



Dietary *Swietenia mahagoni* as Tannin Source to Increase *in-vitro* Nutrients Digestibility

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Abstract | *Swietenia mahagoni* is commonly used in ruminants and a source of condensed tannin (CT) with the potential to improve feed utilization. This study aimed to determine the effect of *Swietenia mahagoni* as a tannin source on *in-vitro* rumen fermentation, rumen hydrolytic enzyme activity, and nutrients digestibility. The levels of tannin were 0, 3, and 6%. Feed fermentation was conducted using gas production Menke and Steingass and two steps Tilley and Terry *in-vitro* digestibility technique for 96 h. Data obtained were analyzed using one-way ANOVA and continued by DMRT. The dietary 6% tannin was able to reduce ($P < 0.05$) pH, ammonia (NH_3) production, microbial protein, and protozoa populations but did not affect the production of volatile fatty acids (VFA). The activity of β -Glucosidase, carboxymethyl cellulose (CMC-ase), amylase, and protease was decreased linearly with tannin concentrations ($P < 0.05$). Rumen digestibility of crude protein (CP), organic matter (OM), and dry matter (DM) decreased by treatment ($P < 0.05$). DM and protein digestibility in post rumen increased linearly with tannin treatments ($P < 0.05$), while OM digestibility increased by 6% of tannin level. Dietary of *Swietenia mahagoni* as a tannin source may be a potential way to improve nutrients utilization in ruminants.

Keywords | Fermentation, Legumes, Protein, Rumen, Tannin, Ruminant

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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 NH_3 , and some that are not degraded are categorized as rumen undegradable protein (Kamalak *et al.*, 2005; Liu *et al.*, 2019). Although NH_3 is indispensable for microbial protein synthesis (Pathak, 2008; Suryani *et*

al., 2020), excess NH_3 of more than 50 mg NH_3 -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* as a tannins source 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.

Feed ingredients	Treatment		
	T1	T2	T3
Concentrate			
Soybean meal	25.58	25.58	25.58
Pollard	4.42	4.42	4.42
Forage			
Elephant grass	70.00	52.50	35.00
Swietenia mahagoni	0.00	17.50	35.00
	100.00	100.00	100.00

DATA ANALYSIS

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

$$Y_{ij} = \mu + \alpha_{ij} + \epsilon_{ij}$$

Where; Y_{ij} is the observation, μ is the overall mean, α_{ij} is the effect of tannin level at 0, 3, and 6%, and ϵ_{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 NH_3 and VFA because microbial activity in the rumen is influenced by pH (Russell and Wilson, 1996; Castillo-González et al., 2014; Harun and Sali, 2019). Rira et al. (2015) examined various tannin-

rich plants such as *Leucaena leucocephala*, *Gliricidia sepium*, and *Manihot esculenta* that affect the pH value of rumen fermentation, which ranges from 6.2 to 6.6. Aguerre et al. (2016) also 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.

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

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

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 NH₃ production (Rimbawanto et al., 2017). The reduced concentration of NH₃ 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 1.8% quebracho-chestnut tannin extracts decreased 22.12% of rumen NH₃ concentrations in dairy cows. Mokhtarpour et al. (2017) showed that pistachio by-product tannin extract reduced 55.78% of the rumen NH₃ concentration of dairy goats.

The use of 3 and 6% *Swietenia mahagoni* significantly (P < 0.05) decreased 10.32% and 13.49% rumen microbial protein synthesis. Microbial protein in this study ranged from 0.109 to 0.126 mg/mL. Although the addition of

Swietenia mahagoni decreased microbial protein, the values were still within the normal range, according to Ramaiyulis et al. (2018) that is 0.059-0.157 mg/mL. Microbial protein is an essential source of protein for ruminants due to supplying more than half of the total protein requirement (Dewhurst et al., 2000; Hackmann and Firkins, 2015). According to Pathak (2008) and Castillo-González et al. (2014), the availability of N-NH₃ and energy is the most influential factor in microbial protein synthesis. The availability of nitrogen and digestible organic matter in large amounts increased microbial protein synthesis. Protein degradation in the rumen provides N to meet the requirement and promote microbial growth (Castillo-Lopez and Domínguez-Ordóñez, 2019). Protein-tannin binding decreases rumen degradation of the protein (Chamadia et al., 2020), resulting in low NH₃ production (Aguerre et al., 2016; Naumann et al., 2017). The decreased ammonia concentration caused low nitrogen availability for microbial growth. Getachew et al. (2000) stated that decreased *in-vitro* microbial fermentation of feed containing tannins could be due to the lower degradation of feed protein limiting the availability of ammonia nitrogen for microbial growth. However, the more significant effect could be related to the inhibitory effect of tannins on microbial cells or enzyme activity. On the other hand, increasing ammonia concentrations of more than 50 mg NH₃-N/L rumen fluid had no effects on microbial protein production (Satter and Slyter, 1974; Neto et al., 2019).

