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Microbial biomass, apparent and true degradability, and Undegradable Dietary Protein (UDP) values of concentrated rations containing white Kabesak Leaf (*Acacia leucophloea*) *In vitro*

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Abstract

The objective of this study was to evaluate the effect of using white kabesak leaves in the concentrate on biomass production, apparent and true degradability, and undegraded protein of rations *In Vitro*. This study used the *In Vitro* gas production method, designed using a group randomized experimental design. The proportion of grass forage and concentrate in the diet was 60:40. The treatment of the use of white kabesak leaves is R0: without white kabesak leaves (0%), R1: 10%, R2 20%, R3 30% and R4 40%. The results showed that the treatment had a very significant effect ($P<0.01$) on microbial biomass production, Apparent and True Degradability, and a considerable impact ($P<0.05$) on undegraded dietary protein rations. Biomass production decreased from 77.30 mg/500 mg DM in R0 to 43.6 mg/500 mg DM in R4. Apparent degradability from 287.25 mg/500 mg DM in R0 to 268.42 mg/500 mg DM in R4, and True degradability from 364.87 mg/500 mg DM in R0 to 312 mg/500 mg DM in R4. Undegraded dietary protein increased from 8.65% in R0 to 13.93% in R3. It was concluded that the use of white kabesak leaves in concentrates *In Vitro* produced a positive effect by decreasing feed protein degradation and increasing undegraded protein, making it more available post-rumen. The use of 20% white kabesak leaves in concentrate is the ideal level for *In Vitro* nutrient acceptance.

Keywords: Apparent and true degradability; Microbial biomass; White kabesak leaves; Undegraded dietary protein; concentrate; *In Vitro*

1. Introduction

The main constraint for ruminants in the tropics is the availability and quality of basal feed to meet production needs during the dry season. One potential way to improve the quality and availability of feed for ruminants is to utilize tree legume leaves as feed supplements. The advantage of tree leguminous leaves is their high protein content ranging from 14-29% which can be used as a feed supplement, but the limitation of their use is the presence of tannin compounds and other secondary compounds. According to Tremblay et al. [1] leguminous forage is an important source of protein for ruminants, but the protein is often poorly utilized because it is extensively degraded during ruminal fermentation, and this is probably the most limiting factor of high-quality leguminous forage. Ruminants require a food supply of protein, sugars, starch, and non-structural polysaccharides for the maintenance and synthesis of microbial biomass, which is the main source of protein required for growth and development [2].

The impact of tannin in tree leguminous leaves can be detrimental or beneficial to ruminant performance depending on the concentration. Based on its molecular structure, tannin exists in the form of hydrolyzed tannin and condensed

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tannin. The concentration of hydrolyzed and condensed tannins strongly influences the imbalance of the proportion of rumen-degradable protein (RDP) to undegradable protein (UDP), thereby reducing microbial protein production, rumen digestibility, and protein availability to livestock. Therefore, feed containing inappropriate proportions of RDP and UDP can cause metabolic changes, especially a decrease in microbial population and activity, digestibility, and energy utilization, which results in decreased livestock productivity. High-producing ruminants need to supplement feed protein that is degraded in the rumen (*Rumen Degradable Protein*) to maximize rumen microbial protein or the addition of feed protein supplements that escape rumen microbial degradation (*Undegraded Protein*) because it will increase the supply of amino acids for livestock [3]. The protein evaluation system assumes that protein requirements for ruminants are met from microbial proteins, and undegraded dietary protein (UDP) digested in the small intestine [4]. Feed that escapes rumen degradation, endogenous protein, and microbial biomass enters the duodenum and is used to supply energy and protein for ruminant tissues. The degradation of feed protein in the rumen is influenced by the type of protein in the feedstuff and its amino acid characteristics, as well as the processing method of the feed [5]. The protective barrier of low or high amounts of condensed tannins in feed as a source of UDP reduces ammonia concentration *In Vitro* [6], as well as microbial protein synthesis *In Sacco* [7] and fiber degradation in the rumen [8], and supplementation of this type of protein can improve productivity in terms of increasing the efficiency of meat, milk, and wool production [9].

