Incremental ratio of urea: Urease in alkaline hydrolysis of dried kume grass (*Sorghum plumosum* var. Timorens) improves its nutritive values

T O D Dato and M L Mullik*

Faculty of Animal Sciences, Nusa Cendana University, Jl. Adisucipto, Kupang NTT 85001, Indonesia

*martin_kpg@yahoo.com.au

**Abstract.** This experiment aimed at assessing effects of alkaline hydrolysis using rice husk ash filtrate (RHAF) with or without urea and urease enzyme on nutrient content of dried kume grass. A 6x3 completely randomized design was employed to test 6 treatments, namely RK1 = dried kume grass sprayed with RHAF (15% w/v) and hydrolysed for 3 hours, RK2 = Like RK1 + urea 4%, RK3 = Like RK2 plus urease for urea: urease ratio of 1:2 or 1:3 (RK4) or 1:4 (RK5) or 1:5 (RK6). Measured variables were dry matter (DM), organic matter (OM), crude protein (CP), and crude lipid (CL). General linear model for CRD was employed in data analysis, and Duncan test for treatment difference. Results showed that increased urea: urease ratio significantly effecting CP and CL content, yet no difference between 1:4 and 1:6 ratios. The lowest improvement was in RK1 (7.41% CP and 41.93% CL) and the highest was in RK6 (22.75% CP and 210.16% CL). In the contrary, all treated groups showed a decline in DM and OM. It is concluded that alkaline hydrolysis using RHAF plus urea: urea ratio at 1:4 is the best treatment to improve the nutritive value of dried kume grass.

1. **Introduction**

Kume grass (*Sorghum plumosum* cv. Timorens) is one of the local productive grasses for ruminants in West Timor, Indonesia due to its high biomass production (up to 12 t DM/ha/year [1]. This grass has a relatively high growth rate (up to 2.5 cm/d) and mature quickly resulting in a sharp increase in fiber content. Kamlasi et al. showed that fiber content increased from 25% on day 20 to 40% on day 120 after growing [2]. Proportion of lignine is slightly high (7.51%) in crude fiber of Kume grass hence surpresses its digestibility when give in dried form to ruminants [3]. Even though cellulose and hemicellulose are two types of digestible structural carbohydrates in Kume grass tissues, yet its digestibility will be surpressed if these digestible structural carbohydrates are bound to lignin fractions to form lignocellulose and lignohemicellulose complex.

To improve availability of nutrients in the dried Kume grass and make them readily to be utilized by ruminants, pre-treatments are required. One of the pre-treatment techniques is hydrolysis using alkaline solution such as rice husk ash filtrate (RHAF) to breakdown lignin binding from cellulose and hemicellulose fractions. Dami Dato and Ghunu used RHAF at a rate of 15% w/v to hydrolyze dried Kume grass for 3 hours and found an increased in digestibility of fiber components (NDF, ADF, hemicellulose, cellulose, and lignin), *in vitro* fiber digestibility, and total digestible nutrients [4]. The same trend was also reported recently by Dami Dato and Mullik [5]. However, the significant reduction
in fiber compounds was not improve nutrient content particular of the substrate. These researchers found that protein content of RHAF hydrolyzed dried Kume grass was merely 1.13% [4]. Based on this, the present experiment was designed to test the effects of combination RHAF with urea and urease at a different ratio on nutrient content of dried kume grass. The urea served as nitrogen source, whilst urease served as catalyst in the hydrolysis of urea to form ammonia (NH₃).

2. Materials and methods

This laboratory experiment was conducted at Ruminant Laboratory of Padjadjaran University, whereas proximate analysis was performed at Balai Penelitian Ternak, Ciawi - Bogor, Indonesia. Substrate used was dried Kume grass (standing hay), freshly collected rumen content as source of urease, urea, and RHAF (15% w/v). A 6 x 3 completely randomized design (CRD) was employed to test 6 treatments namely RK₁ = dried Kume grass sprayed with RHAF (15% w/v) and hydrolyzed for 3 hours; RK₂ = Like RK₁ + urea 4%; RK₃ = Like RK₁ + urea 4% + urease 8% (ratio 1:2); RK₄ = Like RK₁ + urea 4% + urease 12% (ratio 1:3); RK₅ = Like RK₁ + urea 4% + urease 16% (ratio 1:4); RK₆ = Like RK₁ + urea 4% + urease 20% (ratio 1:5).

One kilogram of dried Kume grass was used as substrate in each of the experimental unit. Process in the production of RHAF was based on the method proposed by Dami Dato [3]. Into each litre of RHAF was added 40g urea as nitrogen source, 10g calcium carbonate as calcium source, 18g table salt as source potassium, and 2g sulphur [6]. The hydrolysis procedure was done according to Su'trisno et al. [7]. A three hours’ hydrolysis was applied, and the pH was measured immediately after the hydrolysates was terminated. About 200 grams of the hydrolysed substrate was taken as sample and processed for further chemical analysis. Quantification of nutrient content of the hydrolysed substrate was done according to AOAC [8].

Variables measured were changes in the concentration of dry matter (DM), organic matter (OM), crude protein (CP), and crude lipid (CL) in the hydrolyzed substrate. Collected data were analyzed using general linear model principles for CRD, and Duncan test was employed for treatment difference. SPSS version 23 was used as statistical analysis tool.

