The effects of leguminous supplementation on ammoniated rice straw based completed feed on nutrient digestibility on in vitro microbial protein synthesis

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Abstract. Legume is the best source of protein to ruminant. Tannin content in legumes can provide protein by pass for ruminant. This study was conducted to determine the effect supplementation of legumes (Leucaena leucocephala) as substitute of concentrate on nutrient digestibility and microbial protein synthesis of the completed feed based on ammoniated rice straw. The study was conducted in an in vitro used a randomized block design where there were 3 treatments dan 5 replications. The treatments were A. 40% ammoniated rice straw + 60% concentrate, B. 40% ammoniated rice straw + 50% concentrate + 10% Leucaena leucocephala, C. 40% ammoniated rice straw + 40% concentrate +10, + 20% Leucaena leucocephala. The results showed that the addition of Leucaena leucocephala had increased the digestibility of DM, OM, CP and microbial protein synthesis (p< 0.05). Increased doses of Leucaena leucocephala until 20% decreased digestion of nutrition compared with dose 10% but was still higher than control. In conclusion, the supplementation of Leucaena leucocephala on completed feed based on ammoniated rice straw in vitro can increase nutrient degradability. Supplementation of 10% and 20% Leucaena leucocephala were suitable to be used for further studies, therefore in vivo experiment is required to study the effects on animal production.

Keywords – digestibility, Leucaena leucocephala, completed feed, ammoniated rice straw.

1. Introduction
Feed was one of the determinants of livestock productivity, so the availability of good quality feed was a requirement for livestock development in an area [1]. Cattle breeding systems that were still traditional, where feeding is only dependent on rice straw and native grass, caused the need for nutrients was not fulfilled. Addition of leguminous which had high quality in low quality rations such as rice straw will increase livestock productivity. Legumes such as Leucaena leucocephala was a high-protein content with a good amino acid profile, had mineral content, and low crude fiber [2]. It also had tannin content act as added value because it can function to protect the excess of protein in the rumen (by pass protein). Therefore, the amount of...
protein that can absorb by the small intestine becomes higher and also had the potential to reduce methane production in the rumen [3]. The high use of *Leucaena leucocephala* in the ration can also have a negative effects on livestock due to excess nitrogen in the rumen [4] and the presence of mimosine, antinutrients, that can cause poisoning in livestock [5] [6]. Based on the description above this study was conducted with the aim to determine the effect of *Leucaena leucochepala* as substitute for concentrate in ration of ammoniated straw on nutrient digestibility and microbial protein synthesis.

2. Research methods

This research was carried out in the Laboratory of Ruminant Nutrition, Faculty of Animal Science, Andalas University. This experiment carried out Randomized Block Design with three treatments and five groups as replications. The treatments were A. 40% ammoniated rice straw + 60% concentrate, B. 40% ammoniated rice straw + 50% concentrate + 10% *Leucaena leucocephala*, C. 40% ammoniated rice straw + 40% concentrate + 20% *Leucaena leucocephala*. Concentrate consist of rice brain, palm kernel cake, cassava and mineral. Chemical composition of each treatment can be seen in Table 1. Ruminal fluid was obtained from a cannulated steer. The *in vitro* digestibility measurement was conducted according to [7]. Fermentation tubes contained of 50 ml of rumen fluid and 200 ml McDougall buffer solution. Three fermentation tubes that did not contain substrate were also incubated and used as blanks. Tubes were incubated for 48 h at a temperature of 39ºC in a shaker water bath. After incubation for 48 h, fermentation activity was stopped by immersion in iced water to stop the activities of microbial activity. Rumen fluid pH was measured with pH meter. Tubes were then centrifuged at 1500 rpm for 30 min and the supernatant was removed. Residu samples were oven dried at 60ºC for 24h and stored for later chemical analysis according to [8] for dry matter (DM), organic matter (OM), Crude protein (CP), and microbial protein shynthesis based on [9].

Statistical Analysis: Data were analyzed by ANOVA using a completely randomized design with subsampling. Differences among means were tested using LSD [10].

**Table 1.** The Ingredient composition and the chemical composition (% dry matter).

| Ingredient composition (%) | Diet |
|---------------------------|------|
|                           | A    | B    | C    |
| Ammoniated rice straw     | 40   | 40   | 40   |
| Concentrate               | 60   | 50   | 40   |
| Leucaena leaf meal        | -    | 10   | 20   |
| Total                     | 100  | 100  | 100  |
| Chemical composition      |      |      |      |
| CP                        | 10.93| 12.21| 13.48|
| TDN                       | 67.79| 68.20| 68.60|
| CF                        | 5.07 | 4.91 | 4.74 |
| NDF                       | 46.73| 44.49| 42.26|
| ADF                       | 49.31| 51.53| 52.01|
| Lignin                    | 36.77| 40.04| 43.26|

Notes: TDN = total digestible nutrient, CP = Crude protein, NDF = Neutral Detergent Fiber, ADF = Acid Detergent Fiber, CF = Crude Fat.
3. Result and discussion

3.1. Digestibility nutrient and microbial protein synthesis

Digestibility of nutrient was an indicator of the use of feed by livestock. Table 2 showed that the treatments had a significant effect on the digestibility of dry matter, organic matter, crude protein and microbial protein synthesis (P <0.05).

