# THE EFFECT OF N-$NH_3$ INCLUSION ON THE DEGRADATION OF TANNIN OF SORGHUM GRAIN BY RUMEN MICROBES

**Joelal Achmadi, Surahmanto, Ekha Dewi, and Dewi Arfiyanti**
Faculty of Animal Agriculture, Diponegoro University
Undip Tembalang Campus, Semarang 50275
e-mail : jachmadi@gmail.com

## ABSTRACT

Two experiments were conducted to study the effect of N-$NH_3$ inclusion in the rumen liquor on the degradation of tannin of whole sorghum grain. The first experiment was carried out to determine the effect of N-$NH_3$ inclusion doses in the rumen liquor on the degradation of sorghum grain tannin. The urea as a source of N-$NH_3$ were included in rumen liquor at doses of 0, 0.5, 1.0, and 1.5% from the weight of whole sorghum grain (w/w). The degradation of sorghum grain tannin was increased ($P<0.05$) by the doses of N-$NH_3$ inclusion, but the degradation of sorghum grain tannin began to decrease at 1.5% of N-$NH_3$ inclusion dose. In the second experiment, the 1% of N-$NH_3$ inclusion dose was used to evaluate the duration of fermentation in the rumen liquor. The whole sorghum grain were fermented for 12h, 24h, 48h, and 48h in the rumen liquor. The 48h of fermentation gave a highest production of total protein production. The growth of tannase producing rumen microbes could be enhanced by inclusion of N-$NH_3$.

*Keywords: tannin, tanase, rumen microbes, N-$NH_3$*

## INTRODUCTION

Rumen microbes are well known to have an ability to degrade feed tannin (Murdiati and Mahyudin, 1985). Begovic *et al*. (1978) isolated tanase from goat rumen mucose. On the other hand, tanase activity of rumen microbes are not adequate to degrade most feed tannin (Makkar, 1993). The dry matter of feed and ammonia rumen production remains unchanged in goat fed a *Calliandra calothyrsus* ration, eventhough tanase producing bacteria was innoculated to the goat rumen (Wiryawan *et al*. 1998). This fact showed that the rumen environment needs to be manipulated to enhance the growth of tanase producing microbes.

Rumen microbes use nitrogen non protein as a substrate to build their bodies protein. A proper urea dose could be included together in the ration to maintain the high ruminant production efficiently. It is well known that a 5 mg percent of ruminal ammonia concentration could contribute to the optimum growth of rumen microbes. Therefore some efforts are required to create a better rumen environment for digesting the consumed feed efficiently.

In this study, the tannin of sorghum grain was subjected as a substrate for tanase producing rumen microbes. The urea as N-$NH_3$ source was included to contribute an optimum growth of rumen microbes. It was accomplished by an experimental study adopting the technique of a single batch culture for the *in vitro* feed digestibility test.

## MATERIALS AND METHODS

The first experiment was aimed to determine the N-$NH_3$ inclusion dose in the rumen liquor that contributed to rumen microbes in degrading the tannin of sorghum grain. Four doses of urea [0, 0.5, 1.0, and 1.5% from the weight of whole sorghum grain (w/w)] were included to the rumen liquor. In each dose of urea, the sample of whole sorghum grain was fermented for 48 hours anaerobically. The supernatants were filtered and the residue were analyzed for its tannin and reduced sugar contents. Each dose of the urea treatment was replicated in three times. The contents of tannin and reduced sugar contents from sorghum grain were chemically determined *in duplo*.

From the first experiment, urea dose of 1% gave greatest tannin content. Therefore the aim of second experiment was to determine the fermentation time at urea dose of 1%. Four treatments of fermentation time (12, 24, 48, and 72 hours) were applied to the single batch culture for the *in vitro* feed digestibility test, similar to the first experiment with a inclusion dose of urea at 1%. The supernatants were filtered and the residue were analyzed for its tannin content and its total protein production.

The tannin content of sorghum grain was analyzed using the Lowenthal-Procter procedure of $KMnO_4$ titration (Sudarmadji *et al*., 1989). The total protein content was analyzed using procedure of thio-sulphate precipitation and was continued by procedure of Kjeldahl. The content of reduced sugar was determined using prosedure of AOAC (1984). A completely randomized design was used for experiment 1 and experiment 2, respectively. The one way analyze of varience was utilized to test the significant of treatments, and the Duncan's multiple range test was used to test the different among treatments.

## RESULTS AND DISCUSSION

There are some treatments for reducing tannin content of feed, including physical, chemical, and biological treatments. Recently, Achmadi *et al* (2007) reported that the germination could reduce the tannin content of whole sorghum grain significantly. These efforts contributed to enhance the feed diversity for energy sources. It is well known that energy content of sorghum grain is similar to that of maize grain. The use of tanase producing microbes become more realitistically because in the rumen of ruminants contain some microbes species that able to degrade tannin of feed.

There are two types of feed tannin, hydrolizeable tannin and condensed tannin. Table 1 showed that N-$NH_3$ inclusion to the rumen liquor significantly enhanced ($P<0.05$) the degraded

tannin of whole sorghum grain. The increase in urea dose was followed by the enhance in the degraded tannin. It could be suggested that the gorwth of tanase producing microbes in rumen could be maximized by N-$NH_3$ inclusion. Brooker *et al* (1999) stated that some tanase producing rumen microbes are *Streptococcus caprinus, Streptococcus gallolyticus, Selemonas ruminantium, Aspergillus ficuum, Aspergillus niger*. The inclusion dose of urea at 1.5% may be exceeding the optimum ammonia concentration in rumen above the requirement of microbes, because there was no elimination way in the system of *in vitro* fermentation.

