INTRODUCTION

The primary source of protein for ruminants comes from microbial protein and rumen undegradable protein (Ørskov, 1992; Das et al., 2014; Ruzic-Muslic et al., 2014). About 50-85% of the protein requirements of ruminants are supplied from microbial protein (Dewhurst et al., 2000; Suryapratama and Suhartati, 2012; Hackmann and Firkins, 2015). Rumen microbes can degrade more than 60% of the protein to produce amino acids followed by deamination to NH3, and some that are not degraded are categorized as rumen undegradable protein (Kamalak et al., 2005; Liu et al., 2019). Although NH3 is indispensable for microbial protein synthesis (Pathak, 2008; Suryani et al., 2020), excess NH3 of more than 50 mg NH3-N/L is not utilized for microbial protein production and is excreted in the form of urea (Satter and Slyter, 1974; Fattah et al., 2019; Neto et al., 2019). High-quality protein is needed to provide amino acids to support ruminant productivity. Protein protection is an effort to reduce protein degradation by rumen microbes (Atole and Bestil, 2014). Moreover, protein protection will increase the amount of protein digested in the intestinal tract, which is considered a rumen undegradable protein (Boucher et al., 2009; Arisya et al., 2019). According to Ganai et al. (2019) and Singh et al. (2019), protein protection is carried out by heat treatment, chemical
treatment, encapsulation, and the use of secondary metabolite compounds such as tannins.

Tannin is a natural plant compound that can form complex bonds with proteins (Yusiati et al., 2018; Mahanani et al., 2020). The protein-tannin complex protects the protein from rumen degradation; therefore, it is more readily available in the post-rumen gastrointestinal tract (Arisya et al., 2019; Fitriastuti et al., 2019). One of the plants that contain tannins is Swietenia mahagoni. According to Naveen and Urooj (2015) Swietenia mahagoni contains 94 µg/mg of tannins. Based on the description, this study aims to determine the effect of Swietenia mahagoni on rumen in-vitro fermentation, rumen hydrolytic enzyme activity, and nutrient digestibility.

MATERIALS AND METHODS

ETHICAL APPROVAL

This experimental study was approved by the Research Ethics Committee of the Faculty of Veterinary Medicine, Universitas Gadjah Mada, Yogyakarta, Indonesia with approval number: 00069/EC-FKH/Eks./2021.

SAMPLES COLLECTION AND PREPARATION

Elephant grass (Pennisetum purpureum) and Swietenia mahagoni leaves were dried at 55°C for three days and ground to pass a 1 mm screen for chemical composition and tannin analysis. Proximate analysis was performed using the AOAC (2005) method. Tannin levels were analyzed as reported by Makkar et al. (1993).

In-vitro Fermentation

Two thin-tailed sheep were fed elephant grass, pollard, and soybean meal (forage: concentrate, 70:30) for feed adaptation. Feed was given two times for seven days before the sheep were slaughtered. The rumen fluid was taken by slaughtering sheep. In-vitro fermentation was conducted using gas production (Menke and Steingass, 1988) and two steps Tilley and Terry method for 96 h (Tilley and Terry, 1963). The syringe was filled with a substrate, which was feed materials (elephant grass, pollard, and soybean meal), and in addition to Swietenia mahagoni leaves at a rate of 0, 3, and 6% tannin content. The proportions of forage and concentrate feed are presented in Table 1. At the end of the 48-h incubation, the fermentation product was filtered, and the residue was used to determine nutrient digestibility. Rumen fluid was used for the measurement of pH and protozoa populations (Diaz et al., 1993). The rumen fluid was centrifuged (3000 g/10 min) to determine ammonia concentration (Chaney and Marbach, 1962), microbial protein (Plummer, 1987), and volatile fatty acid (VFA) (Filípek and Dvořák, 2009). The supernatant was then centrifuged (10,000 g/10 min) to separate the microbial cells and the supernatant containing the enzyme.

The measurement of amylase, CMC-ase, β-glucosidase was carried out according to the method of Bergmeyer and Gawehn (1974) and protease using the method of Halliwell (1961). Post-rumen digestibility was measured after 48 h of incubation; three mL of 20% HCL and one mL of 5% pepsin were added and incubated for another 48 h. The syringe was filtered, and the residue was analyzed for DM, OM, and CP to obtain the digestibility of DM, OM, and CP.

Table 1: Proportion of dietary treatment.

