The Effect of Tannin Sources from *Acacia mangium* Willd, *Swietenia mahagoni*, and *Artocarpus heterophyllus* Leaves in Pellets on *In Vitro* Nutrient Digestibility

Rofi Prima Maulana[1] Chusnul Hanim[1] Muhlisin[1] Muhsin Al Anas[1] and Lies Mira Yusiati[1,*]

[1]*Laboratory of Nutritional Biochemistry, Department of Animal Nutrition and Feed Science, Faculty of Animal Science, Universitas Gadjah Mada, Indonesia.*
[*]*Corresponding author. Email: liesmira@ugm.ac.id*

ABSTRACT
The objective of this research was to determine the effect of pellet with a combination leave tannin source from *Acacia mangium* Willd, *Swietenia mahagoni*, dan *Artocarpus heterophyllus* in pellets on *in vitro* nutrient digestibility. This research used three substrate tratments with different tannin levels at 0%, 1%, and 2% with substituting part of the concentrate feed with pellet and still based on dry matter (DM). The ratio of forage and concentrate on the substrate is 60:40. The substrate used for *in vitro* analysis was a two-stage digestibility analysis by Tilley and Terry method. Parameters observed were digestibility of dry matter, organic matter, crude fiber, and crude protein in the rumen and abomasum (total). The results showed that the addition of combination leaves tannin source into pellet with tannin level up to 2% on substrate, reduced the digestibility of crude protein in the rumen ($P<0.05$), but increased the digestibility of crude protein in total ($P<0.05$) without any effects on the digestibility of dry matter, organic matter, and crude fiber. Based on the results of the research, it can be concluded that the addition leaves tannin source from *Acacia mangium* Willd, *Swietenia mahagoni,* and *Artocarpus heterophyllus* into pellet with tannin level up to 2% on substrate, was able to reduce ruminal CPD and increase CPD total without any effects on the DMD, OMD, and CFD.

Keywords: *In Vitro, Nutrient Digestibility, Tannins, Pellets.*

1. INTRODUCTION

Organisms such as ruminants need nutrients for their survival. However, there are several phases that require more protein nutrients, such as the growing phase, the developing phase, the pregnant phase, and the lactation phase. Protein is an important nutrient component in the feed given to livestock. Its roles are very numerous in living systems, such as being involved in the formation of body tissues [1] and being actively involved in all metabolic processes in the body, including protein hormones [2], antibodies [3], and enzymes [1].

Based on the digestive system of ruminants, feed consumed by livestock is not directly utilized by livestock but is first fermented by rumen microbes in the acids (VFA), and microbial protein. Then NH3 will be used by microbes to synthesize microbial protein [4], CO2 will be converted to CH4 with H2 [5], VFA will be absorbed by the body for energy for livestock [6], and microbial protein along with proteins that pass from rumen fermentation is total protein that can be used by livestock for their survival [7]. Therefore, the provision of protein feed for ruminants requires special attention, because the process of transforming feed protein into

A. D. Saputro et al. (Eds.): ICOSEAT 2022, ABSR 26, pp. 538–544, 2023.
https://doi.org/10.2991/978-94-6463-086-2_73

microbial protein requires time and a balance of energy and protein in feed [8], besides that feed protein is also easily degraded in the rumen (such as is soybean meal). The percentage of protein loss after rumen incubation time reached 0, 2, 4, 8, 16, 24, 48 hours was 17.6; 26.7; 35.9; 50.42; 74.9; 91.1; and 99.2 [9]. Based on this, feed protein needs to be protected.

There is a way to protect feed protein from fermentation in the rumen by microbes so that protein can be directly digested in the post-rumen digestive tract and utilized by host animals. That is by utilizing plant bioactive compounds, such as tannins as feed additives. The phenol group of tannins can bind strongly to the carboxyl groups (aromatic and aliphatic) of protein [10] at pH 6 to 7 as in the conditions in the rumen, and the strong bond of tannin with protein will be released again at a pH of less than 3.5 (as in the abomasum) or higher than 8 (as in the duodenum) [11].