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 10⁴ cells/mL to 2.3 x 10⁴ cells/mL and 4.7 x 10⁴ 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

> 0.05) the total volatile fatty acids (VFA) production and the proportion of VFA (acetate, propionate, and butyrate) (Table 2). Muhlisin et al. (2017) and Anas et al. (2015) indicated that the use of 6% tannins from *Calliandra calothyrsus* and *Albazia chinensis*, respectively did not affect VFA production and the proportion of acetate, butyrate, and propionate. Aguerre et al. (2016) recorded that the use of 1.8% quebracho-chestnut tannin extracts did not affect the total or proportion of rumen VFA. Volatile fatty acids are products of microbial fermentation. Different sources of tannins cause different nutritional content, especially carbohydrates such as cellulose and hemicellulose, which affect the total VFA production. The proportion of VFA in rumen fluid varies depending on the type of feed consumed (Kim et al., 2018).

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* Berton 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% of *L. leucocephala* leaves reduced 36.09 and 58.92% of the β -glucosidase activity, respectively. Ikarashi et al. (2011) also reported that the use of *Acacia mearnsii* decreased the activity of the β -glucosidase enzyme. β -glucosidase in the rumen is produced by fungi and bacteria (Ahmed et al., 2017). Tannin has antifungal compounds that can reduce the microbial population that produces β -glucosidase enzymes (Anttila et al., 2013; Zhu et al., 2019). According to Longo et al. (2013), the use of tannin-rich plants such as *Stylobium aterrimum* and *Leucaena leucocephala* can reduce 72-80% of the rumen population of *Fibrobacter succinogenes*. In addition, according to Huang et al. (2019), tannins did not reduce glucosidase content but caused a decrease in enzyme activity through tannin bonding with free enzymes and with enzyme-substrate complexes.

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

Parameters	Tannin levels (%)		
	0	3	6
β -Glucosidase(U/g)	71.45±0.58 ^c	59.46±0.3 ^b	21.12±0.16 ^a
CMC-ase (U/g)	6.10±0.24 ^c	4.00±0.25 ^b	1.96±0.42 ^a
Amylase (U/g)	13.68±0.05 ^b	13.63±0.08 ^b	12.50±0.08 ^a
Protease (U/g)	26.18±0.25 ^c	18.06±0.06 ^b	8.78±0.58 ^a

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

Based on Table 3, the CMC-ase enzyme activity significantly ($P < 0.05$) decreased 34.43 and 67.87% due to the addition of 3 and 6% of *Swietenia mahagoni* tannins. Mahanani et al. (2020) reported that the use of 10 and 25% *L. leucocephala* leaves decreased 12.5 and 62.5% CMC-ase activity. Patra et al. (2006) showed that the addition of 1.67% tannins from *T. chebula* extract and *E. officinalis* reduced CMC-ase activity in Buffalo's rumen up to 26.64 and 31.66%. Carboxymethyl Cellulase is an enzyme produced by cellulolytic bacteria. Tannins can interfere with CMC-ase synthesis by reducing the number of cellulolytic microbes, forming tannin-cellulose complexes, disrupting bacterial adhesion to the substrate, and rumen microbial fibrinolytic activity, causing decreased fiber digestibility (Kumar et al., 2014; Tseu et al., 2020). The use of tannins sourced from *Samanea saman* pod and pomegranate peel has been reported to reduce the population of rumen cellulolytic bacteria (Anantasook et al., 2013; Sharifi et al., 2019).

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 $\mu\text{g/mL}$, the enzyme activity decreased almost linearly with concentration. According to Gonçalves 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 $\mu\text{g/mL}$ 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. 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-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 4: Effects of *Swietenia mahagoni* on in vitro rumen nutrient digestibility.

Digestibility	Tannin levels (%)		
	0	3	6
Crude Protein (%)	53.57±0.08 ^c	42.16±0.47 ^b	35.99±2.42 ^a
Organic Matter (%)	45.27±0.53 ^b	44.36±0.46 ^b	38.03±1.43 ^a
Dry Matter (%)	44.35±0.75 ^b	42.70±0.00 ^b	35.93±0.78 ^a

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

The addition of tannins decreased the digestibility of CP, indicating that the rumen degradable protein content was low (Arisya et al., 2019). Tannins are generally active in protein protection through complex protein mechanisms or inhibiting microbial protein degradation mechanisms. The reduction in CP digestibility may be due to the formation of complex protein-tannin, which may lead to decreased protein solubility or by inhibiting microbial protein degradation mechanisms (Jayanegara and Palupi, 2010; Patra and Saxena, 2011). In addition, the use of tannins also reduces the activity of the protease enzyme, which affects protein digestibility (Mahanani et al., 2020). Min and Hart (2003) stated that condensed tannins in plants could reduce protein digestibility in the rumen and decrease proteolytic activity, furthermore inhibiting rumen microbial extracellular enzymes such as proteases,

cellulases, and hemicellulases.

