Effects of Ammonia, Urea Plus Calcium Hydroxide and Animal Urine Treatments on Chemical Composition and *In sacco* Degradability of Rice Straw

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ABSTRACT : This experiment was conducted to examine the effects on the composition and rumen degradation *in sacco* of rice straw treated with animal urine (1 l of 2.9 g N/kg DM straw) and urea plus calcium hydroxide (2% urea plus 0.5% $Ca(OH)_2$/kg DM straw) as a cheap and relatively safe alternative for ammonia (3% ammonia solution/kg DM straw). Mold occurred in urine treated straw, but other treatments were apparently mold-free. All treatments significantly ($p<0.05$) increased CP content in the straw compared with untreated one. Ammonia-treated straw contained CP at about twice that in urine or urea-calcium hydroxide treated straw. NDF and hemicellulose contents decreased significantly ($p<0.05$) in all treatments, while ADF and cellulose showed no differences compared with untreated straw. The degradable fraction of DM, CP, NDF, hemicellulose and cellulose was significantly ($p<0.05$) increased for ammonia and urea-calcium hydroxide treatments than for urine treated or untreated straw except for CP of urine treated straw. Chemical treatment of rice straw increased the readily degradable fraction of CP, while it decreased the slowly degradable fraction for urine or urea-calcium hydroxide treated rice straw. The degradation rate of hemicellulose was significantly ($p<0.05$) increased for ammonia and urea-calcium hydroxide treatments compared to urine treated or untreated straw. However, no effect on cellulose degradation rate was found by any of the treatments. There was no improvement in the degradation kinetics caused by the urine treatment despite the improvement of the chemical composition. Although the improvement in rumen degradability was less in the urea-calcium hydroxide treatment than in the ammonia treatment, its use may be more desirable because it is less expensive to obtain, less hazardous nature, and readily available. For further improvement it is necessary to investigate the supplementation of slowly degradable nitrogen to urea-calcium hydroxide treated rice straw diet. ***(Asian-Aust. J. Anim. Sci. 2003. Vol 16, No. 3 : 368-373)***

Key Words : Chemical Treatment, Rice Straw, Ruminal Degradation

INTRODUCTION

The majority of the developing countries' ruminants depend on by-products of agriculture or graze forages of relatively poor nutritional value (Leng, 1993). To enhance the utilization of crop residues, various treatment methods have been developed during the past century. Sodium hydroxide (NaOH) has proved to be the most efficient in improving the feeding value of roughages among the many alkalis evaluated. Despite the dramatic improvement in the utilization of NaOH-treated roughage, NaOH is less attractive than before due to the unavailability in developing countries as well as its hazardous nature for farmers and the environment. Another widely used method of alkali treatment is ammoniation, which uses ammonia gas, ammonium hydroxide, urea, or urine as a source of ammonia. Males (1987) stated that the average improvement in in vitro dry matter digestibility (IVDMD) of NaOH-treated straws is 16% higher than that of ammonia-treated straws. Even though the ammoniation has generated considerable interest because of its advantage of adding nitrogen to the treated roughages and its safety compared with NaOH. For developing countries the use of urea as a source of ammonia seems to be a more practical method. However, the cost of the most practiced dose, 4 to 5%, for the treatment is unbearable for the majority of farmers, particularly in remote areas of Asia and Africa. Animal urine, as a source of ammonia, is an old but not often adopted course due to many constraints, mainly urine collection, storage, and handling. Although calcium hydroxide is comparatively cheap and effective, the presence of mold is the main problem. However, ammonia produced during the ammoniation act as a fungicide.

This study examined the effect of animal urine and the synergistic effect of urea plus calcium hydroxide as a cheap and relatively safe alternative to ammonia solution, on the nutritive value of rice straw.

MTERIALS AND METHODS

Rice straw treatment

One kg of chopped rice straw (about 3 cm) patches were placed in polyethylene sacks. Four replicate were used per treatment.

The treatments were: untreated rice straw served as control (Untreated Straw, US), rice straw treated with 1 l of livestock urine (2.9 mg N/ml) collected from cattle (Livestock Urine Treated Straw, LUS), rice straw treated

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with 2% urea plus 0.5% $Ca(OH)_2$ dissolved in 1 l of water (UCS), and rice straw injected with 3% ammonia solution (Ammonium Hydroxide Treated Straw, AHS).

The LUS and UCS were thoroughly mixed and the air was removed from each sack using a vacuum cleaner. For AHS, the air was removed before the injection of the ammonia solution, using a perforated pipe to avoid the loss of ammonia. The sacks were then tightly secured with a rope and incubated for seven weeks enclosed in the shade with an average temperature of 30°C.