Tannin compounds from acacia (*Acacia mangium* Willd), mahogany (*Swietenia mahagoni*), and jackfruit (*Artocarpus* heterophyllus) leaves have been proven to bind to globular proteins in vitro. According to [12], 1 gram of tannin derived from jackfruit leaves can bind 23.149 g of globular protein. According to [13], 1 mg of tannin derived from mahogany leaves can bind 1.217 mg of globular protein. According to [14], feed supplementation with condensed tannin from acacia plants significantly reduced total nutrient digestibility. According to [15], feeding containing tannin compounds with a level of 1% BK can reduce crude protein digestibility in the rumen as much 29.73%.

Tannins have the potential to protect feed protein from fermentation in the rumen and increase the efficiency of protein utilization by livestock, but the level of tannin levels needs to be considered in the diet so as not to interfere with rumen microbial fermentation. According to [16] the level of tannin levels ranging from 0% to 2% of the diet is not dangerous for consumption. According to [17] the ideal level of condensed tannins in the diet does not exceed 5% dry matter. According to [18] adult cattle can tolerate hydrolyzed tannins at the level of 1.5% to 2.5% only.

In addition to tannin levels, the application of processing technology in the use of tannins can also be considered. The application of feed technology such as pelleting can be done to increase feed consumption and palatability [19, 20], as well as an effort to minimize selective uptake by livestock and increase the completeness of the nutritional value consumed by livestock [21]. Thus, it is hoped that pellets from a combination of acacia, mahogany, and jackfruit leaves can be a solution in protecting feed protein from fermentation in the rumen, without affecting the fermentation of other feed substrates in the rumen.

2. MATERIALS AND METHODS

2.1. Experimental Treatments

This study used three substrate treatments which were differentiated based on the tannin content of the substrate (% DW based). The three substrate treatments in question are described as follows:

Tannin level 0%	=	Forage 60% + Concentrate 40% + Pellet 0%
Tannin level 1%	=	Forage 60% + Concentrate 27.6% + Pellet 12.4%
Tannin level 2%	=	Forage 60% + Concentrate 15.2% + Pellet 24.8%

Pellet feed is used as a substitute feed for some concentrate feeds because pellet feed is a feed whose nutrient content is quite similar to concentrate feed. The pellet feed contains a 9% proportion of soybean meal which acts as a protein source feed supplement that will function to increase the availability of protein nutrients (amino acids) in the post-rumen tract.

2.2. Materials

The laboratory equipment used to determine the nutritional content of each feed ingredient is described by [22], the extraction and determination of the tannin content in the leaves is described by [23, 24], in vitro nutrient digestibility analysis has been described by [25], and the field equipment used to manufacture pellets such as pelletizers and mixers. The substrate samples in this study consisted of forage, concentrate, and pellets. The forage chosen for the manufacture of the substrate is elephant grass (*Pennisetum purpura*). Concentrates for the manufacture of substrates consist of soybean meal, pollard, rice bran, palm oil meal, molasses, premix, and corn cobs. The pellets for making the substrate consisted of a combination of acacia leaf flour, mahogany leaf flour, and jackfruit leaf flour added to feed ingredients in the form of soybean meal, molasses, and tapioca.

2.3. Methods

2.3.1 Sample Preparation

Samples of acacia, mahogany, and jackfruit leaves were taken at Kasuran Wetan, Sayegan, Sleman, Yogyakarta. The leaf samples were separated from the stems and then dried in the sun (36^{o}C) for 15 to 20 hours to dry. The leaves used are young to mature leaves. Elephant grass samples were cut into pieces measuring 2 cm to 3 cm in length. Leaves and grass were dried in an oven at 55^{o}C and mashed with a blender to become a powder which then together with the concentrate constituents of feed ingredients will be used forproximate

analysis based on the method that has been carried out by [22].

