The effect of additional tannins source from Mahagony leaves (*Swietenia mahagoni*) to purine derivate exression in urine and synthesis of rumen microbial protein of Merino sheep

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**Abstract.** This study aimed to determine the effect of adding mahogany (*Swietenia mahagoni*) leaves as a source of tannins on purine derivatives (DP) excretion in urine and rumen microbial protein synthesis of merino sheep. This study used 12 sheep with an average body weight of 32.46 ± 5.186 kg which were given additional tannin from mahogany leaves in the feed. The data were analyzed using a completely randomized design analysis unidirectional pattern, with three different levels of tannin treatment (0%, 1.5%, and 3%) and four replications. The feed given during the research process was in the form of forage and concentrate on a ratio of 60% and 40%. The administration was given 2 times a day with a ratio of 60% and 40% in the morning and evening. There was no significant effect (P >0.05) on the addition of Mahogany leaves for the excretion of purine derivatives in urine and microbial protein synthesis of the rumen of Merino sheep.

1. Introduction

Protein is essential for livestock growth and production. Additional feed in the form of a protein source concentrate is a solution to prevent protein deficiency. Whereas protein in ruminants is not only needed by the livestock itself, but also for microbes in the rumen. Feed protein degradation in ruminants also occurs in the rumen for the growth of rumen microbes. In this case, the role of microbes is very important to perform feed protein digestibility in rumen.

However, the high use of protein in the rumen leads to a lack of protein in post-rumen digestion. The use of protein in the rumen and subsequent digestion must be balanced, therefore feed protein needs to be protected from microbial degradation in the rumen but should not interfere with microbial protein synthesis. This protein protection can be done by using secondary metabolites from plants, namely tannins.

Tannins are secondary metabolites found in some plants. Tannins complexes can bind to proteins, so that proteins can be resistant to degradation by protease enzymes in the silo or rumen [1]. Protein tannin complexes are not easily degraded by microbes but can be directly absorbed by the walls of the post-rumen digestive tract as a source of protein which vary from 40% to 60% [2]. A great source of tannins is from mahogany plants. The total amount of tannin content in mahogany leaves extracted with ethanol was calculated to be quite high, namely 325.91 ± 7.28 mg/g of dried mahogany leaves [3].
Thus, this study aimed to determine the effect of mahogany leaves addition as a source of tannins on the excretion of purine derivatives and rumen microbial protein synthesis in merino sheep.

2. Material and methods

2.1. Materials
The livestock used in this study were 12 male merino sheep with an average body weight of 32.46 ± 5.186 kg and 12 months of age. They were fed by king grass and concentrate. Other materials used were mahogany leaves as a source of tannins, distilled water, $\text{H}_2\text{SO}_4$ 10%, and chemicals for purine derivative tests (creatinine, allantoin, uric acid, and xanthine-hypoxanthine).

2.2. Method
Mahogany leaves were dried under the sun for 2 days. The dried mahogany leaves were ground using a grinder to become flour for further analysis.

2.2.1. Adaptation period. The livestock was weighed at the start of the adaptation period. The adaptation period was carried out for 14 days. The first feeding was given with 3% of body weight with a proportion of forage and concentrate of 60% and 40%. The provision of water was ad libitum. The feed was given 2 times a day, namely in the morning at 07.00 and in the afternoon at 15.00.

2.2.2. Collection period. At the beginning of the collection period, livestock was weighed to determine the initial weight during the collection period. The collection period was carried out for 7 days. Morning feed concentrate was added with tannins from mahogany leaves flour with different levels (0%, 1.5%, and 3%). During the collection period, samples of feed (forage and concentrate), feed residue, feces, and urine were taken every day. The samples were weighed of 50 g feed samples, while the remaining feed and feces were 10% of the total excretion. The urine sample were collected once a day (24 hours). The volume of the collected urine was measured, then 10% of the total volume was taken, and added with $\text{H}_2\text{SO}_4$ until the pH changes below 3. Each urine sample was stored in a 50 ml bottle, and kept in the refrigerator until analysis time.

