Effects of *Leucaena leucocephala* Supplementation to Total Mixed Ration Based on Ammoniated Rice Straw Onfiber Digestibility and Rumen Fermentation Characteristics *in vitro*


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Abstract— Legume is a feed ingredient that contains high protein for ruminants. The tannin content in legumes can provide a bypass protein for ruminants. This study was done to find out the effect of legume supplementation (*Leucaena leucocephala*) on fiber digestibility, characteristic rumen, protozoa population and methane production from total mixed ration (TMR) based on ammoniated rice straw. The study was conducted *in vitro* using a randomized block design with 3 treatments and 5 replications. The treatment was A. 40% ammoniated rice straw + 60% concentrate, B. 40% ammoniated rice straw + 50% concentrate + 10% *L. leucocephala*, C. 40% ammoniated rice straw + 40% concentrate + 10%, + 20% *L.leucocephala*.The results showed that the addition of *L. leucocephala* increased digestibility of NDF, ADF, and Cellulose, RUP (rumen undegradable protein) and reduced protozoa population and methane production (p <0.05). Increased doses of *L.leucocephala* up to 20% reduce fiber digestibility of feed substances compared to a dose of 10% but are still higher than controls. The results of this study concluded that *L.leucocephala* supplementation in TMR based on ammoniated rice straw *in vitro* improved digestibility, fermentability, and reduced methane gas production. Supplementation of 10% and 20% *L. leucocephala* needs further research, to see the effect on livestock *in vivo*.

Keywords— digestibility; *Leucaena leucocephala*; completed feed; ammoniated rice straw.

I. INTRODUCTION

Use of rice straw as animal feed in general has several disadvantages, because of the low crude protein content and digestibility. Furthermore Ref. [1] said that rice straw is known to contain nutrient values that are not sufficient for the growth of cattle as a business. Field grass generally contains around 20% dry matter. The crude protein content is around 8.4% and 52% TDN content. Grass if given singly to livestock on a dry matter base around 2.5% of body weight is only expected sufficient energy needs for just the basic life and a little for growth.

Lack of nutrients in animal feed can be overcome by adding feed supplements. In general, feed supplements are beneficial for livestock to meet the nutrients required by the animal body so that there is a balanced composition for production optimally. The optimal composition of feed supplement will increase livestock productivity.

Feed supplement used in this study was *Leucaena leucocephala* leaf. *L. leucocephala* are one type of legume that can be used as an animal feed. *L. leucocephala* has a balanced protein, mineral, amino acid content, has low crude fibre and low tannin content which provides added value because it can function to protect the excess protein degradation in the rumen (by pass protein) so the amount of protein absorbed in the small intestine can be higher [2].

This research was conducted to know the effect of supplementation of *L. leucocephala* on fiber digestibility, characteristic rumen, microbial protein synthesis, and methane production from TMR based on ammoniated rice straw.

II. MATERIAL AND METHODS

This study was done in the Laboratory of Ruminant Nutrition, Faculty of Animal Science, University of Andalas. This experiment assigned in Randomized Block Design with three treatments and five groups as replicates. The treatments were A. 40% rice straw ammoniated + 60% concentrate, B. 40% rice straw ammoniated + 50% concentrate + 10% *L. leucocephala*, C. 40% rice straw ammoniated + 40% concentrate + 20% *L.leucocephala* respectively. Concentrate consist of rice brain, palm kernel cake, cassava and mineral. Chemical composition of each treatment can be seen in Table 1.
Ruminal fluid from a cannulated steer was collected and strained with four layers of cheese cloth. The in vitro digestibility was measured according to [3]. Each of fermentation tubes contained of 50 ml of rumen fluid and 200 ml McDougall buffer solution. Three fermentation tubes containing no substrate as control were also incubated. Tubes were incubated in a shaker water bath for 48 h at a temperature of 39ºC. After 48 h incubation, the fermentation activity was stopped immersed in iced water to stop the activities of microbes. Rumen fluid pH was measured with pH meter. Fermentation tubes were then centrifuged at 1500 rpm for 30 min and the supernatant was retained. Residual samples were dried in oven with the temperature set up at 60ºC for 24h and retained for Neutral Detergent Fiber (NDF), Acid Detergent Fiber (ADF), cellulose digestibility and RUP (rumen undegradable protein) analysis according to [4;5]. The total VFA (volatile fatty acids) concentration, partial VFA and rumen NH₃ were analyzed by distillation according to [6,7], respectively. Population of rumen protozoa was counted by [8] method. Methane gas production was measured based on VFAs production [9]. Data obtained were analyzed by Analysis of Variance using a Completely Randomized Design with sub-samplings. Any differences among means were tested using LSD [10].

