

Assessment of Urea and/or Lime Treatment on Rice Straw Quality Using *in vitro* Gas Fermentation Technique

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Abstract: An experiment was conducted to investigate the effectiveness of urea (NH₂)₂CO and calcium hydroxide [Ca(OH)₂] treatment of straw using a 4×4 factorial arrangement in a completely randomized design. Chopped rice straw was treated with mixtures of urea (0-3 g kg⁻¹ dry matter) and calcium hydroxide [Ca(OH)₂] (0, 0.5, 1 and 1.5 g kg⁻¹ dry matter) by dissolving in 100 mL water g⁻¹ straw and ensiled in a plastic box at room temperature for 14 days. Ensiled rice straws were examined for chemical composition and in *in vitro* gas production. Rumen fluid was collected from two ruminally fistulated native crossbred beef cattle with an average body weight of 230 kg. During the incubations, gas production was recorded at 1, 2, 4, 6, 8, 10, 12, 18, 24, 30, 36, 42, 48, 54, 60, 72 and 96 h after incubation. All gas production volume collected were linearly increased as fermentation time interval proceeded from 0-96 h after incubation. Gas volume from insoluble fraction were significantly altered (p<0.05) by urea level as fermentation time increased while there were no effects by lime treatment. Ammonia nitrogen (NH₃-N) concentration were increased when increasing urea level. The highest NH₃-N was found in 3% urea-treatment (p<0.05) while there were no significant differences in calcium hydroxide treatments under *in vitro* gas production technique.

Key words: Ammonia nitrogen, calcium hydroxide, fermentation, *in vitro* gas, rice straw, urea

INTRODUCTION

Rice straw is the main crop-residue which farmers usually store for use as ruminant feed in tropical areas. However, rice straw is low in nutritive value because of low level of protein (2-5% DM), high fiber and lignin content (70.2% NDF), low DM digestibility (46%) thus resulting in low voluntary feed intake (1.5-2.0%) (Wanapat *et al.*, 1985). Increased utilisation of rice straw as ruminant feed has been of growing interest with efforts devoted towards obtaining most of the potential feeding value of this abundant byproduct. Many chemicals have been screened in laboratory experiments for potential to enhance digestibility (Klopferstein, 1978). Urea treatment is a conventional technique for improving the quality of rice straw, especially increasing the nitrogen content (Sundstol *et al.*, 1979; Wanapat *et al.*, 1985; Zaman and Owen, 1990; Shena *et al.*, 1998). Urea-treated rice straw could increase overall intake, digestibility thus enhanced the performance of ruminant as compared to untreated

rice straw (Wanapat *et al.*, 1985, 1986; Chowdhury and Huque, 1996; Man and Wiktorsson, 2001; Trach *et al.*, 2001b, c). According to Hart and Wanapat (1992) who carried out the experiment to compare effect of urea-ammonia treatment (5%, w/w) of rice straw and untreated rice straw, it was found that there was 46% increase in intake of digestible Organic Matter (OM) in the urea treated rice straw and the digestibility. Although, urea-treated (5%) rice straw has been used as roughage during the dry season but the cost was relatively high due to increasing price of urea (Preston, 1995). Trach *et al.* (2001a) further suggested that when amount of urea was reduced and combined with calcium hydroxide Ca(OH)₂, it could improve rumen degradability and no effect on cellulose degradation rate between ammonia-treated and urea plus calcium hydroxide (Elseid *et al.*, 2003). Currently, combined urea (2.2%) and Ca(OH)₂ (2.2%) has been investigated in lactating dairy cows and resulted in good milk yield and milk composition (Wanapat *et al.*, 2009). Furthermore, urea plus calcium hydroxide is more

desirable because it is less expensive, easy to obtain, no harmful problems to animals or environment. It can also provide as a calcium supplement to the animals (Elseed *et al.*, 2003; Wanapat *et al.*, 2009). Therefore, the objective of this experiment was to determine effects of various chemical-treated rice straw on rice straw quality using *in vitro* gas fermentation technique.

MATERIALS AND METHODS

Treatment preparation and chemical analysis: Rice straw was chopped into about 5 cm length then 500 g was taken to treat with 0-3 (w/w) of urea and 0, 0.5, 1, 1.5 (w/w) of lime, respectively then ensiled in plastic boxes at room temperature for 14 days. The samples were collected and dried by hot air oven at 60°C then ground to pass a 1 mm sieve and for chemical analysis for Dry Mater (DM), Crude Protein (CP), ash according to AOAC (1990); Neutral Detergent Fiber (NDF), Acid Detergent Fiber (ADF), Acid Detergent Lignin (ADL) according to Van Soest *et al.* (1991).