2.3.2 Extraction of a combination of leaves from tannin sources

The method applied for the extraction of acacia, mahogany, and jackfruit leaves is based on the method used by [23]. The extraction process uses the Sonoplus Bandelin Ultrasonic tool. The results of the extraction process are stored in a chiller for further use for the determination of tannin levels.

2.3.3 Determination of total, condensed and hydrolyzed tannin levels

The method applied for the determination of total, condensed, and hydrolyzed tannin content is based on the method used by [23] and [24]. The method uses a sample extraction of each leaf as a source of tannin. The results of the analysis of determining the total, condensed, and hydrolyzed tannin levels in sequence, namely for acacia are 5.68; 5.52; and 0.16; for mahogany is 11.69; 9.83; and 1.86; for jackfruit is 7.8; 5.34; and 2.49.

2.3.4 Production of Pellets from Tannin Sources and Protected Protein Sources

The process of making pellets consists of grinding, mixing, pelleting, and drying processes. Pellets are made by the cold method. The grinding process is carried out using a disc mill with a screen size of 0.1 mm. The mixing process is carried out by prioritizing the largest proportion of feed ingredients. The pelleting process is carried out using a die measuring 1.5 cm. The drying process is carried out using sunlight for 4 to 5 hours.

2.3.5 Technique in vitro nutrient digestibility

The process of preparation of rumen fluid, buffer medium, and substrate was carried out before carrying out the in vitro nutrient digestibility technique by [25]. The rumen fluid used came from the rumen of Bali cattle that had been fistulae and were given feed adaptation for 5 days. The method made in the manufacture of the medium according to the method [25]. The medium is then flowed with carbon dioxide gas to maintain anaerobic conditions. Carbon dioxide gas is flowed before the medium is mixed into the rumen fluid. The feed substrate consisted of 3 treatment levels. The substrate used for dry matter digestibility (DMD), organic matter digestibility (OMD), and crude fiber digestibility (CFD) was 250 mg, while the substrate for crude protein digestibility (CPD) was 500 mg. The in vitro nutrient digestibility technique was carried out based on the method that had been carried out [25] in two stages. The first stage describes the state of fermentation in the rumen, while the second stage describes the state of fermentation in the post-rumen tract (abomasum and intestine). The substrate was incubated for 96 hours (48 hours for the first stage and the next 48 hours for the second stage) under anaerobic conditions at 39oC. Yields at each stage will be analyzed to determine DMD, OMD, CFD, and CPD. Analysis in determining DMD, OMD, CFC, and CPD based on the method that has been carried out [22].

2.4. Parameters Observed

The parameters observed were nutrient digestibility in the rumen and total digestibility. The digestibility of the nutrients included of dry matter digestibility, organic matter digestibility, crude fiber digestibility, and crude protein digestibility.

2.5. Data Analysis

Obtained data were analysed by one-way analysis of variance (ANOVA) continued by Duncan's new multiple range test (DMRT). All statistical analysis calculations using The Statistical Product and Service Solution (SPSS) v.17 software.

3. RESULTS AND DISCUSSION

The effect of adding a combination of leaf sources of tannin from *Acacia mangium* Willd, *Swietenia mahagoni*, and *Artocarpus heterophyllus* in pellet form on the parameters of nutrient digestibility in the rumen and total digestibility can be observed in Table 1.

Table 1. The effect of adding combination leaves tanninsource to pellets on the digestibility of feed nutrients in the rumen and total in vitro.