<table>
<thead>
<tr>
<th>Feed ingredients</th>
<th>Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>T1</td>
</tr>
<tr>
<td>Soybean meal</td>
<td>25.58</td>
</tr>
<tr>
<td>Pollard</td>
<td>4.42</td>
</tr>
<tr>
<td>Forage</td>
<td></td>
</tr>
<tr>
<td>Elephant grass</td>
<td>70.00</td>
</tr>
<tr>
<td>Swietenia mahagoni</td>
<td>0.00</td>
</tr>
<tr>
<td></td>
<td>100.00</td>
</tr>
</tbody>
</table>

DATA ANALYSIS

Data were analyzed using one-way analysis of variance (ANOVA) with the statistical models is as follows:

\[ Y_{ij} = \mu + \alpha_i + \varepsilon_{ij} \]

Where; \( Y_{ij} \) is the observation, \( \mu \) is the overall mean, \( \alpha_i \) is the effect of tannin level at 0, 3, and 6%, and \( \varepsilon_{ij} \) is the residual effects. Different between means value were tested using Duncan’s Multiple Range Test (DMRT) (Gomez and Gomez, 1984) and \( P < 0.05 \) was used to declare the level of statistical difference.

RESULTS AND DISCUSSION

Effect of Swietenia mahagoni on Rumen Fermentation Parameters

The effect of using 3 and 6% Swietenia mahagoni, as a source of tannins, significantly (\( P < 0.05 \)) decreases rumen’s pH (0.43%) ranging from 6.96 to 6.99 (Table 2). Even though it has decreased due to treatment, the pH value is still in the normal range of 6 to 7 (Reis et al., 2014; Sondakh et al., 2017). The pH value of the rumen varies; feed containing much grain will cause a decrease in pH to less than 5.0, while fibrous feed can cause a pH increase to more than 7.0. The pH value of the rumen is influenced by the concentration of feed fiber. The pH value of the rumen affects the production of NH3 and VFA because microbial activity in the rumen is influenced by pH (Russell and Wilson, 1996; Castillo-Gonzalez et al., 2014; Harun and Sali, 2019). Rira et al. (2015) examined various tannin-
Ammonia concentrations using 3 and 6% tannins from *Swietenia mahagoni* significantly (P < 0.05) decreased 17% and 19.88% compared to controls. Ammonia is the result of the degradation of feed protein in the rumen. According to Arisya et al. (2019), 2% tannins from tannic acid, chestnut tannins, *Calliandra calothyrsus*, and *Clidemia hirta* reduced the amount of rumen degradable protein. Tannin compounds in *Swietenia mahagoni* binding feed protein with hydrogen bonds to avoid rumen microbial degradation (Naumann et al., 2017; Chamadia et al., 2020). The feed protein that was not degraded by rumen microbes caused the decrease of NH$_3$ production (Rimbawanto et al., 2017). The reduced concentration of NH$_3$ is evidence of a decrease in the ability of rumen microbes to degrade feed protein, thereby increasing the supply of feed protein to the abomasum and intestines (Addisu, 2016). Aguerre et al. (2016) showed that using tannins from quebracho-chestnut extracts with 1.80% tannins resulted in rumen pH values ranging from 6.44 to 6.38. By providing *Swietenia mahagoni* 3 and 6% tannin levels, the rumen pH value remains optimal for rumen fermentation.

Table 2: Effects of *Swietenia mahagoni* on rumen fermentation parameters.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Tannin levels (%)</th>
<th>0</th>
<th>3</th>
<th>6</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>6.99±0.00°</td>
<td>6.96±0.00°</td>
<td>6.96±0.00°</td>
<td></td>
</tr>
<tr>
<td>NH$_3$ (mg/100 mL)</td>
<td>119.18±0.40°</td>
<td>98.91±0.22°</td>
<td>95.49±0.48°</td>
<td></td>
</tr>
<tr>
<td>Microbial protein (mg/mL)</td>
<td>0.126 ±1.21°</td>
<td>0.113±0.48°</td>
<td>0.109±0.32°</td>
<td></td>
</tr>
<tr>
<td>Protozoa (10$^5$ cells/mL)</td>
<td>9.72±19.84°</td>
<td>52.61±26.25°</td>
<td>36.15±39.69°</td>
<td></td>
</tr>
<tr>
<td>VFA (mMol)</td>
<td>Acetate (C2)</td>
<td>65.58±4.61</td>
<td>65.56±2.10</td>
<td>67.80±4.27</td>
</tr>
<tr>
<td></td>
<td>Propionate (C3)</td>
<td>20.64±1.52</td>
<td>20.43±0.35</td>
<td>21.24±1.44</td>
</tr>
<tr>
<td></td>
<td>Butirat (C4)</td>
<td>10.26±0.92</td>
<td>10.29±0.08</td>
<td>9.43±0.57</td>
</tr>
<tr>
<td></td>
<td>Total VFA</td>
<td>96.49±6.59</td>
<td>96.28±2.29</td>
<td>98.47±5.26</td>
</tr>
<tr>
<td></td>
<td>C2:C3</td>
<td>3.18±0.12</td>
<td>3.21±0.07</td>
<td>3.20±0.11</td>
</tr>
</tbody>
</table>

*a* Different superscript on the same row are differ significantly (P<0.05).