Gas production technique and measurement: The experimental design was a 4×4 factorial arrangement in a Completely Randomized Design (CRD) with triplicates per treatment. Two rumen-fistulated beef cattle crossbreds with average body weight of 230 kg were used as rumen fluid donors. Animals were fed with roughage and concentrate ratio at 60:40 (16.0% CP and 2.6 ME on DM basis). *In vitro* gas production was determined according to Menke *et al.* (1979).

Artificial saliva was prepared and rumen fluid was mixed in a 2:1 ratio to prepare as fermentation solution. The serum bottles with the mixture of substrate treatments were pre-warmed in a hot air oven at 39°C for 1 h before filling with 30 mL of rumen inoculum mixture. During the incubation, the gas production was recorded at 1, 2, 4, 6, 8, 10, 12, 18, 24, 30, 36, 42, 48, 54, 60, 72 and 96 h after inoculations. Sample was used for ammonia nitrogen (NH₃-N) analysis with 5 mL of 1 M H₂SO₄ added to 50 mL of rumen fluid.

The mixture was centrifuged at 16,000×g for 15 min and the supernatant was stored at -20°C before NH₃-N analysis using the Micro-Kjeldahl methods (AOAC, 1990) and Volatile Fatty Acids (VFA) analysis using HPLC (instruments: Controller Water Model 600 E; Water Model 484 UV detector; Novapak C₁₈ column; column size 4×150 mm; mobile phase 10 mmol L⁻¹ H₂PO₄ (pH 2.5)) (Samuel *et al.*, 1997).

Data collection and analysis: During the incubation, the released gas volumes were recorded after 1, 2, 4, 6, 8, 10,

12, 18, 24, 30, 36, 42, 48, 54, 60, 72 and 96 h incubation. Cumulative gas production data were fitted to the model according to Orskov and McDonald (1979) as follows:

$$y = a+b(1-\exp^{-cy})$$

All data obtained from the study were subjected to the analysis of variance of statistical analysis system according the General Linear model of SAS Version 6.12 (SAS, 1998) by 4×4 factorial arrangements in a Completely randomized design. Multiple comparisons among treatment means were performed by Duncan's New Multiple Range Test (DMRT) and orthogonal polynomials (Steel and Torrie, 1980).

RESULTS AND DISCUSSION

Chemical composition and Gas characteristic: The chemical composition of urea and/or lime treatment on rice straw is shown in Table 1. Crude Protein (CP) content of rice straw was increased due to urea addition, especially by 3% urea treatment and CP ranged from 3.8-9.3%, respectively.

According to Wanapat *et al.* (1985) who reported that rice straw treated with 3% urea resulted in increased crude protein to 11.9%. Moreover, Van Soest (2006) reported that ammonia, urea and urine treatment of straw influenced on fiber and lignin fractions, with small decrease in NDF (2-4%) and increase in ADF (3% and lignin 20-50%). The effect was due to urea which was able to cleave lignin to carbohydrate ester bonds.

The fermentation gas parameter characteristics are shown in Table 2. It was found that gas fermentation of soluble fractions ranged from -1.02 to 4.69 mL and the gas fermentation of the insoluble fraction and ranged from

Table 1: Chemical composition of treated rice straw for all treatments

Treatments	DM (%)	Ash	OM	CP	NDF	ADF	ADL
T1 (U0 : L0.0)	90.1	13.1	86.9	3.8	77.1	59.0	12.6
T2 (U0 : L0.5)	49.8	12.9	87.1	4.3	76.4	56.3	13.6
T3 (U0 : L1.0)	46.5	13.8	86.2	3.6	76.1	55.0	13.8
T4 (U0 : L1.5)	48.6	13.7	86.3	4.3	75.4	53.1	12.0
T5 (U1 : L0.0)	36.1	13.2	86.8	5.4	75.9	53.5	12.2
T6 (U1 : L0.5)	43.3	12.6	87.4	5.2	76.6	54.3	12.4
T7 (U1 : L1.0)	45.5	13.4	86.6	5.2	77.3	52.4	11.9
T8 (U1 : L1.5)	45.4	13.2	86.9	5.3	77.0	53.1	12.8
T9 (U2 : L0.0)	50.0	12.7	87.4	7.6	77.7	52.9	11.9
T10 (U2 : L0.5)	46.4	12.6	87.4	7.3	77.5	55.1	11.7
T11 (U2 : L1.0)	46.3	12.9	87.1	7.4	77.1	51.5	11.4
T12 (U2 : L1.5)	45.6	13.2	86.8	8.1	77.4	53.7	11.7
T13 (U3 : L0.0)	48.5	11.9	88.1	8.8	74.2	52.2	10.8
T14 (U3 : L0.5)	43.3	12.6	87.4	9.3	77.4	53.5	11.9
T15 (U3 : L1.0)	47.7	12.9	87.1	8.5	73.8	53.3	15.9
T16 (U3 : L1.5)	40.2	12.8	87.2	9.3	75.4	52.4	11.8