Animals, housing and feeding

Four rumen cannulated Suffolk sheep with a mean body weight of 64 kg were used in this experiment. The animals were housed individually in a metabolic crate. Oat hay was offered twice daily at 08:00 h and 16:00 h for *ad libitum* intake, with free access to water and mineralized salt licks.

***In situ* degradation and chemical analysis**

The *in situ* degradability of the untreated and treated rice straw was conducted using the nylon bag technique as described by Ørskov et al. (1980). The untreated and treated samples were air-dried and ground through a 2 mm sieve before rumen incubation. Duplicate samples of about 5 g air dried test straw were weighed into nylon bags (bag size 80×140 mm; pore size 45 μm) which were then suspended in the rumen of each sheep for 4, 8, 16, 24, 48, 72 and 96 h. After removal from the rumen, the bags were dipped into cold water to stop the microbial activity, then washed with tap water to remove the rumen matter from outside the bags, and then stored to be frozen at -30°C (Kamel et al., 1995). After thawing, the bags were cleaned by rubbing and rinsed under running water for about 25 min. Sample of 0 h, representing water-soluble fraction, were prepared by washing bags, in triplicate, containing test samples for 25 min. The residues were dried to a constant weight at 60°C, ground through 1 mm sieve, and analyzed together with un-incubated samples for a proximate composition according to AOAC (1984). Neutral detergent fiber (NDF), acid detergent fiber (ADF), and acid detergent lignin (ADL) were analyzed according to Georing and Van Soest (1970).

Calculation and statistical analysis

The results from *in situ* studies were fit into the exponential model $\{P=a+b(1-e^{-ct})\}$ of Ørskov and McDonald (1979) to determine the degradation characteristics of the incubated samples. Analysis of variance (ANOVA) was conducted using a general linear model (GLM) of SAS (1993). The model included the sheep and treatment. Mean comparison was carried out by the Tukey's test (Steel and Torrie, 1980).

RESULTS

General appearance

Products of AHS and UCS showed a dark brown color with a strong ammonia smell and that of LUS showed a yellow color with a weak ammonia smell. The mold grew on the outer layer of LUS, which caused a slight loss of the product because the portion covered with mold was discarded. The appearance of LUS was almost unchanged from that at the initial packing time except for the light mold grown on the surface.

Compositional changes

Table 1 shows the contents of crude protein (CP) and fibrous fractions in US, LUS, UCS, and AHS. All treatments significantly ($p<0.05$) increased CP contents in straw compared with US. AHS contained CP at about twice that as LUS or UCS, which showed the same CP level. Nevertheless, when the calculation was made on the increment of the nitrogen content in LUS, UCS, and AHS, a fair amount of nitrogen was lost from UCS and AHS compared with the additional nitrogen supplied from the chemical. Almost all the nitrogen supplied by urine, however, was preserved in LUS. The NDF and hemicellulose contents decreased significantly in LUS, UCS, and AHS compared with US ($p<0.05$), while ADF and cellulose showed no difference among US, LUS, UCS, and AHS. The decrease in content of NDF and hemicellulose for LUS was comparable to those for UCS or AHS.

***In Sacco* nutrients disappearance**

Figure 1 shows the disappearance of CP from US, LUS, UCS, and AHS incubated in the rumen of sheep. A fair amount of CP was disappeared immediately after incubation in the rumen for US, LUS, UCS, and AHS. Among them, more than 50% of the CP for LUS, UCS, and AHS were considered as a readily degradable nitrogen fraction compared to 30% for US. The changes of disappearance patterns, however, showed that the CP degraded in the course of incubation and tended to be smaller than that

Table 1. Chemical composition of treated and untreated rice straw (g/kg DM)

	OM	CP	NDF	ADF	ADL	Hemi-cellulose	Cellulose
US	864	37[c]	772[a]	542	47	229[a]	496
LUS	845	54[b]	731[c]	541	49	189[b]	492
UCS	856	53[b]	747[b]	559	47	189[b]	512
AHS	854	94[a]	728[c]	545	42	183[b]	503
SEM	2	1	4	9	1	7	8

US=Untreated straw; LUS=Urine treated straw; UCS=Urea plus calci hydroxide treated straw; AHS=Ammonia solution treated straw.
SEM=Standard error of mean; Means with different superscripts in the same column were significantly different ($p<0.05$).