The 3 and 6% tannin use of *Swietenia mahagoni* significantly (P < 0.05) reduced the rumen protozoa population by 34% and 54.65%, respectively (Table 2). Protozoa play an essential role in the degradation of microbial proteins and proteins (Bach et al., 2005; Patel and Ambalam, 2018). According to Russell and Hespell (1981) and Belanche et al. (2016), sheep rumen protozoa can digest protein from bacteria 2.4 to 45 g/day. Tannins are polyphenolic compounds that can bind to protein, thereby reducing protein digestibility in the rumen (Tseu et al., 2020; Unnawong et al., 2021). Low protein degradation reduces the supply of nitrogen sources for microbial growth. The results showed that microbial protein synthesis decreased due to the addition of tannins. The reduced protozoa population is caused by low concentrations of microbial protein and protein degradation in the rumen. Makkar et al. (1995) and Cieslak et al. (2016) reported that the population of protozoa in the incubation medium decreased due to tannin supplementation. The use of 10% Gambier leaves waste from Payakumbuh, and Painan reduced the rumen protozoan population from 11.3 x 10$^9$ cells/mL to 2.3 x 10$^9$ cells/mL and 4.7 x 10$^9$ cells/mL (Ningrat et al., 2016). According to Sarnataro and Spanghero (2020) the use of chestnut tannins or *Stevia rebaudiana Bertoni* decreased 34% and 46% of the rumen protozoa population. The use of 3 and 6% *Swietenia mahagoni* did not affect (P
The ratio of C2 and C3 in this study is not significantly different because there was no increase or decrease in fermented acetate and propionate. According to Kim et al. (2018), the proportion of VFA in rumen fluid varies depending on the composition of the feed consumed. Fermentation of carbohydrates in the rumen produces carbon chains used for rumen microbial protein synthesis and produces VFAs consisting of acetic, propionic, and butyric acid (Nafikov and Beitz, 2007). In a previous study conducted by Sarnataro and Spanghero (2020), the use of chestnut tannins or Stevia rebaudiana Bertoni did not affect the acetate and propionate ratio. Aguerre et al. (2016) showed that using 1.8% quebracho-chestnut tannin extracts did not affect the acetate to propionate ratio.

**Effect of Swietenia mahagoni on rumen hydrolytic enzymes activity**

Rumen hydrolytic enzyme activity with the addition of Swietenia mahagoni was observed in Table 3. The use of 3% and 6% Swietenia mahagoni tannins significantly (P < 0.05) reduced the activity of the enzyme β-Glucosidase 16.78 and 70.44% compared to controls. Mahanani et al. (2020) showed that the addition of 10 and 25% L. leucocephala leaves decreased 12.5 and 62.5% CMCase activity. Aguerre et al. (2016) showed that using 1.8% quebracho-chestnut tannin extracts did not affect the acetate to propionate ratio.

Table 3: Effect of Swietenia mahagoni on rumen hydrolytic enzymes activity.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Tannin levels (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td>β-Glukosidase (U/g)</td>
<td>71.45±0.58ab</td>
</tr>
<tr>
<td>CMC-ase (U/g)</td>
<td>6.10±0.24ab</td>
</tr>
<tr>
<td>Amylase (U/g)</td>
<td>13.68±0.05ab</td>
</tr>
<tr>
<td>Protease (U/g)</td>
<td>26.18±0.25ab</td>
</tr>
</tbody>
</table>

abc Different superscript on the same row are differ significantly (P < 0.05).

The use of 6% tannins from Swietenia mahagoni significantly (P < 0.05) decreased 8.6% activity of the amylase enzyme (Table 3). da Silva et al. (2014) showed that using A. mearnsii tannin up to 300 μg/mL, the enzyme activity decreased almost linearly with concentration. According to Goncalves et al. (2011), condensed tannins from grape seeds inhibit the amylase enzyme activity by forming stable interactions between tannins and enzymes. In addition, the decrease in amylase enzyme activity can be caused by a decrease in the population of amylolytic bacteria. According to Carrasco et al. (2017), amylolytic and saccharolytic rumen bacteria decreased with dietary treatment of chestnuts and quebracho tannins, especially the genera Prevotella and Treponema.

