample. This may have occurred because some volatile ammonia escaped when the urea-treated straw was taken from the bag for neutralization. In the case of neutralized straw, the CP loss by drying was only 1.5%, and nitrogen equivalent to 9% CP was fixed and remained in the straw. The rate of ammonia fixation was calculated to be (12.27-3.01)/(13.80-3.01) = 85.8%, which is almost triple that of the non-neutralized straw. If nitrogen was not lost during neutralization process, the CP fixed by neutralization would be nearly 12%, and total CP content of the dried neutralized straw would be about 15%. It is necessary, therefore, to improve the procedure for neutralization and straw preparation.

To compare other components, they were calculated on a CP-free DM basis, as the treatment with urea, and further with sulfuric acid, increased CP content of straw, and the other components would show lower values in the CP-rich samples, even if they were not changed by the treatments. Ash content was apparently increased by the urea treatment, and then decreased by the neutralization to the initial level. When the short-cut straw was used, however, ash content tended to be increased. The changes of EE and NFE seemed to be in the same category. They were reduced by the urea treatment, but recovered by the neutralization. In contrast, CF and NDF were increased by the urea treatment, and recovered to the originl values by the neutralization. ADF content was not affected by treatment, and sulfur content was not affected by urea treatment but significantly increased by neutralization with sulfuric acid. This effect was enhanced when shortly-cut straw was used.

The urea-treated straw lost 9.5% CP by drying (56% of fresh sample), but the neutralized straw lost only 1.5% (11% of fresh sample), indicating that the neutralization well fixed the ammonia (table 2). In the non-neutralized straw, 59.5% of remaining CP was water soluble, and 43% of it was presented as ammonia. Water soluble CP and ammonia CP were significantly increased by neutralization, being 73.5% in the former and 74.2% in the latter. In the water soluble fraction, the major part of ammonia CP can be assumed as ammonium sulfate. Nitrogen as ammonia was increased by the urea treatment, and markedly increased by further neutralization. Since untreated straw was considered not to contain any urea, the non-ammonium fraction of untreated straw would be water soluble protein and some other non-ammonium nitrogenous compounds. Therefore, increased non-ammonium nitrogen due to urea treatment was considered to be residual urea, and the amount was estimated to be 1.69% and 1.45% in the non-neutralized and neutralized urea-treated straw, respectively. The data showed that the amount of urea was not changed by neutralization. In the water insoluble fraction, 61-72% was AD soluble, and this part might be utilized by cattle to some extent. Therefore, although more water insoluble nitrogen appeared in the treated straw, it may have been more utilizable than the CP originally present.

TABLE 2. COMPARISON OF CRUDE PROTEIN FRACTIONS (DM%) OF 6% UREA-TREATED RICE STRAW BETWEEN WITH AND WITHOUT NEUTRALIZATION

Treatment	Untreated	Urea	Urea + neutralization	SEM
Fresh sample	–	17.07^{b}	13.80^{a}	0.30
Dried sample	3.01^{a}	7.56^{b}	12.27^{c}	0.24
Loss by drying		9.51^{a}	1.53^{b}	0.60
(% of fresh sample)	(–)	(55.8)	(11.1)	–
Water soluble	0.88^{a}	4.50^{b}	9.02^{c}	0.14
(% of dried sample)	(29.2)	(59.5)	(73.5)	–
Ammonia form	0.00	1.93^{a}	6.69^{b}	0.02
(% of water soluble)	(0)	(43.0)	(74.2)	–
Non-ammonia form	0.88^{a}	2.57^{b}	2.33^{b}	0.11
Urea	–	1.69	1.45	–
Water insoluble	2.13^{a}	3.06^{b}	3.25^{c}	0.10
AD soluble	1.30^{a}	2.04^{b}	2.34^{c}	0.06
(% of water insoluble)	(61.0)	(66.7)	(72.0)	–
AD insoluble	0.83^{a}	1.02^{b}	0.91^{a}	0.03

Within row, means with different superscripts differ significantly ($p < 0.05$).

Experiment 2

Immediately after the treated straw was dried (0 month-storage), the CP content of 4, 6 and 8 % urea-treated straw was 5.7, 7.2 and 6.7% on DM basis, respectively, whereas neutralization of the 6% urea-treated straw increased CP content to 12.3% (table 3). When the short-cut straw was used, CP content was further increased to 14.6%.

Storage period had a significant effect on the CP content of straw (table 4): CP becoming lower as storage time was lengthened. For the long-cut straw, CP content reduced until 2 months of storage, and thereafter no CP reduction occurred. In contrast, short-cut straw did not show any characteristic changes during storage. Part of the reason could be that the short-cut straw was kept in a bag. Accordingly, it should be better to feed long, dried, treated straw to cattle as early as possible after drying or preferably to keep it in a bag. The effect of urea level on CP content was significant (table 3), showing that the higher the urea level, the higher the CP content remained.

From the result obtained in this experiment, it appears that neutralization of 6% urea-treated straw causes a marked increase in crude protein content. About two-fold percentage unit of CP could be remained in the neutralized straw at all storing periods. Again reducing the cutting length was associated with a further increase in crude protein content, possibly due to keeping the short straw in a bag, instead of in a bale. There was a significant interaction between straw length and storing period (table 4), because changes in CP content were not observed during the storage of

TABLE 3. CRUDE PROTEIN CONTENT (DM%) OF SUN-DRIED UREA-TREATED RICE STRAW STORED FOR DIFFERENT PERIODS

Cutting length (cm)	15-20				2-3
Urea level (%)	4	6	8	6	6
Neutralization	None			Neutralized	
Storage period (months)					
0	5.66	7.24	6.71	12.27	14.58
1	4.58	5.33	6.34	10.76	13.86
2	3.78	4.83	5.83	9.35	14.04
3	3.76	4.85	5.55	9.12	13.05
SEM	0.23	0.21	0.32	0.29	0.34

TABLE 4. STATISTICAL ANALYSIS OF THE RESULTS SHOWN IN TABLE 3

	Urea level	Neutralization	Straw length	Storing period
Main effect	***	***	***	***
Interaction with storing period	NS	NS	***	

***: Significantly different at $p < 0.001$.
NS: Not significant, $p > 0.05$.

TABLE 5. *IN VITRO* DRY MATTER DIGESTIBILITY (%) OF SUN-DRIED 6% UREA-TREATED RICE STRAW WITH AND WITHOUT NEUTRALIZATION KEPT FOR DIFFERENT PERIODS

Cutting length (cm)	15-20		2-3	Mean
Neutralization	None	Neutralized		
Storage period (months)				
0	50.8	55.9	58.6	55.1
1	50.9	53.9	59.3	54.7
2	50.6	54.0	58.5	54.4
3	50.9	53.6	55.4	53.3
MEAN	50.8^a	54.4^b	58.0^c	(1.0)

The figure in the parentheses shows SEM.
Within row, means with different superscripts differ significantly ($p < 0.05$)

short-cut straw (table 3). Although the apparent CP content of neutralized straw was reduced to 9.1% from 12.3% during the three months of storage, the value was still higher than the freshly made non-neutralized straw (7.2%). Storing period did not show any significant effect on *in vitro* DM digestibility (table 5), however, a significant difference was found between the treatments.

It can be concluded that losses of apparent CP of urea-treated straw can be prevented by neutralizing the treated straw with an adequate amount of sulfuric acid. The neutralized straw can be dried and stored for 3 months without a large loss of CP, and the *in vitro* DM digestibility is not changed during 3 months of storage.

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