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% Albazia 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 (Frutos 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) (Mergeduš 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.

Parameters	Tannin levels (%)		
	0	3	6
Rumen and post-rumen			
Crude protein (%)	55.84±0.08 ^a	57.95±0.03 ^b	59.79±0.10 ^c
Organic matter (%)	59.10±1.93 ^a	61.09±1.04 ^a	69.91±1.76 ^b
Dry matter (%)	57.03±0.19 ^a	59.40±0.28 ^b	61.42±0.51 ^c
Post-rumen			
Crude protein (%)	2.26±0.04 ^a	15.79±0.47 ^b	23.79±2.47 ^c
Organic matter (%)	13.82±1.64 ^a	16.72±0.73 ^a	31.87±1.69 ^b
Dry matter (%)	12.68±0.84 ^a	16.70±0.27 ^b	25.49±1.27 ^c

^{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). Mergeduš 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.

REFERENCES

- Addisu S (2016). Effect of dietary tannin source feeds on ruminal fermentation and production of cattle; a review. J. Anim. Feed Res., 6: 45–56. Available at <http://www.science-line.com/index/;%5Cnhttp://www.ojaftr.ir>
- Aguerre MJ, Capozzolo MC, Lencioni P, Cabral C, Wattiaux MA (2016). Effect of quebracho-chestnut tannin extracts at 2 dietary crude protein levels on performance, rumen fermentation, and nitrogen partitioning in dairy cows. J. Dairy Sci., 99: 4476–4486. <https://doi.org/10.3168/jds.2015-10745>
- Ahmed A, Nasim FH, Batool K, Bibi A (2017). Microbial β-Glucosidase: Sources, production and applications. J. Appl. Environ. Microbiol. 5:31–46. <https://doi.org/10.12691/jaem-5-2-2>
- Anantasook N, Wanapat M, Cherdthong A, Gunun P (2013).