Table 2. Nutrient digestibility and microbial protein synthesis.

| No. | Treatments | Digestibility | Microbial protein synthesis (mg/100 ml) |
|-----|------------|---------------|----------------------------------------|
|     |            | DM | OM | CP |                                   | |
| 1   | A          | 58.83<sup>c</sup> | 59.50<sup>b</sup> | 57.83<sup>b</sup> | 74.33<sup>c</sup> |
| 2   | B          | 69.00<sup>a</sup> | 69.70<sup>a</sup> | 71.23<sup>a</sup> | 137.17<sup>a</sup> |
| 3   | C          | 64.33<sup>b</sup> | 66.50<sup>a</sup> | 66.51<sup>b</sup> | 88.04<sup>b</sup> |
| SE  |            | 0.57 | 1.20 | 1.3 | 1.7 | 

Notes: Different superscripts (a,b,c) in the same row indicate statistically significantly different effects (P<0.05).

Supplementation of 10 and 20% *Leucaena leucocephala* in ammoniated rice straw diet significantly increased the digestibility of dry matter, organic matter, crude protein and rumen microbial protein synthesis compared without *Leucaena leucocephala* supplementation. This happens because the content of nutrients from treatment B and C also increases compared to treatment A. So that the availability of nutrients for rumen microbes is also more. Besides that, *Leucaena leucocephala* also contains sulfur and calcium which are needed by rumen microbes to develop, especially cellulolytic bacteria and fungi [11]. [12] [13] said that Leucaena as a protein supplement can improve the quality of feed. Supplementation 10% of *Leucaena leucocephala* provides better digestibility than 20% supplementation (treatment C). The decrease in nutrient digestibility in treatment C is caused more by the high lignin content of the ration. Lignin binds to cellulose and hemicellulose and cannot be digested by enzymes produced by microbes. The higher the lignin content in the diet, the nutrient digestibility of diet will decreased as explained by [14]. Besides lignin, the tannin content which is slightly higher in this treatment could reduced digestibility because tannin will bind protein and carbohydrate feed so it cannot be degraded in the rumen. Tannin bond with protein can be released in an acidic condition according to the opinion of [15] also states that tannins in the rumen are able to bind proteins and carbohydrates so that the digestibility in the rumen can be reduced.

Table 2 also showed that the addition of *Leucaena leucocephala* significantly affects the digestibility of the crude protein. Protein degradation described a number of proteins that are degraded in the rumen which is a supply of feed protein as a source of N for rumen microbes as well as an indication of a number of diet protein that are not degraded in the rumen entering the small intestine. Protein digestibility increased with the addition of *Leucaena leucocephala* in the ration. This increment is caused by the increase in diet protein content because *Leucaena leucocephala* is a feed ingredient with good protein content [16]. Protein digestibility in the rumen decreases with the addition of 20% *Leucaena luecocephala*. This decrease was caused by protein ration in treatment C mostly came from *Leucaena luecocephala* which was bound with tannin so that it was not degraded in the rumen as stated by [2] that Leucaena has tannin content which can provide added value because it can protect the over-protein overhaul in rumen (by pass protein) so that the amount of protein that can be absorbed by the small intestine becomes higher.

Microbial protein synthesis was a description of microbial growth in the rumen. Increased microbial protein synthesis means that the microbial population in the rumen also increases. Table 2 showed that the addition of *Leucaena luecocephala* increases significantly microbial protein synthesis in the rumen. This was due to the availability of nutrients for optimal
microbial growth and the presence of the most important microbial growth factors such as sulfur and calcium minerals in *Leucaena leucocephala* especially for cellulolytic microbial growth and fungi [11]. Increased microbial protein synthesis was also associated with a decrease in the population of protozoa in the rumen. The presence of saponins and tannins in legumes including *Leucaena leucocephala* could reduce the number of protozoa in the rumen [17]. The reduced number of protozoa in the rumen will increase the bacterial population so that microbial protein synthesis will also increase. This is in line with research conducted by [18] [19] and [20].

4. Conclusion

Supplementation of *Leucaena leucocephala* as concentrate substitution on completed feed based on ammoniated rice straw *in vitro* can increase degradability and microbial protein synthesis. Supplementation of 10% and 20% *Leucaena leucocephala* were suitable to be used for further studies, therefore *in vivo* experiment is required to study the effects on animal production.

5. References

[1] Retnani Y, Kamesworo S, Khotidjah L, Saenab A. 2010. Pemanfaatan Wafer Limbah Sayuran Pasar Untuk Ternak Domba. Seminar Nasional Teknologi Peternakan dan Veteriner, 2010 Agustus 2-3; Bogor, Indonesia. Bogor (ID): Pusat Penelitian dan Pengembangan Peternakan. 503-510.