U = Urea, L = Lime, DM = Dry Matter, OM = Organic Matter, CP = Crude Protein, NDF = Neutral Detergent Fiber, ADF = Acid Detergent Fiber, ADL = Acid Detergent Lignin

Table 2: Effect of urea and/or lime treatment of rice straw on gas production characteristic *in vitro* digestibility and gas volume after 96 h incubation for all treatments

Treatment	Gas kinetics			IVDMD at 48 h	IVOMD at 48 h	Gas (96 h) mL/0.2 g DM substrate
	a	b	c			
T1 (U 0:L 0)	-3.17	67.01	0.010	49.76	62.35	39.33
T2 (U 0:L 0.5)	-1.02	64.40	0.018	66.72	75.79	49.68
T3 (U 0:L 1.0)	-3.26	60.26	0.016	52.18	60.25	42.16
T4 (U 0:L 1.5)	-2.19	62.27	0.014	50.13	64.27	42.07
T5 (U 1:L 0)	-2.79	66.02	0.017	62.31	73.65	47.15
T6 (U 1:L 0.5)	-3.45	63.76	0.011	54.45	68.08	37.03
T7 (U 1:L 1.0)	-2.07	65.09	0.018	58.40	73.21	49.30
T8 (U 1:L 1.5)	-4.19	79.29	0.018	63.57	72.58	58.81
T9 (U 2:L 0)	-4.69	77.55	0.015	57.02	69.53	52.84
T10 (U 2:L 0.5)	-3.68	83.01	0.014	64.08	74.04	55.03
T11 (U 2:L 1.0)	-4.33	77.66	0.014	55.41	68.27	52.53
T12 (U 2:L 1.5)	-2.17	71.49	0.014	58.31	70.74	49.01
T13 (U 3:L 0)	-2.33	79.47	0.014	58.70	72.18	54.30
T14 (U 3:L 0.5)	-1.22	79.50	0.012	58.66	70.87	51.61
T15 (U 3:L 1.0)	-2.51	78.53	0.013	54.29	67.36	51.74
T16 (U 3:L 1.5)	-3.44	81.98	0.013	68.23	77.53	52.26
SEM	0.31	2.02	0.001	1.46	1.32	2.61
Comparison						
Urea	*	**	NS	NS	**	NS
Lime	NS	NS	NS	NS	NS	NS
Interaction	*	NS	NS	*	NS	NS

a = The gas production from the immediately soluble fraction (mL), b = The gas production from the insoluble fraction (mL), c = The gas production rate constant for the insoluble fraction (mL h⁻¹), *p<0.05, **p< 0.01, NS = Non-Significant, SEM = Standard Error of the Mean

60.26-83.21 mL. However, the gas fermentation of the insoluble fraction increased when level of urea increased (p<0.05). According to Van Soest (2006) who pointed out that ammonia and urea can disrupt the silicified cuticular barrier in leaves. The rise in digestibility likely results from this effect as well as cleavage of some lignin-carbohydrate bonds. Whereas, the rate of gas production ranged from 0.010-0.018 mL h⁻¹ (p>0.05) which was lower than that reported by Khejornsart and Wanapat (2010).

Cumulative gas production for each of the substrate treatments were presented as gas production curves and are shown in Fig. 1. The gas volumes showed increases as fermentation time interval proceeded from 0-96 h after incubation. While there were no influences by lime treatment (p>0.05) under this study, this could be due to a lower dissociation constant for calcium hydroxide treatment and a longer reaction period for complete effectiveness may be required (Rounds *et al.*, 1976). Under this recent study, a comparison of cumulative gas production at 96 h showed no significant differences among treatments (p>0.05). However, cumulative gas production tended to be increased when level of urea was increased. According to Wanapat *et al.* (2009) who reported that ammonium hydroxide (NH₄OH) formed in urea treated rice straw influenced ruminal pH and affected by swelling the hemicelluloses-lignin complex of rice straw hence, digestion of DM was increased from 46-55%.