Nutrient Digestibility	Level of Tannin (% DW based)		
	0%	1%	2%
In Rumen			
DMD ^{ns}	52.43±2.29	54.32±0.94	53.33±3.27
OMD ^{ns}	51.69±1.28	53.77±0.35	52.38±2.93
CFD ^{ns}	46.85±4.84	48.70±0.80	54.72±0.38
CPD	63.82^{c}±3.49	51.73^{b}±2.99	40.51^{a}±1.01
Total			
DMD ^{ns}	55.59±2.52	57.49±1.86	59.67±2.99
OMD ^{ns}	54.64±2.84	55.30±0.31	56.48±0.12
CFD ^{ns}	49.32±3.19	50.36±1.85	55.33±2.60
CPD	62.89^{a}±2.62	69.75^{a}±3.66	82.10^{b}±4.11

^{ns} : Non-significant (P>0.05),

^{abc} : Different superscripts in the same column showedsignificant differences (P<0.05),

[1)]DMD, dry matter digestibility; OMD, organic matter digestibility; CFD, crude fiber digestibility; CPD, crude protein digestibility.

3.1. Digestibility of Nutrients in the Rumen

The addition of combination leaves tannin sources in pellet with tannin treatment level of up to 2% did not significantly affect DMD, OMD, and CFD in the rumen (P>0.05) and was significantly able to reduce CPD in the

rumen (P<0.05). The absence of significant changes in DMD, OMD, and CFD was suspected because the level of addition of tannins up to a level of 2% could still be tolerated by rumen microbes to degrade other organic compounds (except protein). According to [26], tannin supplementation can provide two different responses.The positive response is that when tannins at optimum levels from certain plants can increase the activity of the glutamate ammonium ligase enzyme and also suppress the protozoan population, so that the degradation of fibrous feed will increase. The negative response is when the addition of high levels of tannins will cause digestive disorders and decreased digestibility. The absence of a significant effect on DMD and OMD also occurred in a study [27] that used pelleted tannins from acacia, mahogany, and jackfruit leaves with a tannin level of up to 2% in the gast test method. The same study was also able to reduce the number of protozoa in the rumen by up to 35%. This can be the basic factor for the incidence of increasing CFD number at the addition of 1% tannin level by 3.95% from 0% tannin level (control), and at increasing 2% tannin level by 16.8% from control. Although there was no significant change from CFD when the tannin pellet was added to a level of 2%.

The results of the analysis showed that there was a significant decrease in CPD in the rumen significantly. The addition of 1% tannin level decreased CPD by 18.94% from the control, while the addition of 2% tannin level decreased CPD by 36.52% from control. This is due to polyphenolic tannin compounds that can bind strongly to feed protein in rumen conditions [28], so that the work of protease enzymes from proteolytic bacteria will decrease in degrading feed protein in the rumen. Research results [27] using 1% and 2% tannins from a mixture of acacia, mahogany and jackfruit leaves were able to significantly reduce the activity of protease enzymes by 6.88% and 36.31%, respectively. The decrease in CPD also occurred in a study [29] that used tannin compounds from the leaves of *Psidium guajava*, *Ficus bengalensis*, and *Ficus infectoria* from levels 1 to 3% added to the feed to reduce CPD in the rumen by 13% to 49% in vitro. According to [28], the formation of a strong bond of tannin with protein is influenced by several factors, namely the concentration of tannin, the isoelectric point of the protein, pH, and protein chemistry (the higher the proline content in the protein, the more easily it reacts with tannin).

The use of tannins from a combination of acacia, mahogany, and jackfruit leaves in pellets up to a tannin level of 2% in the diet is still in a good range to be given to livestock. The addition of tannin pellets was able to effectively bind protein without disturbing the degradation of the feed substrate in the rumen by the rumen microbial population. [30] argues that a balanced microbial population in the rumen is important for optimizing the use of organic matter in feed.

3.2. Total Nutrient Digestibility

The addition of combination leaves tannin sources in pellet with tannin treatment level of up to 2% did not significantly affect DMD, OMD, and CFD in total (P>0.05) and significantly increased CPD in total (P<0.05). The results of the total digestibility of DMD and OMD in this study showed that the effort to protect protein by tannin compounds in pellets gave a positive response. This positive response is illustrated by the protection of specific nutrients (amino acids from soybean meal) without reducing the level of degradation of feed organic matter, so that the microbial population in the rumen is not disturbed and absorption of specific nutrients from the digestive processes in the rumen and post-rumen can be optimally absorbed. [28] stated that in general the enzyme response to tannins varies greatly depending on the affinity of the enzyme for tannins, so it cannot be predicted.