The condensed tannins in leguminous leaves are widely utilized for their antinutritional effects and potential ability to increase post-rumen protein supply. The potential white kabesak leaves (*Acacia leucophloea*, Roxb.)Willd can be utilized as a supplementary feed for ruminants because it has a fairly high crude protein and has not been widely used by farmers. White kabesak leaves contain crude protein 16.20%, crude fiber 24.56%, Ether Extract 24.56%, total ash 8.22%, and NFE 49.03% [10]. The white kabesak plant is a leguminous tree plant that has an important role as a source of feed for ruminants on the island of Timor because it can produce forage throughout the year. This plant not only provides feed in the dry season but is also useful as protection for livestock in pastures and the leaves, shoots, and pods are easily consumed by livestock [11]. In addition, white kabesak leaves contain secondary compounds, namely tannin, which can protect feed protein. The tannin content in white kabesak leaves will be beneficial in concentrate feed if it can protect crude protein and other feed components in the concentrate. Therefore, this study was conducted to evaluate the effect of adding white kabesak leaves in the concentrate on microbial biomass value, apparent and true degradability, and undegradable dietary protein *In Vitro*.

2. Material and methods

2.1. Sample Preparation

The concentrate feeds used were: Soybean meal, coconut meal, corn meal, and rice bran while white kabesak leaves were collected from around Kupang City. The white kabesak plant is endemic to the island of Timor, East Nusa Tenggara province, Indonesia. White kabesak leaves were dried by aerating and then ground into flour and then mixed with other concentrate feed based on the percentage in each treatment. The ration was prepared with a ratio of 60% natural grass and 40% concentrate (in DM) with a CP content of 11.5-12.5%. White kabesak leaf contains crude protein of 14.72%, crude fiber of 30.40%, total tannin of 0.97%, condensed tannin of 0.49%, total phenol of 3.52%, and NDF and ADF contents of 46.88% and 34.89%. The composition of feed ingredients in each treatment is listed in Table 1. The nutrient content of each treatment is listed in Table 2.

Table 1 Composition of Concentrate Ingredients Containing White Kabesak Leaf

Feed Ingredients (%)	**Treatments**				
	R0	**R1**	**R2**	**R3**	**R4**
Soybean meal	12.5	10.0	7.5	5.0	0.0
Coconut meal	20.0	15.0	12.5	10.0	0.0
Rice bran	37.5	25.0	17.5	5.0	0.0
Fine corns	30.0	25.0	12.5	5.0	0.0
White kabesak leaf	0.0	25.0	50.0	75.0	100.0
	100	100	100	100	100

Table 2 Nutrient Content of Feed Treatment

Nutrient content (%)	Treatments				
	R0	**R1**	**R2**	**R3**	**R4**
Dry matter (DM)	88.97	89.89	89.84	89.88	90.06
Organic matter (OM)	87.89	87.86	88.19	88.47	88.50
Crude Protein (CP)	12.14	12.13	12.25	12.38	11.72
Crude Fiber (CF)	27.90	28.32	28.64	31.21	34.54

2.2. Research Methods

The ration was prepared with a ratio of 60% natural grass (RA) and 40% concentrate—the percentage of white kabesak leaf in concentrate according to treatment.