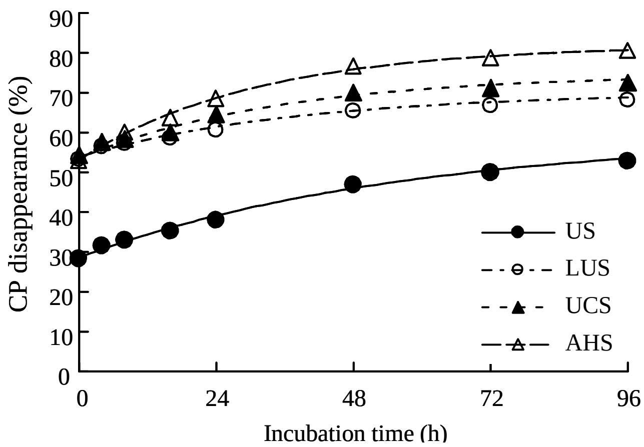

Figure 1. Disappearance of CP from treated and untreated rice straw during rumen incubation for different times in sheep.
* See the text for abbrevation notation

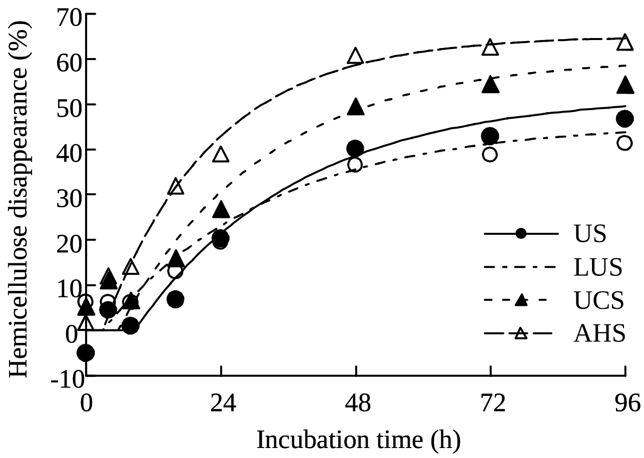

Figure 2. Disappearance of hemicellulose from treated and untreated rice straw during rumen incubation for different times in sheep * See the text for abbrevation notation

immediately degraded after incubation. The slope of curvilinear change in the course of incubation showed a slower disappearance of the nitrogen even in a 24 hr period.

The disappearance patterns of hemicellulose were presented in Figure 2. US showed a longer time for the initiation of the degradation compared with LUS, UCS, and AHS. The degradation rate of hemicellulose tended to be greater in the first 24 h than that in the latter period of incubation for all the straw. Among them, the rates for UCS and AHS tended to be greater than for US and LUS. The degradation patterns of cellulose, however, were similar between US and LUS, but a slightly higher for UCS or AHS, with the tendency for being slightly higher in AHS, as shown in Figure 3. The rates of cellulose degradation tended to be greater in the first 48 h than in the latter period of incubation for all the straw and were a little higher for UCS and AHS than with US and LUS.

Table 2 shows the *in sacco* degradation characteristics for US, LUS, UCS, and AHS. The degradable fraction together with the slowly degradable fraction of DM were significantly increased for UCS and AHS compared with US or LUS ($p<0.05$). The increased degradable fraction for UCS and AHS was also observed in NDF, hemicellulose, and cellulose fractions ($p<0.05$). The degradable fraction for LUS was similar to that for US in DM, NDF, and cellulose. The rates of degradation of NDF and cellulose did not show a significant difference among the treatments. The degradation rate of hemicellulose, however, was significantly ($p<0.05$) increased for UCS and AHS compared with LUS or US. There was no difference in lag time (LT) for cellulose among the treatments. The degradation of hemicellulose for LUS and AHS, however, began significantly ($p<0.05$) faster than that for US, and that for UCS tended to be faster than US. The LT of NDF for LUS tended to be smaller than for UCS and US. The AHS had a significantly ($p<0.05$) shorter LT of NDF than US and UCS without a significant difference with LUS.

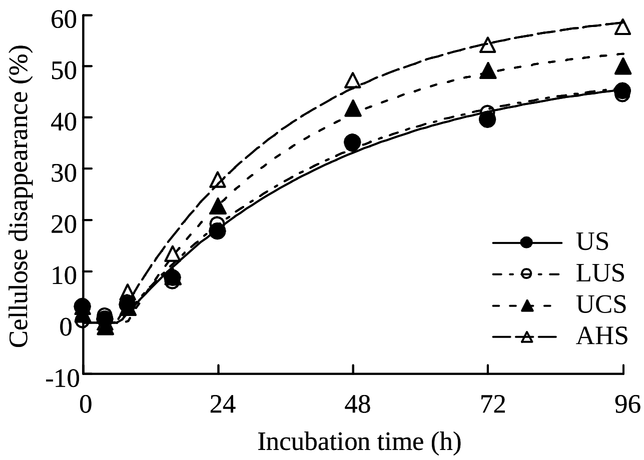

Figure 3. Disappearance of cellulose from treated and untreated rice straw during rumen incubation for different times in sheep "See the text for abbrevation notation"