Table 1. Results of experiment 1

| | Dose inclusion of urea (% of whole sorghum grain) | | | |
|---|---|---|---|---|
| | 0.0 | 0.5 | 1.0 | 1.5 |
| Tannin[1], % | 10.51[d] | 28.93[c] | 63.08[a] | 46.14[b] |
| Reduced sugar[2], % | 4.13 | 4.43 | 5.38 | 4.73 |

[1]Degraded tannin was based on % dry matter.
[2]Content of reduced sugar was based on % dry matter.
[a,b,c,d] P<0.05.

Table 2. Results of experiment 2

| | Fermentation time (hours) | | | |
|---|---|---|---|---|
| | 12 | 24 | 48 | 72 |
| Tannin[1], % | 3.90[d] | 13.21[b] | 60.09[a] | 8.86[c] |
| Total protein[2], % | 2.05[b] | 4.32[a] | 4.97[a] | 1.06[c] |

[1]Degraded tannin was based on % dry matter.
[2]Total protein production was based on % dry matter.
[a,b,c,d] P<0.05.

The inclusion of N-$NH_3$ at 1% was an optimum dose for the growth of tanase producing microbes, because the fermentation time for 48 hours caused the highest tannin degradation (Table 2). This result was contributed by the highest production of total protein also at the fermentation time for 48 hours. The single batch culture for the *in vitro* feed digestibility test may give an optimum result at the fermentation time for 48 hours, and the result of *in vitro* feed digestibility test starts to decrease at the fermentation time for 72 hours (Tillman *et al*, 1985).

It is well known that an enzyme will act on its substrate specifically. The tanase secreted from rumen microbes has highly specify to tannin of sorghum grain. The tannin then will be converted into galic acid and glucose. The galic acid and glucose then will be utilized as readily availaible carbohydrate by the microbes (Kumar and Singh, 1984). The starch content of sorghum grain may not be utilized by the rumen microbes during the fermentation because this experiment used the whole sorghum grain. The content of reduced sugar remained unchanged among treatments (Table 1).

## CONCLUSION

The fermentation of whole sorghum grain in rumen liquor for 48 hours with inclusion of urea at 1% resulted in the highest tannin degradation. This single batch culture from fermentation might be a mixed culture for tanase producing prebiotic. Further study is needed to identify and

isolate the tanase producing microbes from the mixed culture, thus the elimination of tannin during feed preparation become more realistically.

## REFERENCES


Achmadi, J., E. Pangestu, and F. Wahyono. 2007. Glucose tolerance and insulin response to glucose load in sheep fed on germinated sorghum grain. Asian-Aust. J. Anim. Sci. 10: 1575 – 1579.

AOAC, 1984. Official Methods of The Association Official Agricultural Chemist, AOAC, Arlington.

Begovic, S., E. Duzic, A. Sacirbegovic, and A. Tafro, 1978. Examination of variations of tannase activity in ruminal content and mucosa of goats on oak leaf diet and during intraruminal administration of 3 and 10% tannic acid. Veterinaria, Sarajevo 27 : 445 - 457.

Brooker, J.D., L. O'Donovan, I. Skene dan G. Sellick. 1999. Mechanism of tannin resistance and detoxification in the rumen. Proc. of The 8th International Symposium on Microbial Ecology, Canada.

Kumar, R., dan M. Singh. 1984. Tannins, their adverse role in ruminant nutrition. J. Agric. Food Chem. 32 : 447 – 453.

Makkar, H. P. S., 1993. Antinutritional factors in foods for livestock. In : Animal Production in Development Country, M. Gill, E. Owen, G. E. Pollott and T. L. J. lawrence (eds.). British Society of Animal Production, Occasional Publ. No. 16 : 69 - 85.

Murdiati, T. B. and P. Mahyudin, 1985. The residual tannin and crude protein of *Calliandra callothyrus* and *Albizia falcatoria* following incubation in heated and unheated rumen fluid. Proc. Of The 3rd Australasian Association for Animal Production Animal Congress, Seoul, Korean Republic, Vol. 2A, p. 814 - 816.

Sudarmadji, S., B. Haryono, dan Suhardi. 1989. Analisa Bahan Makanan dan Pertanian. Liperty, Yogyakarta.

Tillman, A.D., H. Hartadi, S. Reksohadiprodjo, S. Prawirokusumo dan S. Lebdosukojo. 1985. Ilmu Makanan Ternak Dasar. Gadjah Mada University Press, Yogyakarta.

Wiryawan, K.G., Surhayadi, B. Tangendjaja, S. Syahrir, J.D. Brooker. 1998. The effect of tannin degrading bacterial innoculation on the performance of calliandra (*Calliandra calothyrsus*) fed goats. Bulletin Peternakan, Edisi Tambahan : 126 – 135.

| | |
|---|---|
| Filename: | Sem_Rum_Nas |
| Directory: | E:\Documents |
| Template: | C:\Users\Owner\AppData\Roaming\Microsoft\Templates\Normal.dot |
| Title: | THE EFFECT OF N-NH3 INCLUSION ON THE DEGRADATION OF |
| Subject: | |
| Author: | Faculty of Anim Agric |
| Keywords: | |
| Comments: | |
| Creation Date: | 16/09/2010 10:57:00 |
| Change Number: | 5 |
| Last Saved On: | 30/12/2010 11:01:00 |
| Last Saved By: | Faculty of Anim Agric |
| Total Editing Time: | 45 Minutes |
| Last Printed On: | 30/12/2010 11:02:00 |

As of Last Complete Printing

| | |
|---|---|
| Number of Pages: | 4 |
| Number of Words: | 1.512 (approx.) |
| Number of Characters: | 8.621 (approx.) |