- R0 = 60%: 40% Concentrate (without white kabesak leaves)
- R1 = 60%: 30% Concentrate + 10% white kabesak leaf
- R2= 60%: 20% Concentrate + 20% white kabesak leaves
- R3 = 60%: 10% Concentrate + 30% white kabesak leaves
- R4 = 60%: 40% White kabesak leaves (without concentrate)

2.3. Variable Measurement

This *In Vitro* study uses the *In Vitro* Gas Production (IVGP) method according to Makkar et al. [12]. Rumen fluid was filtered through a 100 μm nylon filter and added with diluted buffer. The rumen buffer was saturated with CO2 for 10 min before being put into a glass syringe to ensure anaerobic conditions in the reaction. A total of 5g of DM sample was put into the syringe and closed with a piston lubricated with Vaseline. A total of 30 mL of buffered rumen fluid was put into each syringe through the inlet channel, then the syringe was immediately put into a water bath at 39°C. Samples in the syringe were incubated from 0 to 48 hours.

2.3.1. Determination of Microbial Biomass, Apparent undegradable and True undegradable and undegradable Dietary Protein (UDP)

Microbial biomass was determined according to Blummel et al. [13] namely, truly degraded substrate - apparently degraded substrate.

Determination of apparent undegradable was done at 48 hours incubation time by 3 syringes that had been removed from the water bath were immersed in ice water to stop the fermentation process then the sample in the syringe was transferred into a test tube and centrifuged at 10.000 rpm for 15 minutes. The residue obtained was put into the oven at 105^0C for 6 hours, then weighed first for 1 hour in a desiccator. The weight of the residue obtained is the apparent undegradable. Apparent degradability (g) obtained from the incubated substrate minus the centrifuged residue.

True undegradable was done at 48 hours incubation time by 3 syringes removed from the incubator were immersed in ice water to stop the fermentation process, followed by boiling using NDS solution. A total of 100 mL of NDS solution was used in the boiling for 1 hour. The solution was filtered with a crucible, and washed with hot water 3 times and acetone 3 times. The residue in the crucible was baked at 105^0C for 6 hours, cooled in a desiccator for 1 hour, and then weighed. The weight obtained is the true undegradable. True degradability (g) was obtained from the incubated substrate minus the residue that had been treated with NDS solution.

2.3.2. Determination of Undegradable Dietary Protein (UDP)

Determination of UDP using the method of Makkar et al. [12] that is, the sample was weighed as much as 0.5 g for each duplo. The water bath was filled with enough water and prepared at a temperature of 39^0 C. The weighed sample was put into a syringe tube which was placed in a rack and was in a water bath with a constant temperature of 39^0C. Into the syringe tube was added a mixture of McDougall solution and 50 mL rumen fluid. Rumen fluid was collected from one Frisian Holland breeding cow (PFH). The tube containing the sample and the mixture of McDoughall's solution and rumen fluid is supplied with CO2 to create an aerobic atmosphere, then the syringe tube is closed tightly. The syringe

tubes were incubated in a water bath for 48 hours. Every 6 hours, it was shaken and CO2 was added. After incubation was complete, the tubes were removed from the water bath and placed in cold water to stop fermentation. After incubation is complete, the residue is filtered with Whatman 40 filter paper that has been measured by weight (g), the filtering process is carried out with the help of a vacuum pump. The tube was then washed using distilled water. The residue was dried in an oven at 105^0C for 6 hours, then cooled in a desiccator for 15 minutes, then weighed to obtain the dry matter weight (DM) of the residue. Destruction, distillation, and titration followed to measure the proportion of undegraded protein.

2.4. Statistical Analysis

The data were tabulated and analyzed statistically using Analysis of Variance (ANOVA) according to a randomized group design. Duncan's test was used to see the differences between treatments. This data analysis used the General Linear Procedure (GLM) Statistical Analysis System [14].

3. Results and discussion

The average data of research variables using white kabesak leaves in concentrates incubated *In Vitro* are listed in Table 3.