The measurement of purine derivatives carried out was in the form of allantoin, uric acid, xanthine-hypoxanthine using spectrophotometric method by adopting the Yang and Conway method [4]. The amount of microbial DP absorbed ($X$, mmol/day) related to the excretion of DP in urine ($Y$, mmol/day) was calculated based on the equation [5]:

$$ Y = 0.84X + (0.150W^{0.75} e^{-0.25X}) $$

Equation $W^{0.75}$ is a metabolic live weight. The estimation of microorganism nitrogen synthesis in g/day was calculated according to this equation [5]:

$$ \text{EMNS} = \frac{70X}{0.116 \times 0.83 \times 1000} = 0.727X $$

3. Results and discussion
In this experiment, merino sheep were given fed including king grass, concentrate, and tannins from mahogany leaves with different levels (0%, 1.5%, and 3%). The chemical composition of king grass were 17.96% of dry matter (DM), 6.85% of crude protein (CP), 32.26% of crude fiber (CF) and 42.53% of total digestible nutrient (TDN). The chemical composition of concentrate were 89.32% of dry matter (DM), 15.18 % of crude protein (CP), 18.06% of crude fiber (CF) and 61.44% of total digestible nutrient (TDN). The chemical composition mahagony leaves were 88.59% of dry matter (DM), 12.61% of crude protein (CP), 26.56% of crude fiber (CF), 47.35% of total digestible nutrient (TDN), and 10.62% tannins.
3.1. Urinary purine derivatives excretion

Table 1 presents the amount of excretion of purine derivatives and levels of purine derivatives of merino sheep which were given different levels of tannins. The analysis of purine derivatives in the urine of merino sheep showed that there was no significant difference (P > 0.05) between the tannin treatments at 0%, 1.5%, and 3% levels. The application of 1.5% tannins gave the greatest effect amongst other level of 0% and 3% tannins addition. Purine derivatives consist of allantoin, uric acid, and xanthine-hypoxanthine. Tannins at the 1.5% level showed the highest value on allantoin and xanthine-hypoxanthine levels, while tannins at the 0% level performed the highest value on uric acid levels.

Tannin influences the digestion process of protein due to its ability form complex bonds with proteins during neutral conditions (tend to be the same as rumen pH). The complex bonds between protein and tannins are difficult to degrade by the protease enzyme in the rumen, causing digestibility in the rumen to decrease and increase post-rumen digestibility (protein by-pass). The higher the tannins are given, the lower the levels of purine derivatives produced. This happens due to tannins can inhibit microbial protein synthesis, so that the levels of purine that are absorbed and excreted through urine will decrease. The absorbed purines derived from microbes are extensively degraded and subsequently excreted as a product. Nitrogen from microbes in livestock in ruminants can be estimated from the excretion of purine derivatives in urine, which assumes that the purine absorbed in the duodenum with the excreted purine derivatives is constant [6].

The 1.5% and 3% tannins addition showed non-significant (P >0.05) purine derivatives. This happened because of giving too high tannins can reduce the level of livestock consumption. Tannins have bitter taste which then lead to lower livestock’s palatability. Tannins are polyphenol group compounds that have anti-nutritional abilities where the effect of tannins on ruminants begins with the mastication process. Tannins tends to bind salivary protein so that feed is not preferred, thus feed consumption decreases [2]. One of the factors that influence rumen microbial protein synthesis is feed consumption [5].