The degradable fraction of CP was significantly ($p<0.05$) increased for LUS, UCS, and AHS compared with US. The degradable CP was more than 700 g/kg CP for LUS, UCS, and AHS and about 600 g/kg CP for US. The treated rice straw also showed a significant ($p<0.05$) increase in the readily degradable fraction of CP being more than 500 g/kg CP compared with the untreated straw being about 300 g/kg CP. While the slowly degradable fraction was significantly ($p<0.05$) decreased for LUS and UCS compared with US, except for AHS. The rate of degradation of the CP was significantly ($p<0.05$) greater for LUS, UCS, and AHS compared with US.

Table 2. Degradation characteristics of treated and untreated rice straw (g/kg)

		US	LUS	UCS	AHS	SEM
DM	D	567[c]	572[c]	628[b]	683[a]	12
	RD	120[b]	155[a]	116[b]	147[a]	9
	SD	447[a]	417[a]	512[b]	536[b]	12
	k/h	0.024	0.03	0.03	0.033	0
	ED (0.03)	319[d]	349[c]	372[b]	428[a]	11
CP	D	592[d]	701[c]	747[b]	820[a]	9
	RD	286[b]	540[a]	536[a]	531[a]	6
	SD	306[a]	161[b]	211[b]	289[a]	11
	k/h	0.018[b]	0.03[a]	0.03[a]	0.032[a]	0
	ED (0.03)	401[d]	616[b]	640[b]	680[a]	10
NDF	D	491[b]	467[b]	555[a]	612[a]	15
	k/h	0.028	0.03	0.03	0.036	0
	LT	6.9[b]	5.1[ab]	6.7[b]	3.7[a]	0.5
	ED (0.03)	237[b]	230[b]	295[a]	334[a]	15
Hemi-cellulose	D	521[b]	459[c]	604[a]	651[a]	27
	k/h	0.033[c]	0.03[c]	0.04[b]	0.051[a]	0
	LT	8.6[b]	2.9[a]	5.7[ab]	2.9[a]	1.2
	ED (0.03)	273[c]	237[d]	345[b]	410[a]	27
cellulose	D	505[c]	502[c]	555[b]	621[a]	15
	k/h	0.028	0.03	0.03	0.032	0
	LT	6.5	6.3	7.8	5.9	0.5
	ED (0.03)	244[b]	238[b]	291[a]	321[a]	16

D=Degradable fractions; RD=Readily degradable fractions; SD=Slowly degradable fractions; k=Degradation rate; LT=Lag time; ED=Effective degradability.

US=Untreated straw; LUS=Urine treated straw; UCS= Urea plus calcium hydroxide treated straw; AHS=Ammonia solution treated straw.

SEM=Standard error of mean; Means with different superscripts in the same row were significantly different ($p<0.05$).

DISCUSSION

The appearance of mold on the surface of the straw was not observed in UCS and AHS treatments. A strong ammonia smell suggested a higher ammonia concentration in UCS and AHS, which may have prevented the growth of mold in UCS and AHS by acting as a fungicide. The ammonia generated in LUS, however, may not be enough to prevent the growth of mold as observed by Saadullah et al. (1980). An increased in CP concentration for LUS, UCS, and AHS was contributed by the addition of nitrogenous substances as reported by Hadjipanayiotou et al. (1993), Hasan et al. (1993), and Sirohi and Rai (1999).

The hemicellulose content for LUS, UCS, and AHS was significantly lower than US, which contributed to a significantly lower NDF for LUS, UCS, and AHS than US. The treatment of nitrogenous substances such as urine, urea, and ammonia may enhance the partial solubility of hemicellulose as described by Buettner et al. (1982) and Chaudhry (1998). The results of the present study show that the urine containing 2.9 mg N/mlis as effective as ammonia solution or urea treatment for the solubilization of hemicellulose in the rice straw, resulting in the reduction of NDF content. The same trend was observed by Saadullah et al. (1980, 1981) when urea or urine was used as a source of ammonia.

The CP for LUS, UCS, and AHS was highly degradable, but the proportion of readily degradable fraction increased for all the treatments. The disappearance of CP was greater in LUS, UCS, and AHS than US at the beginning of ruminal incubation (Figure 1). In addition, the slowly degradable fraction for LUS and UCS was significantly lower than US, although the rate of degradation was faster for LUS, UCS, and AHS than US. The nitrogen supplemented, therefore, is considered to be a readily degradable fraction, although the slowly degradable fraction is degraded faster for LUS, UCS, and AHS than US.