Table 3 Average microbial biomass (mg/500 mg DM), Apparent degradability (mg/500 mg DM), True degradability (mg/500mg DM), and UDP (%) of concentrate containing kabesak leaves

Variables measured	Treatments				
	R0	R1	R2	R3	R4
Microbial biomass	77.30±3.0c	72.85±2.65c	57.5±5.5b	45.85±0.85a	43.6±10.0a
Apparent degradability	287.25±7.45c	280.56±5.55b	283.78±4.8b	282.83±3.14b	268.42±6.2a
True degradability	364.87±2.6c	353.41±1.85c	342.28±6.6c	328.68±5.74b	312.02±6.37a
UDP	8.65±0.31a	11.60±1.10b	13.44±0.53bc	13.93±1.83c	12.62±0.14b

Notes: Different superscripts in the same row indicate significant differences (P≤0.05) according to Duncan's test.

3.1. Microbial Biomass

Microbial biomass as a product of substrate fermentation in this study was significantly (P<0.01) affected by the use of white kabesak leaves in the concentrate. The higher percentage of white kabesak leaves used in the concentrate resulted in a decrease in microbial biomass production. There is a relationship between microbial biomass production and feed degradability, where a decrease in microbial biomass production is followed by a decrease in feed degradability. The increasing amount of tannin inhibits nutrient degradation by rumen microbes so that fewer nutrients are obtained to break them down into NH3 and VFA for microbial biomass formation. The decrease in microbial biomass production *In Vitro* with increasing use of white kabesak leaves, especially in treatment R4 (40% white kabesak leaves) was due to the decreased synergy between NH3 produced with energy in the fermentation medium. In this study, the NH3 concentration of treatment R4 at 48 hours incubation was 6.29 mg/100 mL and the highest in treatment R0 (7.31 mg/100 mL) and R2 (7.06 mg/100 mL) while the total VFA concentration of treatment R4 (83.07 mM/l) was not different from other treatments, R1 (84.66 mM/l), R2 (83.52 mM/l) and R3 (84.01 mM/l) except R0 (91.96 mM/l) which was significantly the highest of all treatments.

The R0 treatment (concentrate without white kabesak leaf) produced the highest microbial biomass but was not different from the R1 treatment (10% white kabesak leaf). Microbial biomass production of R2 (20% white kabesak leaf) was in the middle, lower than R0 and R1 but higher than R3 (30% white kabesak leaf) and R4 (40% white kabesak leaf). The decrease in microbial biomass is in line with the increase in the percentage of white kabesak leaves in concentrate when compared to the control feed. This indicates that the provision of white kabesak leaves with concentrate produces a positive effect on reducing feed degradation, especially protein so it is expected that feed protein can be more beneficial to the landlady through the process of nutrient absorption in the small intestine without disturbing rumen microbial activity and N excretion in urine and feces decreases. According to Santos et al. [15], high amounts of easily degradable protein will reduce the rate of microbial protein in the small intestine.

Microbial biomass production in treatment R4 was the lowest (43.6 mg/500 mg DM), this means that the use of white kabesak leaves as a single concentrate produces low microbial biomass compared to the use of 10%, and without white kabesak leaves (0%). The high amount of CT due to the increased amount of white kabesak leaf is thought to decrease protein degradation. According to Jayanegara et al. [16], secondary compounds in the form of tannins and phenols contained in white kabesak leaves affect the degradation of protein and other nutrients from microbial degradation and fermentation in the rumen. In addition, the concentration of other components such as NDF and ADF also increased until the 48-hour incubation period, resulting in lower microbial biomass production. The high fiber content of NDF and ADF in kabesak leaves is one of the factors that cause a decrease in degradability so that microbial biomass production also decreases. Concentrate feed containing white kabesak leaves as fermented samples produced ammonia and energy needed by microbes to form biomass according to the treatment level applied.