### Table 1. Excretion and concentration purine derivatives of Merino sheep with different levels of tannins addition.

| Parameter                                      | Tannins level (%) |
|------------------------------------------------|-------------------|
| Excretion of purine derivatives (μ/BW^{0.75}/head/day) |                   |
| Allantoin ns                                   | 18.88±4.54        |
| Uric acid ns                                   | 13.25±3.50        |
| Xanthine-hypoxanthine ns                       | 3.77±0.95         |
| Purine derivatives ns                          | 35.90±8.67        |
|                                           | 16.42±7.08        |
|                                           | 9.04±1.77         |
|                                           | 3.08±1.06         |
|                                           | 28.54±9.07        |
|                                           | 17.58±12.71       |
|                                           | 9.37±6.33         |
|                                           | 3.26±2.15         |
|                                           | 30.20±21.09       |
| Excretion of purine derivatives (μ/BW^{0.75}/L) |                   |
| Allantoin ns                                   | 11.33±1.58        |
| Uric acid ns                                   | 7.91±0.96         |
| Xanthine-hypoxanthine ns                       | 2.24±0.09         |
| Purine derivatives ns                          | 21.48±2.23        |
|                                           | 13.49±4.18        |
|                                           | 7.56±1.52         |
|                                           | 2.44±0.15         |
|                                           | 23.49±5.06        |
|                                           | 17.58±12.71       |
|                                           | 9.37±6.33         |
|                                           | 3.26±2.15         |
|                                           | 30.20±21.09       |
|                                           | 12.17±1.95        |
|                                           | 6.68±0.86         |
|                                           | 2.34±0.39         |
|                                           | 21.19±2.21        |

ns Not significantly different.

3.2. Rumen microbial protein synthesis

Table 2 presents the results of rumen microbial protein synthesis which were estimated through excretion of purine derivatives in the urine of merino which were given different levels of tannins. The results of rumen microbial protein synthesis which were estimated through excretion of purine derivatives in the urine of merino sheep showed that there was no significant difference (P > 0.05) between the treatment of tannins at the level of 0%, 1.5%, and 3%. Application of tannins at the 0%
level had the greatest effect on rumen microbial protein synthesis than the application of tannins at the 1.5% and 3% levels.

Giving too high tannins level addition can reduce rumen microbial protein synthesis. Protein is the source of nitrogen for microbial protein synthesis, therefore when the protein are protected by tannins, rumen microbes are prone to the condition. Microbial protein synthesis requires the availability of nitrogen in the form of ammonia (a result of protein degradation in the rumen) and energy (derived from carbohydrate sources). The feed protein is a very decisive component for the production of microbial protein synthesis because feed protein indicates the availability of elemental N for rumen microbes [7].

Purine derivatives can be used to estimate rumen microbial protein synthesis. In principle, the nucleic acids that in the post-rumen are originally from rumen microbes. All feed nucleic acids will be degraded in the rumen as a result of microbial fermentation in the form of microbe biomass. The nucleic acid which mainly absorbed is purine. Later, purine will then be excreted as its derivatives, namely xanthine, hypoxanthine, uric acid, and allantoin. Therefore, the excretion of purine derivatives is directly related to the absorbed purine so that it can be used to estimate the microbial protein synthesized [5].

Table 2. Rumen microbial protein synthesis which were estimated through excretion of purine derivatives (EMNS) with different levels of tannins.

| Parameter         | Tannins level (%) |
|-------------------|-------------------|
|                  | 0                 | 1.5               | 3                 |
| DOM (g/day)       | 477.13 ± 74.80    | 308.06 ± 101.43   | 417.88 ± 48.52    |
| DOMR (g/day)      | 310.13 ± 48.62    | 200.24 ± 65.93    | 271.62 ± 31.54    |
| EMNS (mmol/day)   | 0.22 ± 0.11       | 0.12 ± 0.10       | 0.13 ± 0.25       |
| EMNS/DOMR (g N/kg DOMR) | 0.71 ± 0.39 | 0.52 ± 0.45 | 0.47 ± 0.88 |

Not significantly different.

4. Conclusion
Based on the research, it can be concluded that there was no significant effect on the addition of mahogany leaves (Swietenia mahagoni) as a source of tannins for the excretion of purine derivatives in urine and microbial protein synthesis of the rumen of merino sheep. Further research is needed on the factors that can influence the excretion of purine derivatives and rumen microbial protein synthesis.

Acknowledgement
The authors would like to thank to Universitas Gadjah Mada for the grant under the scheme of Rekognisi Tugas Akhir (RTA) 2020.

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