The addition of leaf combination pellets from tannin sources up to a level of 2% did not significantly reduce the total CFD. This result is directly proportional to the CFD in the rumen. This is presumably because the administration of tannins up to a level of 2% in this study can still be tolerated by the fiber-digesting enzymes so that they do not interfere with the hydrolysis of the fiber-digesting enzymes. [28] in his research showed that low concentrations of tannins will increase the circular structure of the enzyme thereby increasing its catalytic activity. Enzymes vary greatly in their affinity for tannins so that the potential effect of tannins on enzymes cannot be predicted.

The results of the analysis shown in Table 1. indicate that the addition of a mixture of acacia, mahogany and jackfruit leaf pellets as a source of tannins with a level of 1% did not increase the total CPD. Giving pellets with 1% tannin level increased the total CPD, which was 10.91% from the control. Meanwhile, the 2% tannin level significantly increased the total CPD value (P<0.05) against the control. Giving pellets with a tannin level of 2% can increase the total CPD as much as 30.55% of the control (62.89%). This is due to the hydrolysis of strong bonds between tannins and feed protein at a pH of less than 3.5 (as in the abomasum) or greater than 8 (as in the duodenum) [11]. In this study, the administration of a mixture of leaf pellets with a tannin source of up to 2% in this study still had a beneficial effect on livestock because it did not reduce the hydrolysis ability of enzymes in the post-rumen tract.

4. CONCLUSION

In conclusion addition leaves tannin source from *Acacia mangium* Willd, *Swietenia mahagoni,* and *Artocarpus heterophyllus* into pellet with tannin level up to 2% on substrate, was able to reduce ruminal CPD and

increase CPD total without any effects on the DMD, OMD, and CFD.

AUTHORS' CONTRIBUTIONS

Lies Mira Yusiati: conceived and designed the experiments and study. Chusnul Hanim, Muhlisin, dan Muhsin Al Anas: designed the experiments and analysed the paper. Rofi Prima Maulana: performed the experiments, analyzed the data, wrote and presented the paper.

ACKNOWLEDGMENTS

The author acknowledges the Ministry of Research and Technology-National Research and Innovation Agency of the Republic of Indonesia for financial support in the decentralization program of "*Penelitian Terapan Unggulan Perguruan Tinggi* (PTUPT)"

REFERENCES

[1] H. M. G. P. Herath, S. J. Pain, P. R. Kenyon, H. T. Blair, and P. C. H. Morel, Effect of dietery protein to energy ratio of milk replacer on growth and body composition of pre-weaned lambs reared artificially, Animal feed science and technology, volume 264, 2020. DOI: https://doi.org/10.1016/j.anifeedsci.2020.114478

[2] Y. Elfassy, J. P. Bastard, C. McAvoy, S. Fellahi, J. Dupont, and R. Levy, Adipokines in semen: physiopathology and effects on spermatozoa, Int J Endocrinol, Hindawi, London, United Kingdom, 2018, pp. 1-11. DOI: https://doi.org/10.1155/2018/3906490

[3] K. M. McRae, M. J. Stear, B. Good, and O. M. Keane, "The host immune response to gastrointestinal nematode infection in sheep", Parasite Immunology 37(12) (2015) 605-613. DOI: http://doi.org/10.1111/pim.12290

[4] P. McDonald, R. A. Edward, and J. F. D. Greenhalgh, Animal Nutrition. 4th Edition, John Wiley and Sons,New York, USA, 2002.