- Changes of microbial population in the rumen of dairy steers as influenced by plant containing tannins and saponins and roughage to concentrate ratio. *Asian-Australasian J. Anim. Sci.*, 26: 1583–1591. <https://doi.org/10.5713/ajas.2013.13182>
- Anas MA, Yusiati LM, Kurniawati A, Hanim C (2015). Evaluation of *Albazia chinensis* as tannins source for in vitro methane production inhibitor agents sheep rumen liquor. 6th Int. Semin. Trop. Anim. Prod. Yogyakarta, Indonesia. pp. 261–265.
 - Anttila AK, Pirttilä AM, Häggman H, Harju A, Venäläinen M, Haapala A, Holmbom B, Julkunen-Tiitto R (2013). Condensed conifer tannins as antifungal agents in liquid culture. *Holzforschung*, 67: 825–832. <https://doi.org/10.1515/hf-2012-0154>
 - AOAC (2005). Official methods of analysis. 20th ed. Association of Official Analytical Chemists, Washington DC, USA.
 - Arisya W, Ridwan R, Ridla M, Jayanegara A (2019). Tannin treatment for protecting feed protein degradation in the rumen *in vitro*. *IOP Conf. Ser. J. Phys.*, 1360: 012022. <https://doi.org/10.1088/1742-6596/1360/1/012022>
 - Atole AFF, Bestil LC (2014). Extrapolating bypass protein potential of treated soybean meal by in situ degradation in rumen-fistulated brahman cattle. *Ann. Trop. Res.*, 36: 50–62. <https://doi.org/10.32945/atr3614.2014>
 - Attia MFA, El-din ANN, El-Shazyl KA, Sallam SM (2013). Effect of quebracho tannins supplementation on nutrients utilization and rumen fermentation characteristics in sheep. *Alex. J. Agric. Res.*, 58: 165–171.
 - Bach A, Calsamiglia S, Stern MD (2005). Nitrogen metabolism in the rumen. *J. Dairy Sci.*, 88: E9–E21. [https://doi.org/10.3168/jds.S0022-0302\(05\)73133-7](https://doi.org/10.3168/jds.S0022-0302(05)73133-7)
 - Belanche A, Pinloche E, Newbold CJ, Preskett D (2016). Effects and mode of action of chitosan and ivy fruit saponins on the microbiome, fermentation and methanogenesis in the rumen simulation technique. *FEMS Microbiol. Ecol.*, 92: fiv160. <https://doi.org/10.1093/femsec/fiv160>
 - Bergmeyer HU, Gawehn K (1974). Methods of enzymatic analysis. Verlag Chemie, Weinheim.
 - Boucher SE, Calsamiglia S, Parsons CM, Stein HH, Stern MD, Erickson PS, Utterback PL, Schwab CG (2009). Intestinal digestibility of amino acids in rumen-undegraded protein estimated using a precision-fed cecectomized rooster bioassay: II. Distillers dried grains with solubles and fish meal. *J. Dairy Sci.*, 92: 6056–6067. <https://doi.org/10.3168/jds.2008-1885>
 - Bunglavan S, Dutta N (2013). Use of tannins as organic protectants of proteins in digestion of ruminants. *J. Livest. Sci.*, 4: 67–77.
 - Buyukcapar HM, Atalay AI, Kamalak A (2011). Growth performance of Nile tilapia (*Oreochromis niloticus*) fed with diets containing different levels of hydrolysable and condensed tannin. *J. Agric. Sci. Technol.*, 13: 1045–1051.
 - Carrasco JMD, Cabral C, Redondo LM, Viso NDP, Colombatto D, Farber MD, Miyakawa MEF (2017). Impact of chestnut and quebracho tannins on rumen microbiota of bovines. *Biomed Res. Int.*, 2017: 9610810. <https://doi.org/10.1155/2017/9610810>
 - Castillo-González AR, Burrola-Barraza ME, Domínguez-Viveros J, Chávez-Martínez A (2014). Rumen microorganisms and fermentation. *Arch. Med. Vet.*, 46: 349–361. <https://doi.org/10.4067/S0301-732X2014000300003>