The protease enzyme activity significantly (P < 0.05) decreased by 31 and 66% with the addition of 3 and 6% tannins from Swietenia mahagoni (Table 3), respectively. Previous studies using 400 μg/mL tannins reduced the protease enzyme activity by 86% (Zaidi-Yahiaoui et al., 2008). The loss of protease enzyme activity is caused by tannins inhibiting the binding site of the substrate, catalytic site, or both, thereby reducing their proteolytic activity.
activity. Additionally, tannins caused enzyme inhibition via allosteric denaturation rather than single-site inhibition, where multiple allosteric binding causes conformational changes and leads to loss of active conformation (Velickovic and Stanic-Vucinic, 2018). The reduced population of a decrease in the activity of the protease enzyme. Molan et al. (2001) and Smith et al. (2005) explained that the use of Lotus corniculatus condensed tannins reduced the population of four proteolytic bacteria. According to Min et al. (2002), bacteria can be directly inhibited by tannins that interact with membranes, cell walls, extracellular proteins, but tannins have an indirect effect by making nutrients unavailable.

**Effects of Swietenia mahagoni on in-vitro rumen nutrient digestibility**

The effect of *Swietenia mahagoni* on the digestibility of crude protein, organic matter, and dry matter in in-vitro rumen was shown in Table 4. The use of 3 and 6% tannins levels significantly (P < 0.05) reduced the digestibility of CP by about 21.30 and 32.57%, respectively compared to control in the rumen. Tseu et al. (2020) reported that the addition of 2.25% tannins from *Acacia mearnsii* linearly decreased the CP digestibility of cows by about 16.50%. Unnawong et al. (2021) showed that the use of 0.6% *Sesbania grandiflora* as a tannin source also reduced CP digestibility by 5.39%.

<table>
<thead>
<tr>
<th>Digestibility</th>
<th>Tannin levels (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td>Crude Protein (%)</td>
<td>53.57±0.08a</td>
</tr>
<tr>
<td>Organic Matter (%)</td>
<td>45.27±0.53a</td>
</tr>
<tr>
<td>Dry Matter (%)</td>
<td>44.35±0.75a</td>
</tr>
</tbody>
</table>

abc Different superscript on the same row are differ significantly (P<0.05).

Based on the data in Table 4, the digestibility of OM and DM in the rumen significantly (P < 0.05) decreased with the addition of *Swietenia mahagoni* at the 6% tannin level of 15.99 and 18.98%. Tseu et al. (2020) showed that cows’ OM and DM digestibility decreased significantly with the addition of 2.25% tannins from *Acacia mearnsii*. Other studies have also shown that the addition of tannins from various sources such as *Quercus persica*, *Pistachio vera*, *Acacia mearnsii*, and *Quebracho* reduces OM and DM digestibility in feed fermentation in the rumen (Kozloski et al., 2012; Mohammadabadi and Chaji, 2012; Attia et al., 2013). Anas et al. (2015) showed that the addition of 6% Albizia chinensis as a source of tannins decreased 16.08% of OM digestibility and 17.51% DM digestibility.

Decreased digestibility of OM and DM is associated with decreased digestibility of proteins, which are parts of dry matter and organic matter. The decreased digestibility of DM and OM was caused by reducing the digestibility of dry matter and organic matter of other compounds such as carbohydrates and fats. In addition, tannins can form strong complexes with proteins, and other macromolecules such carbohydrates and lipids become unusable by rumen microbes; otherwise, tannins bind to microbial enzymes modulating their activity causing a decrease in digestibility (Spencer et al., 1988; Naumann et al., 2017). Tannins form complex bonds with molecules such as carbohydrates, proteins, polysaccharides, bacterial cell membranes, and enzymes through hydrogen binding mechanisms, hydrophobic, covalent, precipitates, dissolved complexes, and insoluble complexes (Fruutos et al., 2004; Smith et al., 2005). The availability of tannins to form complex protein–tannin bonds may lead it difficult to degrade by rumen microbes, causing a decrease in the digestibility of DM and OM.

**Effects of Swietenia mahagoni on in-vitro post-rumen nutrient digestibility**

The effect of *Swietenia mahagoni* on the total and post-rumen nutrient digestibility is presented in Table 5. The total crude protein digestibility significantly (P < 0.05) increased 3.78 and 7.07% compared to controls due to the addition of 3 and 6% tannin *Swietenia mahagoni*, respectively. Post-rumen CP digestibility was obtained from total digestibility minus rumen digestibility. The addition of 3 and 6% tannin *Swietenia mahagoni* increased post-rumen digestibility of CP 7 and 10 fold, respectively compared to controls.