[5] B. K. Ahring, N. Murali, and K. Srinivas, "Fermentationof cellulose with a mixed microbial rumen culture with and without methanogenesis", Fermentation Technology 7(1) (2018) 1-7. DOI: http://doi.org/10.4172/2167-7972.1000152

[6] X. Gao and M. Oba, "Effect of increasing dietary nonfiber carbohydrate with starch, sucrose or lactose on rumen fermentation and productivity of lactating dairy cows", Journal of Dairy Science 99(1) (2016) 291-300. DOI: https://doi.org/10.3168/jds.2015-9871

[7] L. Hanun, A. Muktiani, and L. K. Nuswantara, "The effect of using complete feed silage made from water hyacinth on protein digestibility in sheep", MEDIAGRO 15(1) (2019) 12-19, DOI: http://dx.doi.org/10.31942/mediagro.v15i1.3066

[8] A. Belance, M. Doreau, J. E. Edwards, J. M. Moorby, Eric Pinloche, and C. J. Newbold, "Shifts in the rumen microbiota due to the type of carbohydrate and level of protein ingested by dairy cattle are associated with changes in rumen fermentation", The Journal of Nutrition 142(9) (2012) 1684-1692.

[9] N. Todorov, M. Simeonov, and E. Yildiz, "Rumen degradability of dry matter and protein in four protein sources and their relationships with milk protein yield in dairy cows", Bulgarian Journal of Agricultural Science 22(2) (2016) 278-285. URL: http://www.agrojournal.org/22/02- 18.pdf

[10] I. Mueller-Harvey, Unravelling the conundrum of tannins in animal nutrition and health, Lecture presented at the XXII International Conference on Polyphenols, Helsinki, Finland, 2006, pp. 2010-2037, DOI: https://doi.org/10.1002/jsfa.2577

[11] S. J. Bunglavan and N. Dutta, "Use of tannins as organic protectants of proteins in digestion of ruminants", J. Livestock Science volume 4 (2013) 67-77. URL: http://livestockscience.in/wp-content/uploads/Buglavan_tannin.pdf

[12] W. T. Sasongko, L. M. Yusiati, and Z. Bachruddin, Mugiono, "Optimalisation binding of jackfruit leavestannin with bovine serum albumin protein", Buletin Peternakan 34(3) (2010) 154-158. DOI: https://doi.org/10.21059/buletinpeternak.v34i3.84

[13] L. M. Yusiati, A. Kurniawati, C. Hanim, and M. A. Anas, "Protein binding capacity of different forages tannin", IOP Conference Series: Earth and Environmental Science 199 (2018). 1-5. DOI: https://doi.org/10.1088/1755-1315/119/1/012007

[14] J. Zhang, X. Xu, Z. Cao, Y. Wang, H. Yang, A. Azarfar, and S. Li, "Effect of different tannin sources on nutrient intake, digestibility, performance, nitrogen utilization, and blood parameters in dairy cows", Animals 9(8) 2019 507. DOI: https://doi.org/10.3390/ani9080507

[15] Nugroho, Black tea waste (*Camelia sinensis*) as a source of tannins for protein protection from rumen microbial degradation in vitro, Thesis of Animal Husbandry, Faculty of Animal Husbandry, Universitas Gadjah Mada, Yogyakarta, 2011

[16] B. W. Norton, Tree legumes as dietary supplements for ruminants, In: Forage Tree Legumes in Tropical Agriculture, Wallingford, Oxford: CAB International, 1994, pp.192-201. URL: https://www.cabdirect.org/cabdirect/abstract/19941400418

[17] H. D. Naumann, L. O. Tedeschi, W. E. Zeller, and N. F.Huntly, "The role of condensed tannins in ruminant animal production: advances, limitation, and future directions", Brazilian Journal of Animal Science 46(2) (2017) 929-949. DOI: https://doi.org/10.1590/s1806-92902017001200009

[18] C. Lu, X. Luo, L. Lu, H. Li, X. Chen, and Y. Ji, "Preliminary extraction of tannins by 1-butyl 3-methylimidazole bromide and its subsequent removal from *Galla chinensis* extract using macroprouns resins", Journal Animal Science 46(5) (2013) 959-964. DOI: https://doi.org/10.1002/jssc.201200679

