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Effect of Supplementation with Green (*Piper betle* L.), Red (*Piper crocatum*) and Yellow (*Epipremnum aureum*. L.) Betel Leaves on Feed Digestibility

Rifa Ratna Sari¹, Mardiaty Zain^{2*}, Elihasridas², Despal³, Bella Veliana Utami¹, dan Laras Sukma Sucitra¹

¹ Postgraduate Program, Faculty of Animal Science Institution, Andalas University, Padang, West Sumatra 25163, Indonesia

² Department of Nutrition and Feed Technology, Faculty of Animal Science, Andalas University, Limau Manis Campus, Padang, West Sumatra 25163, Indonesia

³ Department of Animal Nutrition and Feed Technology, Faculty of Animal Science, IPB University, Bogor, West Java, Indonesia.

*E-mail: mardiaty@ansci.unand.ac.id

Abstract. Betel leaves comprise several types commonly used as herbal supplements, namely green betel (*Piper betle* L.), red betel (*Piper crocatum*), and yellow betel (*Epipremnum aureum* L.). This study aimed to evaluate the effects of supplementation with green, red, and yellow betel leaves in ruminant feed *in vitro* on rumen pH, dry matter digestibility (DMD), and organic matter digestibility (OMD). The experiment was arranged in a Completely Randomized factorial Design (3 × 5) with three replications. The first factor was betel leaf type (green, red, and yellow) and the second factor was supplementation level (0, 2.5, 5, 7.5, and 10%). The results showed that betel leaf supplementation did not affect rumen pH but significantly affected both DMD and OMD across the three leaf types. DMD values ranged from 69.49 to 75.11%, while OMD values ranged from 69.92 to 77.73%. Based on the observed digestibility responses, the recommended inclusion levels are 7.5% for green betel leaf, 5% for red betel leaf, and 2.5% for yellow betel leaf. In conclusion, betel leaf supplementation improved *in vitro* feed digestibility without altering rumen pH, indicating potential as a natural feed additive for ruminants.

1. Introduction

Ruminant farming plays a very important role as a source of animal protein for human consumption, especially from the milk and meat produced. However, the primary challenge in ruminant farming is the effective utilization of feed by livestock. Feed is the most expensive component of livestock production process, therefore efforts are needed to improve feed digestibility as a key strategy to increase yields while reducing production costs. Rumen feed digestibility is greatly influenced by rumen microbial activity, which plays an important role in the degradation of fiber and production of *volatile fatty acids* (VFA) as the main source of energy for ruminant livestock [1].



In recent years, the use of natural additives as feed supplements to replace chemical ones has been widely developed. One of the additives that has been extensively researched is bioactive compounds derived from plants. Several plant bioactive compounds have been shown to influence rumen fermentation and improve nutrient utilization efficiency in livestock [2][3][4]. Betel leaf is a plant native to Indonesia and has been utilized in alternative medicine for an extended period due to its richness in bioactive elements, including phenolic compounds, flavonoids, tannins, and essential oils [5][6][7]. The bioactive compounds in betel leaf are known to have antimicrobial and antioxidant properties [8][9] and can affect microbial activity in the rumen. In Indonesia, there are several types of betel leaves, namely green (*Piper betle*. L.), red (*Piper crocatum*), and yellow (*Epipremnum aureum*. L.) betel leaves. Each type of betel leaf has different morphology and bioactive compound content, so their potential to influence the rumen fermentation process may also differ. However, research on the use of various types of betel leaves as feed supplements for ruminants is still limited. Most studies have focused on green betel leaves, while information on the use of red and yellow betel leaves is still very limited, especially regarding their effect on nutrient digestibility in the rumen [6][8][9][10][11].

Based on the above explanation, this research was conducted to evaluate the effect of green, red and yellow betel leaf as supplements in ruminant feed rations in vitro on feed digestibility. It is hoped that the results of this research will provide scientific knowledge about the potential of green, red and yellow betel leaves as supplements in ruminant feed, as well as open up opportunities to utilize local biodiversity in Indonesia to support increased ruminant livestock productivity.

2. Materials and Methods

This research was conducted at the Ruminant Nutrition Laboratory, Faculty of Animal Science, Andalas University. The experiment was arranged as a Completely Randomized factorial Design (3×5) with three replications. The first factor (A) was the type of betel leaf, consisting of green (*Piper betle* .L.), red (*Piper crocatum*) and yellow (*Epipremnum aureum* L.) betel leaf. The second factor (B) was the betel leaf supplementation dose, consisting of 0, 2.5, 5, 7.5, and 10%. This study used feed consisting of forage and concentrate in a ratio of 60:40. The parameters measured in this study were dry matter digestibility (DMD), organic matter digestibility (OMD), and rumen pH. The feed composition is shown in Table 1.