The main effect of tannins on protein is their ability to form stable hydrogen bonds between pH 3.5 and 8 (approximately). These complexes are stable at rumen...
pH but dissociate when the pH falls below 3.5 (such as abomasum, pH 2.5–3) or greater than 8 (for example, in the duodenum, pH 8) (Mergetuš et al., 2018). Tannins protect the protein from rumen microbial degradation, causing increased post-rumen protein availability for digestion and absorption by ruminants. Bunglavan and Dutta (2013) stated that stable complexes increased the total number of dietary amino acids available for post-ruminal absorption. Protein–tannin complexes are then available in the abomasum and digested in the intestine. The use of condensed tannins from various forage legumes increased the post-ruminal amino acid flux due to a greater proportion of rumen undegradable protein and improved intestinal amino acid availability (Naumann et al., 2017). According to Arisya et al. (2019), utilization of tannin sources increases rumen undegradable protein. Riswandi et al. (2015) reported that the use of 30% *Leucaena leucocephala* increased 11.83% protein digestibility of Bali cattle compared to control.

### Table 5: Effects of Swietenia mahagoni on in vitro post-rumen nutrient digestibility.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Tannin levels (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td>Rumen and post-rumen</td>
<td></td>
</tr>
<tr>
<td>Crude protein (%)</td>
<td>55.84±0.08</td>
</tr>
<tr>
<td>Organic matter (%)</td>
<td>59.10±1.93</td>
</tr>
<tr>
<td>Dry matter (%)</td>
<td>57.03±0.19</td>
</tr>
<tr>
<td>Post-rumen</td>
<td></td>
</tr>
<tr>
<td>Crude protein (%)</td>
<td>2.26±0.04</td>
</tr>
<tr>
<td>Organic matter (%)</td>
<td>13.82±1.64</td>
</tr>
<tr>
<td>Dry matter (%)</td>
<td>12.68±0.84</td>
</tr>
</tbody>
</table>

abc Different superscript on the same row are differ significantly (P<0.05).

The use of *Swietenia mahagoni* tannins significantly (P < 0.05) improved rumen and post-rumen OM and DM digestibility (Table 5). The addition of 6% *Swietenia mahagoni* tannins significantly (P < 0.05) increased OM's total and post-rumen digestibility by 18.29 and 130.61%, respectively. The total DM digestibility with the addition of 3 and 6% *Swietenia mahagoni* significantly (P < 0.05) increased by 4.15 and 7.70% respectively, while post-rumen digestibility increased by 31.70 and 101%.

The digestibility of feed nutrients strongly influences the digestibility of OM and DM. The increase in digestibility of OM and DM was in proportion to the increase in the rumen and post-rumen CP digestibility. Tannin–protein binding prevents rumen microbial degradation, increasing the number of amino acids absorbed by ruminants (Hidayah, 2016). Proteins are degraded by enzymes in the abomasum, increasing the post-rumen digestibility of dry matter, organic matter, and crude protein. Supplementation of that 0.02% Cashew Nutshell Oil supplementation increased the digestibility of DM and OM post-rumen by 4.6 and 3 times (Fitriastuti et al., 2019). In addition, tannins could form complex bonds with feed components such as protein, fat, minerals, vitamins, and carbohydrates to influence digestibility (Buyukcapar et al., 2011; Yao et al., 2019). Mergetuš et al. (2018) stated that tannins from *Lotus pedunculatus* decreased rumen digestibility of carbohydrates and hemicellulose but improved post-rumen digestibility.

### CONCLUSIONS AND RECOMMENDATIONS

Dietary *Swietenia mahagoni*, as a source of tannins up to 6%, reduces rumen hydrolytic enzyme activity and rumen nutrient digestibility without the negative effect on rumen VFA production, while it improves post-rumen nutrient digestibility. The use of *Swietenia mahogany* may have the potential to increase nutrient utilization in ruminants.

### AUTHOR’S CONTRIBUTION

Achmad Chairul Basri, Wahyu Prambudi Yustanto, Chusnul Hanim, and Lies Mira Yusiati designed the concept of the research. Achmad Chairul Basri and Wahyu Prambudi Yustanto performed the experiment, laboratory analysis and collected the data. Achmad Chairul Basri, Wahyu Prambudi Yustanto, and Muhsin Al Anas analyzed the data and wrote the manuscript. Asih Kurniawati, Chusnul Hanim, Muhsin Al Anas, and Lies Mira Yusiati supervised the studies and revised the manuscript. All authors read and approved the final manuscript for publication.

### CONFLICT OF INTEREST

The authors have declared no conflict of interest.

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Advances in Animal and Veterinary Sciences

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