Ammonia nitrogen (NH₃-N), Volatile Fatty Acids (VFA) concentration and *in vitro* digestibility: Ruminal ammonia

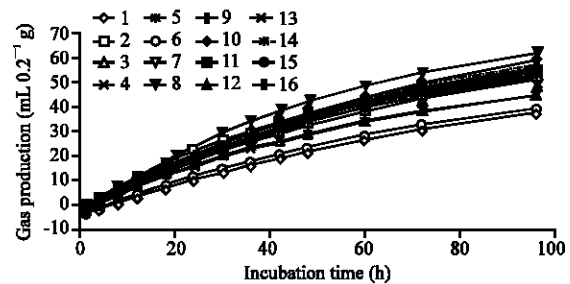


Fig. 1: Effect of urea and/or lime treatment of rice straw on cumulative gas production at different time of incubation

nitrogen and volatile fatty acid concentrations are shown in Table 3. The rumen ammonia nitrogen varied from 6.4-7.6 mg/100 mL and was increased when the level of urea supplementation increased (p<0.05). However, Satter and Slyter (1974) reported that 5 mg% ruminal ammonia nitrogen was optimum for microbial fermentation in mixed culture in a closed system. Under this study, gas production was subsequently increased by urea-treatment and it could be due to the effect of ammonia nitrogen formed from urea as an alkaline affecting on fibrous fractions of rice straw (Wanapat, 1995; Wanapat *et al.*, 1996). Based on this study, ammonia nitrogen concentration was highest in urea 3% treatment and agreed with Khejornsart and Wanapat (2010) who reported that higher nitrogen content from 3% urea treatment and 2% urea-lime treatment contributed to

Table 3: The effect of urea and/or lime treatment of rice straw on *in vitro* VFA (mmol L⁻¹), NH₃-N (mg/100 mL) and DM disappearance characteristics for all treatments

Treatments	VFAs (mmol L ⁻¹)			NH ₃ -N (mg dL ⁻¹)
	C2	C3	C2:C3	
T1 (U 0:L 0)	17.70	5.43	3.10	6.65
T2 (U 0:L 0.5)	17.46	5.77	3.06	6.85
T3 (U 0:L 1.0)	14.20	5.30	3.48	6.40
T4 (U 0:L 1.5)	19.53	5.77	3.44	6.75
T5 (U 1:L 0)	17.26	5.70	3.07	7.60
T6 (U 1:L 0.5)	18.05	5.64	3.14	6.80
T7 (U 1:L 1.0)	18.58	5.77	3.21	7.00
T8 (U 1:L 1.5)	18.41	5.73	3.18	7.60
T9 (U 2:L 0)	18.23	5.62	3.19	6.85
T10 (U 2:L 0.5)	16.67	5.50	3.06	6.45
T11 (U 2:L 1.0)	17.68	5.51	3.19	7.60
T12 (U 2:L 1.5)	17.22	5.38	3.25	6.65
T13 (U 3:L 0)	17.94	5.40	3.30	7.55
T14 (U 3:L 0.5)	17.03	5.79	3.01	7.10
T15 (U 3:L 1.0)	17.87	5.59	3.25	7.20
T16 (U 3:L 1.5)	15.51	5.32	2.91	7.20
SEM	0.40	0.10	0.11	0.14
Comparison				
Urea	NS	NS	NS	*
Lime	NS	NS	NS	NS
Interaction	NS	NS	NS	NS

*p<0.05, NS = Non-Significant, SEM= Standard Error of the Mean

the increased in ammonia nitrogen concentration in the culture media. As reported previously (Sriskandarajah and Kellaway, 1984; Haddad *et al.*, 1998), ruminal ammonia concentration was not significantly different between wheat straw treated with 3% sodium hydroxide plus 2% calcium hydroxide [Ca(OH)₂] and untreated wheat straw. Under this study, ruminal volatile fatty acid is shown in Table 3. It was found that acetate, propionate concentrations and acetate to propionate ratio were not significantly different among treatments (p>0.05), this result was similar to the research of Khejornsart and Wanapat (2010) who found the acetate to propionate ratio of untreated rice straw and NaOH treated, urea or lime treated straw ranged from 3.4-4.4, respectively.

The *In vitro* Dry Matter Digestibility (IVDMD) and *In vitro* Organic Matter Digestibility (IVOMD) at 48 h after incubation ranged from 49.8-68.2 and 62.3-77.5%, respectively (Table 2). This result was similar with Wanapat *et al.* (2009) who found that dry matter and organic matter digestibility of untreated rice straw in *in vivo* trial were 49.5 and 53.3%, respectively.

CONCLUSION

Based on this study, it could be concluded that 3% urea treatment could be used as a treatment to improve the quality of rice straw in order to enhance its rumen degradability and utilization in the ruminants. Moreover, the cost of the treated rice straw was dramatically reduced from using 5% urea-treatment. However further researches

are required using *in vivo* feeding trials in productive ruminants to investigate together with particle size on its practicality and economics.

ACKNOWLEDGEMENTS

The researchers would like to express their most sincere gratitude and appreciation to The Norwegian Programme for Development Research and Education (NUFU) project; The Tropical Feed Resources Research and Development Center (TROFREC), Department of Animal Science, Faculty of Agriculture, Khon Kaen University, Thailand; Department of Livestock and Fisheries Science, Faculty of Agriculture, National University of Laos, Lao PDR., for all of their financial and the research facilities support.

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