Table 1. Chemical composition of the research ration

Chemical Composition of Rations	Value (%)
Dry Matter (DM)	90.93
Organic Matter (OM)	88.97
Crude Protein	13.15
Crude Fat	3.19
Neutral Detergent Fiber	58.08
Acid Detergent Fiber	34.36
Hemicellulose	23.72
Cellulose	23.97

Source: Ruminant Nutrition Laboratory Analysis, Faculty of Animal Science, Andalas University (2025)

2.1 Ethical approval

This study did not require ethical approval because no live animals were used. The method of collecting rumen fluid from slaughterhouses complies with institutional and national ethical guidelines, which exempt the use of biological material from slaughterhouses from ethical review. This procedure ensures humane collection while maintaining the safety and integrity of the samples.

2.2 In vitro and Measurement

Rumen fluid used as rumen microbe inoculum was obtained from a slaughterhouse. In vitro testing was conducted using method [12]. A sample of 2.5 grams was used, to which 200 mL of buffer solution and 50 mL of rumen fluid were added at a ratio of 4:1. The mixture was sealed and incubated at 39°C for 48 hours in a shaker incubator. After 48 hours, the fermentation process was stopped and the pH of the rumen fluid was measured using a pH meter.

After that, the clear supernatant at the top was separated from the sediment residue at the bottom. The separation was carried out using a centrifuge for 30 minutes at a speed of 3000 rpm. The residue was filtered using Whatman No. 41 paper and oven-dried at 60°C for 48 hours for analysis of DMD and OMD. The sediment on the filter paper was placed in a weighed porcelain dish, then placed in an oven at 105°C for 8 hours and weighed again to obtain the DM content value. After weighing, it was placed in a furnace for 6 hours at a temperature of 450-600°C to obtain the OM value.

Digestibility of DM

$$\frac{(\text{Sample weight} \times \text{DM}) - (\text{Residual weight} \times \text{DM} - \text{Blank weight} \times \text{DM})}{\text{Sample weight} \times \text{DM}} \times 100\% \quad (1)$$

Digestibility of OM

$$\frac{(\text{Sample weight} \times \text{OM}) - (\text{Residual weight} \times \text{OM} - \text{Blank weight} \times \text{OM})}{\text{Sample weight} \times \text{OM}} \times 100\% \quad (2)$$

2.3 Statistical analysis

The research data were analysed by one-way analysis of variance (ANOVA). Significant differences in treatment mean values were assessed using Duncan's Multiple Range Test (DMRT). Data analysis was performed using Statistical Package for the Social Sciences (SPSS) version 27.0.

3. Result and Discussion

The DMD, ODM, and rumen pH values are shown in Table 2, Table 3, and Table 4, respectively.

Table 2. Dry Matter Digestibility data values

Factor A	Factor B					Average
	B1(0%)	B2(2.5%)	B3(5%)	B4(7.5%)	B5(10%)	
A1 (green)	69.49 ^{bc}	70.24 ^{bc}	69.56 ^{bc}	75.11 ^a	70.55 ^{bc}	70.99
A2 (red)	69.49 ^{bc}	69.96 ^{bc}	74.50 ^a	68.47 ^c	68.58 ^c	70.20
A3 (yellow)	69.49 ^{bc}	74.98 ^a	72.30 ^{abc}	73.00 ^{ab}	71.70 ^{abc}	72.29
Average	69.49	71.73	72.12	72.20	70.28	

Means in the same row with different letter are significant ($P < 0,01$).

Table 3. Organic Matter Digestibility data values

Factor A	Factor B					Average
	B1(0%)	B2(2.5%)	B3(5%)	B4(7.5%)	B5(10%)	
A1 (green)	71.73 ^{cde}	72.73 ^{bcde}	71.11 ^{de}	77.73 ^a	71.69 ^{cde}	73.00
A2 (red)	71.73 ^{cde}	71.27 ^{de}	75.24 ^{abc}	69.92 ^e	70.14 ^e	71.66
A3 (yellow)	71.73 ^{cde}	75.85 ^{ab}	73.82 ^{bcd}	72.93 ^{bcde}	72.76 ^{bcde}	73.42
Average	71.73	73.28	73.39	73.53	71.53	

Means in the same row with different letter are significant ($P < 0.01$).