The degradable fractions of DM, NDF, hemicellulose, and cellulose were significantly increased for UCS and AHS compared with LUS or US. This increase may have been partially caused by an increased degradation of CP for the treated rice straw. The degradation rate of the fibrous fraction tended to be greater in the first 24 and 48 h than that in the later period (Figures 2 and 3), which may have resulted from high amount of nitrogen supply immediately after incubation. A greater nitrogen supply at the initial stage of incubation is also supported by an increase in the readily degradable fraction of CP for LUS, UCS, and AHS compared with US.

An increased degradable fraction of DM may be contributed by an increase in the slowly degradable fraction of DM. Mason et al. (1990) suggested that the increase in the cell wall degradability after ammoniation could have resulted from the effect of the treatment on lignin itself or its linkage. Wuliji and McManus (1988) and Grenet and Barry (1990), using electronic microscopy, have observed an increased susceptibility of alkaline treated-roughage to rumen microorganisms. An increased slowly degradable fraction of DM, therefore, may have been attributed to an increased degradability of structural carbohydrates such as hemicellulose and cellulose for UCS and AHS compared with LUS and US. This is comparable to the 6.6 units increases reported by Orden et al. (2000). The increased degradable fractions of hemicellulose and cellulose may have resulted in the increased degradable fraction of NDF for UCS and AHS. Thus, the synergetic effect of urea and calcium hydroxide treatment appears to be as effective as ammonia treatment for the improvement of the degradability of the fibrous fraction in the rice straw.

The disappearance pattern, however, showed a slightly lower disappearance for UCS than for AHS, both in hemicellulose and cellulose (Figures 2 and 3). The reduction in the slowly degradable fraction of CP for UCS may have resulted in this difference in degradation of the fibrous fraction between UCS and AHS because of the shortage of nitrogen supply to the rumen microbes.

Furthermore, even though the alkaline treatment with the rice straw may have increased the susceptibility to microorganisms by the effect on the lignin linkages, the LT of NDF was not shortened for UCS but AHS. This difference may be attributed to a lower CP content for UCS compared to AHS, even though the readily degradable fraction of CP was similar for both UCS and AHS.

Urine treatment improved the chemical composition compared with the untreated straw, but the improvement was not extended to the degradation kinetics except for CP. The unrinary nitrogen level of 2.9 mg/ml may have failed to generate enough ammonia affecting the structural carbohydrate in straw. This may be supported by the lower intensity of ammonia smell at the opening of the package and the failure to prevent mold growth on the surface of the straw. Although the urine pH of herbivores is generally alkaline, the urine collected from cattle in the present study may not have satisfied the nitrogen supply for affecting the structure of the rice straw. In addition, under ruminant production by using grazing systems, it may encounter a great difficulty for collecting urine from an individual animal.

It has been well documented that ammonia treatment of forage improves its digestibility and its nutritive value (Buettner et al., 1982; Orden et al., 2000). The improvement of DM degradation for UCS in the present study is comparable to what was reported for 3% urea-treated wheat straw (Sirohi and Rai, 1999) and for 4% calcium hydroxide treated straw (Pradhan et al., 1997). The results of the present study show that ammonia treatment is promising for the improvement of low quality forage such as rice straw. Its treatment has, however, some drawbacks as described earlier, that is, its cost for treatment and consideration on safety. On the other hand, the treatment with urea together with calcium hydroxide represented a comparable result on the improvement of the degradation of the fibrous fraction in the in sacco experiment, although the slowly degradable fraction of CP decreased compared with ammonia treatment, and the LT for NDF was not improved. The decrease in the slowly degradable fraction of CP may have partially caused a slightly lower degradation of the fibrous fraction for UCS compared with AHS. It is, therefore, speculated that the supplementation with slowly degradable fraction of CP to UCS may enhance the growth of rumen microbes, which in turn may expect the release of more energy from the fibrous fraction.

In conclusion, despite of the improvements in chemical composition, there would be little advantage for the use of ruminant urine as a method of treatment for rice straw. Ammonia treatment is promising when its drawbacks have been overcome. The improvement in rumen degradability was somewhat smaller in the urea-calcium hydroxide treatment than with the ammonia treatment. The advantages may give additional values for the treatment of low quality forage such as rice straw by urea and calcium hydroxide because of availability, less expensive to obtain and less hazardous nature. For further improvement, however, there is a need for the investigation on the supplementation of slowly degradable nitrogen to a rice straw diet treated with urea and calcium hydroxide.

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