 - Castillo-Lopez E, Domínguez-Ordóñez MG (2019). Review: Factors affecting the ruminal microbial composition and methods to determine microbial protein yield. *Rev. Mex. Ciencias Pecu.*, 10: 120–148. <https://doi.org/10.22319/rmcv.v10i1.4547>
 - Chamadia B, Grewal RS, Lamba JS, Kaur J, Kashyap N (2020). Effect of varying levels of tannins treatment on in vitro degradability of soybean meal. *Int. J. Curr. Microbiol. Appl. Sci.*, 9: 3991–4000. <https://doi.org/10.20546/ijcmas.2020.907.469>
 - Chaney AL, Marbach EP (1962). Modified reagents for determination of urea and ammonia. *Clin. Chem.*, 8: 130–132. <https://doi.org/10.1093/clinchem/8.2.130>
 - Cieslak A, Zmora P, Matkowski A, Nawrot-Hadzik I, Pers-Kamczyc E, El-Sherbiny M, Bryszak M, Szumacher-Strabel M (2016). Tannins from *sanguisorba officinalis* affect *in vitro* rumen methane production and fermentation. *J. Anim. Plant Sci.*, 26: 54–62.
 - da Silva SM, Koehnlein EA, Bracht A, Castoldi R, de Moraes GR, Baesso ML, Peralta RA, de Souza CGM, de Sá-Nakanishi AB, Peralta RM (2014). Inhibition of salivary and pancreatic α -amylases by a pinhão coat (*Araucaria angustifolia*) extract rich in condensed tannin. *Food Res. Int.*, 56: 1–8. <https://doi.org/10.1016/j.foodres.2013.12.004>
 - Das LK, Kundu SS, Kumar D, Datt C (2014). Metabolizable protein systems in ruminant nutrition: A review. *Vet. World*, 7: 622–629. <https://doi.org/10.14202/vetworld.2014.622-629>
 - Dewhurst RJ, Davies DR, Merry RJ (2000). Microbial protein supply from the rumen. *Anim. Feed Sci. Technol.*, 85: 1–21. [https://doi.org/10.1016/S0377-8401\(00\)00139-5](https://doi.org/10.1016/S0377-8401(00)00139-5)
 - Diaz A, Avendano M, Escobar A (1993). Evaluation of *Sapindus saponaria* as a defaunating agent and its effects on different ruminal digestion parameters. *Livest. Res. Rural Dev.*, 5: 1–6.
 - Fattah AH, Syamsu JA, Natsir A, Garantjang S (2019). VFA concentration, ammonia and pH value in vivo rumen content system made from rice fermentation of Lamtoro leaves, Gamal leaves and Indigofera leaves. *Adv. Environ. Biol.*, 13: 4–9.
 - Filípek J, Dvořák R (2009). Determination of the volatile fatty acid content in the rumen liquid: Comparison of gas chromatography and capillary isotachopheresis. *Acta Vet. Brno.*, 78: 627–633. <https://doi.org/10.2754/avb200978040627>
 - Fitriastuti R, Yusiati LM, Widobroto BP, Bachruddin Z, Hanim C (2019). Effect of cashew nutshell oil supplementation as phenol source for protein protection on *in vitro* nutrient digestibility. *Bul. Peternak*. 43: 225–230. <https://doi.org/10.21059/buletinpeternek.v43i4.35591>
 - Frutos P, Hervás G, Giráldez FJ, Mantecón AR (2004). Review: Tannins and ruminant nutrition. *Spanish J. Agric. Res.*, 2: 191–202. <https://doi.org/10.5424/sjar/2004022-73>
 - Ganai AM, Haq Z, Beigh YA, Sheikh GG (2019). Bypass nutrient technology with recent advances for enhancing animal production: A review. *J. Pharmacogn. Phytochem.*, 5: 269–275.
 - Getachew G, Makkar HPS, Becker K (2000). Tannins in tropical browses: Effects on *in vitro* microbial fermentation and microbial protein synthesis in media containing different amounts of nitrogen. *J. Agric. Food Chem.*, 48: 3581–3588. <https://doi.org/10.1021/jf990740v>
 - Gomez KA, Gomez AA (1984). Statistical procedure for agricultural research. John Wiley and Sons, USA.
 - Gonçalves R, Mateus N, de Freitas V (2011). Inhibition of