Table 2 shows that supplementation with betel leaves, namely green, red and yellow betel leaves, significantly affects ($P < 0.01$) the increase in DMD. Supplementation with green betel leaves increased DMD by 8.09%, red betel leaves by 7.21%, and yellow betel leaves by 7.9% compared to the control. The highest DMD in green betel leaves was found at a dose of 7.5%, red betel leaves at 5%, and yellow betel leaves at 2.5%. DMD represents true digestibility, so the increase in DMD indicates an increase in rumen microbial activity [13]. High DMD means that more nutrients are available for utilization by rumen microbes. In addition, increased nutrient digestibility is also influenced by the nutrient content of the feed. According to [10], betel leaves also have a dry matter content of 89%, organic matter 74.62%, and crude protein 18.13%, thereby helping to increase the nutrients in the feed required by livestock. The increase in DMD due to betel leaf supplementation is also possible because of the secondary metabolite compounds in betel leaves that can modulate optimal conditions for rumen microbes to increase digestibility [5][6][7]. [5] states that betel leaves can also act as a source of carbohydrates and have many hydroxyl groups that can increase fermentable substrates.

Meanwhile, supplementation with green, red, and yellow betel leaves significantly affected ($P < 0.01$) the increase in OMD (Table 3). The increase in OMD in green betel leaves was 8.36%, red betel leaves 4.9%, and yellow betel leaves 5.74% compared to without supplementation. OMD is closely related to DMD because OM is part of DM [11][14]. Therefore, OMD will be in line with DMD. The highest increase in OMD was also found in green betel leaves at a dose of 7.5%, red betel leaves at 5%, and yellow betel leaves at 2.5%. The OMD value with betel leaf supplementation was higher than without supplementation.

One factor that affects DMD and OMD is the rumen microbial population and its activity in the rumen. According to [15], betel leaves contain up to 4.2% essential oils, phenylpropanoid, tannins, and saponins. These active compounds, particularly saponins, can influence the rumen microbial population by reducing the population of protozoa, whose presence is less beneficial, as protozoa are predators of bacteria. [16] states that the active compounds in betel leaves act as antiprotozoals. The balance of rumen microbial populations produced by betel leaves results in optimal rumen conditions and an increase in DMD and OMD. The presence of antiprotozoal compounds in feed can improve feed digestibility, as the reduced protozoa population allows bacteria in the rumen to degrade feed more effectively [17]. In addition, the eugenol content in betel leaves can reduce pathogenic bacteria in the digestive tract, thereby increasing feed nutrient absorption. Betel leaves contain up to 20.37% eugenol using aqueous and ethanol extracts [18], and this eugenol content can suppress the growth of pathogenic bacteria in the digestive tract [19]. Betel leaves are also known as a plant with antioxidant properties, so that with their antioxidant ability, betel leaves can maintain optimal rumen conditions from free radicals that can interfere with rumen microbial activity [8][18].

Table 4. Rumen pH values

Factor A	Factor B					Average
	B1(0%)	B2(2.5%)	B3(5%)	B4(7.5%)	B5(10%)	
A1 (green)	6.91	6.89	6.93	6.92	6.93	6.91
A2 (red)	6.91	6.92	6.89	6.95	6.99	6.93
A3 (yellow)	6.91	6.96	6.91	6.90	6.88	6.91
Average	6.91	6.92	6.91	6.92	6.93	

Means in the same row with different letter are not significant ($P>0,05$).

Table 4 shows that supplementation with green, red and yellow betel leaves did not affect ($P>0.05$) rumen pH values. Rumen pH describes the condition of the rumen and is one of the key factors that can affect the population and activity of rumen microbes and the products produced from rumen fermentation [13][19]. The performance of rumen microbes in digestion will be optimal if the rumen pH value is normal in accordance with rumen conditions [20]. According to [1], the neutral pH of the rumen is 6.5-7.0, while this study found a range of 6.88-6.99, which is still within the neutral range. Low rumen pH values (<6) can have a negative effect on fibrolitic bacteria in the rumen and reduce the population of amylolytic bacteria [19]. Rumen bacterial activity will be disrupted if the pH value is low, and the bacteria's ability to bind cellulose or catabolize hydrolytic products will be impaired, making it difficult for the bacteria to survive.

4. Conclusion

This study shows that green, red and yellow betel leaves have potential as feed supplements for ruminant livestock, producing a very significant effect ($P<0.01$) on DMD and OMD without affecting rumen pH. Differences in betel leaf morphology result in differences in the optimal dosage for DMD and OMD. The optimal dosage of green betel leaf supplementation is 7.5%, red betel leaf is 5%, and yellow betel leaf is 2.5% for DMD and OMD. Further research is needed in vivo, as more complex factors such as feed consumption behavior and long term rumen microbial adaptation also need to be considered.

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