[2] Panjaitan, T. S., 2002. Mengenal Potensi Lamtoro Hibrida F2 sebagai Sumber Pakan Hijauan Ternak. BPTP Nusa Tenggara Barat.

[3] Jayanegara, A., Wina, E., Soliva, C. R., Marquardt, S., Kreuzer, M., Leiber, F. 2011. Dependence of forage quality and methanogenic potential of tropical plants on their phenolic fractions as determined by principal component analysis. Animal Feed Science and Technology. 163: 231-243.

[4] Calsamiglia, S., Ferret, A., Reynolds, C., Kristensen, N., & Van Vuuren, A. 2010. Strategies for optimizing nitrogen use by ruminants. *Animal. 4*(7), 1184-1196.

[5] Ghosh, M. K., Samiran, B. 2007. Mimosine toxicity a problem of *Leucaena* feeding in ruminants. Asian Journal of Animal and Veterinary Advances. 2: 63-73.

[6] Dalzell, S. A., Burnett, D. J., Dowsett, J. E., Forbes, V. E., Shelton, H. M. 2012. Prevalence of mimosine and DHP toxicity in cattle grazing *Leucaena leucocephala* pastures in Queensland, Australia. Animal Production Science. 52: 365-372.

[7] Tilley J M A & Terry R A. 1963. A two-stage technique for the in vitro digestion of forage crops. J. Brit. Grassland Soc. 18:104-11.

[8] AOAC, 2007.Official Method of Analysis.Association of Official Analytical Chemist, Washington D.C.

[9] Makkar, H. P. S., O. P. Sharma, R.K. Dawra and S. S. Negi. 1981. Simple determination of microbial protein in rumen liquor. J. Dairy Sci 65: 2170-2173.

[10] Steel, R. G. D. & J. H. Torrie.1993. Principles and Procedures of Statistics: A Biometrical Approach. McGraw-Hill, New York.

[11] Aregheore, E. M. 1999. Nutritive and antinutritive value of some tree legumes used in ruminant livestock nutrition in Pacific island countries. Journal of South Pacific Agriculture. 6: 50- 61.

[12] Jabbar, M. A., L. Reynolds, A. Larbi and J. Smith, 1997. Nutritional and economic benefits of *Leucaena* and *Gliricidia* as feed supplements for small ruminants in humid West Africa. Trop. Anim. Health Prod., 29: 35-47. PMID: 9090017.

[13] Aregheore, E. M. and D. Perera. 2004. Effect of supplementation of a basal diet of maize stover with *Erythrina variegata*, *Gliricidia sepium* or *Leucaena leucocephala* on feed intake and digestibility by goats. Trop. Anim. Health Prod., 36: 175-189. PMID: 14998316.
[14] Hariadi BT, Santoso B. 2010. Evaluation of tropical plants containing tannin on in-vitro methanogenesis and fermentation parameters using rumen fluid. J Sci Food Agri. 90:456–461.

[15] Patra, A. K & J. Saxena. 2010. Review A new perspective on the use of plant secondary metabolites to inhibit methanogenesis in the rumen. J. Phytochem. 71: 1198–1222.

[16] Galindo, J., Marrero, Y., Ruiz, T., González, N., Díaz, A., Aldama, A., Moreira, O., Hernández, J., Torres V., Sarduy, L. 2009. Efecto de una mezcla múltiple de leguminosas herbáceas y Leucaena leucocephala en la población microbiana y productos fermentativos del rumen de añojos mestizos de Cebú. Revista Cubana de Ciencia Agrícola. 43: 256-264.

[17] Ningrat, R. W. S., Mardiati Zain, Erpomen and Heny Suryani. 2016. Effects of doses and different sources of tannins on in vitro ruminal methane, volatile fatty acids production and on bacteria and protozoa populations. Asian J. AnimSci 11: 47-53.

[18] Carulla, J. E., M. Kreuzer, A. Machmüller & H. D. Hess.2005. Supplementation of Acacia mearnsii tannins decreases methanogenesis and urinary nitrogen in forage-fed sheep. Aust. J. Agric. Res. 56: 961-970.

[19] Herawaty, R., N. Jamarun, M. Zain, Arnim and R. W. S. Ningrat. 2013. Effect of Supplementation Sacharomyces cerevisiae and Leucaena leucocephala on Low Quality Roughage Feed in Beef Cattle Diet. Pakistan. J. Nut. 12 (2): 182-184.

[20] Zain M, J. Rahman, Khasrad and Erpomen, 2016. Supplementation of Saccharomyces cerevisiae and Sapindus rarak in diet based of oil palm frond (OPF) on nutrient digestibility and daily weight gain of goat. Asian J. Anim. Vet. Adv., 11: 314-318.

Acknowledgments
This study was supported by Professor Research Grant Cluster by BOPTN Andalas University contract No. 33/UN.16.17/PP.KP.RGB/LPPM/2018 The research would not have been possible without the cooperation of my post graduated students and technical assistance of Laboratory of Ruminant Nutrition, Faculty of Animal Science of Andalas University.