[19] Winarto, N. Irwani, and S. Kaffi, "Optimization on manufacturing of the elephant grass (*Pennisetum purpurium*) pellets as export opportunities for ruminant feed", Jurnal Ilmiah Teknik Pertanian 6(2) (2014) 71-142. DOI: https://doi.org/10.25181/tektan.v6i2.892

[20] K. C. Behnke, "Feed manufacturing technology: current issues and challenges, Pasific northwest animal nutrition conference", Anim. Feed Sci. Tech, Elsevier 62(1) (1996) 49-57. DOI: https://doi.org/10.1016/S0377-8401(96)01005-X

[21] T. Nurhayatin and M. Puspitasari, "The effect of processing method of arrow root tuber (*Maranta arundinacea*) as binder and length of storage time on physical quality pellet feed for chicken broiler", JANHUS 2(1) (2017) 32-40. DOI: http://dx.doi.org/10.52434/janhus.v2i1

[22] AOAC, Official method of analysis of the association of official analitycal chemist, 18th edition, Maryland: AOAC International. William Harwitz (ed.), United Stated of America, 2005. DOI: https://doi.org/10.1002/0471740039.vec0284

[23] Food and Agriculture Organization (FAO_/IAEA, Working document (lab manual0, Quantification oftannins in tree foliage, Vienna, IAEA, 2000. https://pdf4pro.com/amp/view/quantification-of-tannins-in-tree-foliage-iaea-1f6412.html

[24] L. J. Porter, L. N. Hrstich, and B. G. Chan, "The conversion of procyanidins and prodelphinidins to cyaniding and delphinidin", Phytochemistry, no.25, (1986) 223-230. DOI: http://dx.doi.org/10.1016/S0031-9422(00)94533-3

[25] J. M. A. Tilley and R. A. Terry, "A two-stage techniquefor the in vitro digestion of forage crops", The journalof the british grassland society 18(2) (1963) 104-111. DOI: http://dx.doi.org/10.1111/j.1365-2494.1963.tb00335.x

[26] A. Jayanegara, M. Ridla, E. B. Laconi, and Nahrowi, Komponen Antinutrisi pada Pakan, IPB Press, Bogor, 2019.

[27] W. A. Setiawan, L. M Yusiati, C. Hanim, Muhlisin. The effect of mixed leaves tannin sources (*Acacia mangium* Willd, *Swietenia mahagoni* and *Artocarpus heterophyllus*) in pellets on in vitro methane production, Proceedings of the 6th International Seminar of Animal Nutrition and Feed Science (ISANFS 2021), Atlantis press, volume 21,2022, pp. 270-274. DOI: https://doi.org/10.2991/absr.k.220401.055

[28] B. Adamczyk, J. Simon, and V. Kitunen, "Tannins and their complex interaction with different organic nitrogen compounds and enzymes: old paradigms versus recent advances", Chemistry Open 6(5) (2017) 610-614. DOI: https://doi.org/10.1002/open.201700113

[29] D. Manish, D. Narayan, K. Sharma, A. K. Pattanaik, P. S. Banerjee, M. Singh, Effect of condensed tannins supplementation from tanniferous tree leaves on in vitro nitrogen and substratedegradation, Animal Nutrition and Feed Technology, volume 11, issue 1, 2011, pp. 115-122. URL: https://www.researchgate.net/publication/259327170_Effect_of_Condensed_Tannins_Supplementation_from_Tanniferous_Tree_leaves_on_In_vitro_Nitrogen_and_Substrate_Degradation

[30] M. Wodzicka, A. Tomaszewska, A. Djajanegara, S. Gardines, T. R. Wiradarya, and I. M. Mastika, Sheep production in Indonesia, UNS Press Surakarta, Surakarta, 1993.