- α -amylase activity by condensed tannins. Food Chem., 125: 665–672. <https://doi.org/10.1016/j.foodchem.2010.09.061>
- Hackmann TJ, Firkins JL (2015). Maximizing efficiency of rumen microbial protein production. Front. Microbiol., 6: 465. <https://doi.org/10.3389/fmicb.2015.00465>
 - Halliwell G (1961). The action of cellulolytic enzymes from *Myrothecium verrucaria*. Biochem. J., 79: 185–192. <https://doi.org/10.1042/bj0790185>
 - Harun AY, Sali K (2019). Factors affecting rumen microbial protein synthesis: A review. Vet. Med., 4: 27–35. <https://doi.org/10.17140/VMOJ-4-133>
 - Hidayah N (2016). Utilization of plant secondary metabolites compounds (tannin and saponin) to reduce methane emissions from ruminant livestock. J. Sain Peternak. Indones., 11: 89–98. <https://doi.org/10.31186/jspi.id.11.2.89-98>
 - Huang Q, Chai WM, Ma ZY, Ou-Yang C, Wei QM, Song S, Zou ZR, Peng YY (2019). Inhibition of α -glucosidase activity and non-enzymatic glycation by tannic acid: Inhibitory activity and molecular mechanism. Int. J. Biol. Macromol., 141: 358–368. <https://doi.org/10.1016/j.ijbiomac.2019.09.010>
 - Ikarashi N, Takeda R, Ito K, Ochiai W, Sugiyama K (2011). The inhibition of lipase and glucosidase activities by acacia polyphenol. Evidence-based Complement. Altern. Med., 2011: 272075. <https://doi.org/10.1093/ecam/neq043>
 - Jayanegara A, Palupi E (2010). Condensed tannin effects on nitrogen digestion in ruminants: A meta-analysis from *in vitro* and *in vivo* studies. Media Peternak. 33: 176–181. <https://doi.org/10.5398/medpet.2010.33.3.176>
 - Kamalak A, Canbolat Ö, Gürbüz Y, Özay O (2005). Protected protein and amino acids in ruminant nutrition. J. Sci. Eng., 8: 84–87.
 - Kim SH, Mamuad LL, Kim EJ, Sung HG, Bae GS, Cho KK, Lee C, Lee SS (2018). Effect of different concentrate diet levels on rumen fluid inoculum used for determination of *in vitro* rumen fermentation, methane concentration, and methanogen abundance and diversity. Ital. J. Anim. Sci., 17: 359–367. <https://doi.org/10.1080/1828051X.2017.1394170>
 - Kozloski GV, Härter CJ, Hentz F, de ávila SC, Orlandi T, Stefanello CM (2012). Intake, digestibility and nutrients supply to wethers fed ryegrass and intraruminally infused with levels of *Acacia mearnsii* tannin extract. Small Rumin. Res., 106: 125–130. <https://doi.org/10.1016/j.smallrumres.2012.06.005>
 - Kumar K, Chaudhary LC, Kumar S (2014). Exploitation of tannins to modulate rumen ecosystem and ruminants performance: A review. Indian J. Anim. Sci., 84: 609–618.
 - Liu C, Li D, Chen W, Li Y, Wu H, Meng Q, Zhou Z (2019). Estimating ruminal crude protein degradation from beef cattle feedstuff. Sci. Rep., 9: 1–8. <https://doi.org/10.1038/s41598-019-47768-3>
 - Longo C, Abdalla AL, Liebich J, Janzik I, Hummel J, Correa PS, Südekum KH, Buraue P (2013). Evaluation of the effects of tropical tanniferous plants on rumen microbiota using qRT PCR and DGGE analysis. Czech J. Anim. Sci., 58: 106–116. <https://doi.org/10.17221/6667-CJAS>
 - Mahanani MMP, Kurniawati A, Hanim C, Anas MA, Yusiati LM (2020). Effect of (*Leucaena leucocephala*) leaves as tannin source on rumen microbial enzyme activities and *in vitro* gas production kinetics. IOP Conf. Ser. Earth Environ. Sci., 478: 012088. <https://doi.org/10.1088/1755-1315/478/1/012088>
 - Makkar HPS, Blümmel M, Becker K (1995). *In vitro* effects of and interactions between tannins and saponins and fate of tannins in the rumen. J. Sci. Food Agric., 69: 481–493. <https://doi.org/10.1002/jsfa.2740690413>
 - Makkar HPS, Blümmel M, Borowy NK, Becker K (1993). Gravimetric determination of tannins and their correlations with chemical and protein precipitation methods. J. Sci. Food Agric., 61: 161–165. <https://doi.org/10.1002/jsfa.2740610205>
 - Menke KH, Steingass H (1988). Estimation of energetic feed value obtained from chemical analysis and *in vitro* gas production using rumen fluid. Anim. Res. Dev., 28: 7–55.
 - Mergeduš A, Pšenková M, Janžeković M (2018). Tannins and their effect on production efficiency of ruminants. Agricultura, 15: 1–11.
 - Min BR, Attwood GT, Reilly K, Sun W, Peters JS, Barry TN, McNabb WC (2002). Lotus corniculatus condensed tannins decrease *in vivo* populations of proteolytic bacteria and affect nitrogen metabolism in the rumen of sheep. Can. J. Microbiol., 48: 911–921. <https://doi.org/10.1139/w02-087>
 - Min BR, Hart SP (2003). Tannins for suppression of internal parasites. J. Anim. Sci., 81: E102–E109.