3. Results and discussion

Hydrolyzing dried kume grass with alkaline solution such as RHAF alone or added with urea and urease significantly affecting nutrient content of the substrate. The mean change in the nutrient content is presented in Table 1. The higher the ratio of urea: urease, the higher the concentration of the CP and CL. The highest CP was recorded in treatment RK6 (415.8%) and the lowest value was detected in the hydrolyzed dried kume grass was merely 1.13%.

Statistical analysis showed that increasing the ratio of urea and urease from 1:2 to 1:5 had a significant effect on CP and CL content, yet reduced DM and OM significantly. The effect was plateau when the urea: urease was increased from 1:4 to 1:5. This suggests that at a ratio of 1:4 the biochemical reactions has reach the equilibrium state; hence addition of urease will not have a significant effect in the hydrolyses processes.

Table 1. Changes in nutrient content of dried Kume grass after hydrolyzed with rice husk ash filtrate alone (RK₁) or added with 4% urea (RK₃) or added with 4% urea + urease at a ratio of 1:2 (RK₄) or 1:3 (RK₅) or 1:4 (RK₆) or 1:5 (RK₆).

| Variable            | Treatment | RK₁ | RK₂ | RK₃ | RK₄ | RK₅ | RK₆ |
|---------------------|-----------|-----|-----|-----|-----|-----|-----|
| Dry matter          |           | -18.27  a | -18.61 b | -18.99 c | -19.69 d | -20.37 e | -20.52 e |
| Organic matter      |           | -19.35  a | -19.98 b | -20.61 c | -21.65 d | -22.56 e | -22.75 e |
| Crude protein       |           | 7.41 a   | 148.41 b | 294.71 c | 339.42 d | 409.52 e | 415.08 e |
| Crude lipid         |           | 41.03 a  | 70.51 b | 106.41 c | 135.90 d | 205.13 e | 210.26 e |

Different superscript in the same row indicates a significant difference (P<0.05)
An increase in crude protein and crude lipid concentration in all treatments which received urea and urease is predictable since extra nitrogen from urea and also the action of urease to hydrolyze urea to form ammonia (NH$_3$). The released NH$_3$ is then fixed into the tissues of the substrate (dried kume grass). Bo Göhl reported that ammonization of sugar cane bagasse and given additional urea accelerated ammonization process and fixation of the ammonia into the tissues of the fiber compounds in substrate [9]. An increased in CP content of the hydrolyzed substrate was also affected by the formation of N-ammonium hydroxide, which is an alkaline substrate, that degrading lignocellulose and legnohemicellulose complex to lignin, cellulose and hemicellulose [10]. The released ammonia is trapped in the tissues of the substrate and is not escapable causing an increase in CP concentration of the substrate [11,12]. A similar trend was also reported by Ahmed et al. who found an increased in protein content of rice straw from 2.68% to 8.7% when 4% urea was applied [13]. These researchers also observed that protein content of the straw continues to increase and reach 12.21% when soybean meal was added (6%) into the substrate which already received 4% urea [13].

Data in table 1 showed that all nutrient variables were not affected when urea: urease ratio was increased from 1:4 (KR$_3$) to 1:5 (KR$_6$). This clearly suggests that the optimum urea: urease ratio in the alkaline hydrolysis of dried Kume grass sprayed with RHAF (15% w/v) is 1:4. This could be related to an equilibrium of urea: urease or could be due to equilibrium in the ability of substrate tissues to absorb addition ammonia released from urea. Whilst at lower urea: urease ratio (1:2-1:3) the respond was observed when the urea: urease ratio was changed.

Crude lipid concentration in the hydrolyzed dried kume grass in the present study was improved by 70.7% at urea: urease ratio of 1.2 (RK$_3$) and reached 210.26% improvement when the ration was increased to 1:5 (RK$_6$). However, the rate of improvement in the CL concentration was not different between the treatments with urea: urea ratio of 1:4 and 1:5. This suggests that the optimum urea: urease ratio for this experimental setting was 1:4. An improvement trend in CL concentration in hydrolyzed rice straw was also reported by Ahmed et al. who showed that addition of 4% urea plus soybean meal at a rate of 4% and 6% increased CL concentration by 14% and 22% respectively [12]. Improvement in lipid content in the present experiment could be related to inductive effects of RHAF and urea-urease on Maillard reaction in carbohydrate and protein fractions thus releasing some free fatty acids in the substrate. Maillard reaction involving protein and carbohydrate fractions could be a stimulant to the formation of certain fatty acids [10]. As a result, lipid concentration in the substrate increases. Crude lipid improvement in the present study might also be related to the hydrolysis of acetyl groups in polysaccharides fractions in the substrate tissues as suggested by Ismadi [14].

A significant reduction in the DM and OM in the current study by up to 22% is predictable since some organic materials were used up in the hydrolysis processes to form other compounds and to provide energy for biochemical reactions in the system.

4. Conclusion
Nutrient quality of the dried Kume grass is improved significantly by alkaline hydrolysis using rice husk ash filtrate (15% w/v) and combined with 4% urea and urease at certain ratio. The best results were achieved at urea: urease ratio of 1:4.

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