3.2. Apparent Degradability and True Degradability

The apparent degradability and true degradability values were significantly ($P<0.01$) influenced by the increased use of white kabesak leaf levels, where there was a decrease with increasing percentage of white kabesak leaves. This shows that the higher amount of white kabesak leaves in the concentrate produces a higher content of total tannin, CT, total phenol, and fiber components in inhibiting the fermentation process of the substrate by rumen microorganisms so that the degradability of the substrate decreases. The highest apparent degradability was produced by the feed treatment without the addition of white kabesak leaves (R0) and was significantly ($P<0.05$) higher than the other treatments, indicating low or no fermentation-inhibiting components. Between R1, R2, and R3 there was no significant difference, and treatment R4 produced the lowest apparent degradability. This shows that supplementation of white kabesak leaves alone (100%) as a concentrate is not able to increase rumen microbial activity to digest feed nutrients due to high crude fiber content and high CT content. According to Jayanegara and Sofyan [17], high amounts of tannin in plants can cause carbohydrates and proteins to be difficult to degrade by rumen microorganisms, and enzyme activity is inhibited. In this study, the amount of tannin present in the treatment ration increased with the level of use of kabesak leaves in the concentrate.

The same phenomenon is shown in the true degradability parameter where the highest R0 treatment is followed by R1, R2, R3, and the lowest R4. However, R0, R1, and R2 did not show a significant difference. This shows that the tannin content in white kabesak leaves up to the level of 30% use in concentrates cannot inhibit the activity of microorganisms to break down the nutrient components in the substrate. The combination of the use of white kabesak leaves with other concentrate feed as a supplement has no negative impact on true degradability. The impact of using white kabesak leaves as a sole concentrate on true degradability is shown in R4. The lowest value in R4 illustrates the true digestibility of the white kabesak leaves themselves. The decrease in true degradability is due to the increased CT concentration and the high content of crude fiber fraction, which inhibits the fermentation process of microorganisms on the substrate. Olivares-Perez et al. [18] reported that the content of total phenolics, CT, and ADF was negatively correlated with digestibility in forage where the higher content of these secondary compounds in forage resulted in decreased nutrient digestibility.

3.3. Undegraded Dietary Protein (UDP)

Undegraded Dietary Protein (UDP) was significantly ($P<0.05$) influenced by the use of white kabesak leaves in the concentrate. The higher level of white kabesak leaves used showed an increase in undegraded protein produced in the 48-hour incubation period. This indicates that the presence of a complex between tannin and protein in the forage reduces protein degradation in the substrate so that the undegraded protein becomes higher. This result is by Alcaide et al. [19] that feeding proteins that are slow to degrade in the rumen due to protection, generally fail to supply enough nitrogen in the rumen for microbial production. According to Jolazadeh, et al. [20] several treatments have been carried out to convert highly degradable proteins in soybean meal into less degradable proteins (protein bypass) through the use of tannin. The utilization of white kabesak leaves as a local plant that has a not-too-high tannin content of 0.97% and condensed tannin (CT) of 0.49% can be relied upon as part of supplements in concentrates.

The results showed that the UDP values of R2 and R3 treatments (13.44% and 13.93%) were highest compared to R0 (8.65%), R1 (11.60%) and R4 (12.62%). According to Orskov [21], to some extent, tannin can increase N absorption in the post-rumen digestive tract compared to unprotected protein source feed. The increased UDP in R2 and R3 is in line with Norton [22] that low amounts of tannin in forage can increase livestock production through binding of feed protein during mastication and protection of protein from microbial attack in the rumen. In line with that, Mustafa et al. [23] stated that UDP requirements increase with livestock performance and this protein can be supplied by reducing rumen degradation and thus increasing the amount of post-rumen digested protein.

4. Conclusion

The use of white kabesak leaves in concentrates in ruminant rations *In Vitro* produces positive effects on reducing feed degradation, especially protein which is indicated by decreased degradability and microbial biomass production and increased undegraded protein (UDP) so that it is more available after the rumen. The use of 20% kabesak leaves in concentrate is an ideal level for nutrient acceptance *In Vitro*.

Compliance with ethical standards

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Disclosure of conflict of interest

The authors declare no competing interest.

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