 - Mohammadabadi T, Chaji M (2012). The influence of the plant tannins on *in vitro* ruminal degradation and improving nutritive value of Sunflower meal in ruminants. Pak. Vet. J., 32: 225–228. Available at http://www.pvj.com.pk/pdf-files/31_3/192-194.pdf
 - Mokhtarpour A, Naserian AA, Pourmollae F, Safa S (2017). Effects of two sources of tannins on performance, nitrogen utilization and efficiency of microbial nitrogen synthesis in dairy goats. Iran. J. Appl. Anim. Sci., 7: 61–68.
 - Molan AL, Attwood GT, Min BR, McNabb WC (2001). The effect of condensed tannins from *Lotus pedunculatus* and *Lotus corniculatus* on the growth of proteolytic rumen bacteria *in vitro* and their possible mode of action. Can. J. Microbiol., 47: 626–633. <https://doi.org/10.1139/cjm-47-7-626>
 - Muhlisin MA, Anas, Hanim C, Yusiati LM (2017). Calliandra calothyrsus as tannins source for *in vitro* methane production inhibitor agents. Int. Semin. Trop. Anim. Prod. (ISTAP): Contr. Livest. Prod. Food Sovereignty Trop. Count. Yogyakarta, Indonesia. pp. 133–136.
 - Nafikov RA, Beitz DC (2007). Carbohydrate and lipid metabolism in farm animals. J. Nutr., 137: 702–705. <https://doi.org/10.1093/jn/137.3.702>
 - Naumann HD, Tedeschi LO, Zeller WE, Huntley NF (2017). The role of condensed tannins in ruminant animal production: Advances, limitations and future directions. Rev. Bras. Zootec., 46: 929–949. <https://doi.org/10.1590/s1806-92902017001200009>
 - Naveen YP, Urooj A (2015). Phytochemical, proximate composition and antioxidant potential of swietenia mahagoni leaves. Asian J. Pharm. Res., 5: 161–166 Available at http://www.ajprjournal.com/view_content.php?quat=5&date=2015&issue=3
 - Neto JAS, Oliveira JS, Oliveira CJB, Santos EM, Costa ECB, Saraiva CAS, Pinho RMA (2019). Ammonia levels on *in vitro* degradation of fibrous carbohydrates from buffel grass. South Afr. J. Anim. Sci., 49: 585–597. <https://doi.org/10.4314/sajas.v49i3.19>
 - Ningrat RWS, Zain M, Erpomen, Suryani H (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. Anim. Sci.*, 11: 47–53. <https://doi.org/10.3923/ajas.2017.47.53>
- Ørskov E (1992). Protein nutrition in ruminants. Second. Academic Press Ltd., London.
 - Patel S, Ambalam P (2018). Role of rumen protozoa: Metabolic and fibrolytic. *Adv. Biotechnol. Microbiol.*, 10: 555793. <https://doi.org/10.19080/AIBM.2018.10.555793>
 - Pathak AK (2008). Various factors affecting microbial protein synthesis in the rumen. *Vet. World*, 1: 186–189.
 - Patra AK, Kamra DN, Agarwal N (2006). Effect of plant extracts on *in vitro* methanogenesis, enzyme activities and fermentation of feed in rumen liquor of buffalo. *Anim. Feed Sci. Technol.*, 128: 276–291. <https://doi.org/10.1016/j.anifeedsci.2005.11.001>
 - Patra AK, Saxena J (2011). Exploitation of dietary tannins to improve rumen metabolism and ruminant nutrition. *J. Sci. Food Agric.*, 91: 24–37. <https://doi.org/10.1002/jsfa.4152>
 - Plummer DT (1987). An introduction to practical biochemistry. Third. Mc. Graw-Hill Book Company, New Delhi.
 - Ramaiyulis RWS, Ningrat, Zain M, Warly L (2018). Optimization of rumen microbial protein synthesis by addition of Gambier leaf residue to cattle feed supplement. *Pak. J. Nutr.*, 18: 12–19. <https://doi.org/10.3923/pjn.2019.12.19>
 - Reis LF, Minervino AHH, Araújo CASC, Sousa RS, Oliveira FLC, Rodrigues FAML, Meira-Júnior EBS, Barrêto-Júnior RA, Mori CS, Ortolani EL (2014). Comparison of rumen fluid pH by continuous telemetry system and bench pH meter in sheep with different ranges of ruminal pH. *Sci. World J.*, 2014: 195782. <https://doi.org/10.1155/2014/195782>
 - Rimbawanto EA, Suhermiyati S, Hartoyo B (2017). Effects of slow release urea supplementation of sheep protein source feed protected with condensed tannin from *Leucaena* on protein degradation in rumen and post-rumen *in vitro*. *Anim. Prod.*, 19: 119–126. <https://doi.org/10.20884/1.jap.2017.19.2.624>
 - Rira M, Morgavi DP, Archimède H, Marie-Magdeleine C, Popova M, Bousseboua H, Doreau M (2015). Potential of tannin-rich plants for modulating ruminal microbes and ruminal fermentation in sheep. *J. Anim. Sci.*, 93: 334–347. <https://doi.org/10.2527/jas.2014-7961>
 - Riswandi AI, Ali M, Muhakka, Syaifudin Y, Akbar I (2015). Nutrient digestibility and productivity of Bali cattle fed fermented *Hymenachne Amplexicalis* based rations supplemented with *Leucaena Leucocephala*. *Media Peternak*, 38: 156–162. <https://doi.org/10.5398/medpet.2015.38.3.156>
 - Russell JB, Hespell RB (1981). Microbial rumen fermentation. *J. Dairy Sci.*, 64: 1153–1169. [https://doi.org/10.3168/jds.S0022-0302\(81\)82694-X](https://doi.org/10.3168/jds.S0022-0302(81)82694-X)
 - Russell JB, Wilson DB (1996). Why are ruminal cellulolytic bacteria unable to digest cellulose at low pH? *J. Dairy Sci.*, 79: 1503–1509. [https://doi.org/10.3168/jds.S0022-0302\(96\)76510-4](https://doi.org/10.3168/jds.S0022-0302(96)76510-4)
 - Ruzic-Music D, Petrovic MP, Petrovic MM, Bijelic Z, Caro-Petrovic V, Maksimovic N, Mandic V (2014). Protein source in diets for ruminant nutrition. *Biotechnol. Anim. Husband.*, 30: 175–184. <https://doi.org/10.2298/BAH1402175R>
 - Sarnataro C, Spanghero M (2020). *In vitro* rumen fermentation of feed substrates added with chestnut tannins or an extract from *Stevia rebaudiana* Bertoni. *Anim. Nutr.*, 6: 54–60. <https://doi.org/10.1016/j.aninu.2019.11.009>
 - Satter LD, Slyter LL (1974). Effect of ammonia concentration on rumen microbial protein production *in vitro*. *Br. J. Nutr.*, 32: 199–208. <https://doi.org/10.1079/BJN19740073>
 - Sharifi A, Chaji M, Vakili A (2019). Effect of treating recycled poultry bedding with tannin extracted from pomegranate peel on rumen fermentation parameters and cellulolytic bacterial population in Arabian fattening lambs. *Vet. Res. Forum*, 10: 145–152.
 - Singh A, Sidhu S, Singh P (2019). Bypass protein technology: A review. *Pharma. Innov. J.*, 8: 150–153. Available at <http://epubs.icar.org.in/ejournal/index.php/IJAnS/article>.
 - Smith AH, Zoetendal E, Mackie RI (2005). Bacterial mechanisms to overcome inhibitory effects of dietary tannins. *Microb. Ecol.*, 50: 197–205. <https://doi.org/10.1007/s00248-004-0180-x>
 - Sondakh EHB, Waani MR, Kalele JAD (2017). Changes in *in vitro* methane production and fatty acid profiles in response to cakalang fish oil supplementation. *Media Peternak*, 40: 188–193. <https://doi.org/10.5398/medpet.2017.40.3.188>
 - Spencer CM, Cai Y, Martin R, Gaffney SH, Goulding PN, Magnolato D, Lilley TH, Haslam E (1988). Polyphenol complexation some thoughts and observations. *Phytochemistry*, 27: 2397–2409. [https://doi.org/10.1016/0031-9422\(88\)87004-3](https://doi.org/10.1016/0031-9422(88)87004-3)
 - Suryani NN, IW Suarna, IG Mahardika, NP Sarini (2020). Rumen fermentation and microbial protein synthesis of Bali Cattle Heifers (*Bos sondaicus*) fed ration containing different energy protein level. *J. Sain Peternakan Indonesia*. 15:187–194. <https://doi.org/10.31186/jspi.id.15.2.187-194>.
 - Suryapratama W, Suhartati FM (2012). Increasing rumen microbial protein synthesis with additional dietary substrate of *Saccharomyces cerevisiae* and soybean oil. *Anim. Prod.*, 14: 155–159.
 - Tilley JMA, Terry RA (1963). A two-stage technique for the *in vitro* digestion of forage crops. *Grass Forage Sci.*, 18: 104–111. <https://doi.org/10.1111/j.1365-2494.1963.tb00335.x>
 - Tseu RJ, Junior FP, Carvalho RF, Sene GA, Tropaldi CB, Peres AH, Rodrigues PHM (2020). Effect of tannins and monensin on feeding behaviour, feed intake, digestive parameters and microbial efficiency of Nelore cows. *Ital. J. Anim. Sci.*, 19: 262–273. <https://doi.org/10.1080/1828051X.2020.1729667>
 - Unnawong N, Cherdthong A, So S (2021). Influence of supplementing *Sesbania grandiflora* pod meal at two dietary crude protein levels on feed intake, fermentation characteristics, and methane mitigation in Thai purebred beef cattle. *Vet. Sci.*, 8: 35. <https://doi.org/10.3390/vetsci8020035>
 - Velickovic TDC, Stanic-Vucinic DJ (2018). The role of dietary phenolic compounds in protein digestion and processing technology to improve their antinutritive properties. *Comp. Rev. Food Sci. Food Saf.*, 17: 82–103. <https://doi.org/10.1111/1541-4337.12320>
 - Yao J, Chen P, Apraku A, Zhang G, Huang Z, Hua X (2019). Hydrolysable tannin supplementation alters digestibility and utilization of dietary protein, lipid, and carbohydrate in Grass Carp (*Ctenopharyngodon idellus*). *Front. Nutr.*, 6: 183. <https://doi.org/10.3389/fnut.2019.00183>
 - Yusiati LM, Kurniawati A, Hanim C, Anas MA (2018). Protein binding capacity of different forages tannin. *IOP Conf. Ser. Earth Environ. Sci.*, 119: 012007. <https://doi.org/10.1088/1755-1315/119/1/012007>
 - Zaidi-Yahiaoui R, Zaidi F, Bessai AA (2008). Influence of gallic and tannic acids on enzymatic activity and growth of

Pectobacterium chrysanthemi (*Dickeya chrysanthemi* bv. *chrysanthemi*). *Afr. J. Biotechnol.*, 7: 482–486.

•Zhu C, Lei M, Andargie M, Zeng J, Li J (2019). Antifungal

activity and mechanism of action of tannic acid against *Penicillium digitatum*. *Physiol. Mol. Plant Pathol.*, 107: 46–50. <https://doi.org/10.1016/j.pmpp.2019.04.009>