### TABLE I
THE INGREDIENT AND THE CHEMICAL COMPOSITION (% DRY MATTER)

<table>
<thead>
<tr>
<th>Ingredient composition (%)</th>
<th>Diet</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ammoniated straw rice</td>
<td>A 40</td>
</tr>
<tr>
<td>Concentrate</td>
<td>60</td>
</tr>
<tr>
<td>Leucana leaf meal</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>100</td>
</tr>
<tr>
<td>Chemical composition (%)</td>
<td>10.93</td>
</tr>
<tr>
<td>Protein</td>
<td>67.79</td>
</tr>
<tr>
<td>TDN</td>
<td>5.07</td>
</tr>
<tr>
<td>NDF</td>
<td>46.73</td>
</tr>
<tr>
<td>ADF</td>
<td>49.31</td>
</tr>
<tr>
<td>Lignin</td>
<td>36.77</td>
</tr>
</tbody>
</table>

III. RESULT AND DISCUSSION

A. Fiber Digestibility and Rumen Undegradable Protein (RUP)

The results showed that the treatments had a significant effect on the fibre fraction digestibility (NDF, ADF and cellulose) and RUP (P <0.05) (Table 2).

### TABLE II
FIBER FRACTION DIGESTIBILITY AND RUP

<table>
<thead>
<tr>
<th>No</th>
<th>Treatments</th>
<th>Digestibility</th>
<th>RUP (g/100g sample DM)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>NDF</td>
<td>ADF</td>
</tr>
<tr>
<td>1</td>
<td>A</td>
<td>50.66°</td>
<td>47.33°</td>
</tr>
<tr>
<td>2</td>
<td>B</td>
<td>60.54°</td>
<td>53.66°</td>
</tr>
<tr>
<td>3</td>
<td>C</td>
<td>58.50°</td>
<td>47.83°</td>
</tr>
<tr>
<td>SE</td>
<td></td>
<td>2.4</td>
<td>2.2</td>
</tr>
</tbody>
</table>

Description: Means within row bearing different superscripts differ significantly (p <0.05).

Supplementation of 10% and 20% L. leucochepala in ammoniated rice straw ration significantly increased digestibility (NDF, ADF, cellulose) and RUP compared to treatment without supplementation of L. leucochepala. This could occur because the nutrient content of treatments B and C also increased compared to treatment A, hence more nutrients available for rumen microbes. Besides, L. leucochepala also contains sulfur and phosphorous required by rumen microbes to develop mainly cellulolytic bacteria and fungi [11]. Supplementation of 10% L. leucochepala provides better digestion than supplementation of 20%. The decrease in nutrient digestion in treatment C was due to the high content of lignin in the ration. Lignin binds to cellulose and hemicellulose and cannot be digested by enzymes produced by microbes. The higher the lignin contents in the ration, the lower the digestibility of the ration as explained by [12].

Beside lignin, the tannin content which was slightly higher in this treatment can reduce digestibility because the tannin would bind protein and carbohydrate so that it cannot be degraded in the rumen. Complex tannins with protein can be released in acidic conditions in accordance to [13] that tannins in the rumen can bind proteins and carbohydrates so that the digestion in the rumen reduced.

As can be seen in Table 2, the addition of L. leucochepala significantly affected the amount of by-pass protein (RUP). This increase was caused by an increase in the protein content of TMR because L. leucochepala is a feed ingredient with a good quality protein content [14] and as stated by [2] that L. leucochepala has its tannin content which can provide added value as it can protect the degradation of the protein inside rumen (bypass protein), hence more protein can be absorbed in the small intestine. This is also shown by the increase in the post-rumen protein digestibility and the amount of bypass protein.

B. Concentration of Total VFA and Partial VFA

Volatile Fatty Acids (VFA) is the result of carbohydrate degradation in the rumen. VFA is the main energy source for ruminants. VFA production will increase if the digestibility of food substances also increases. The production of individual VFA and total VFA in this study was presented in Table 3. From Table 3, it can be seen that the treatment had significant effects (P <0.05) on total VFA, propionate production and acetate to propionate ratio but not significantly on acetate and butyrate production.

### TABLE III
EFFECT OF TREATMENTS ON VFA PRODUCTION IN THE RUMEN

<table>
<thead>
<tr>
<th>Diet</th>
<th>Acetate</th>
<th>Propionate</th>
<th>Butyrate</th>
<th>Valerate + iso-valerate + iso-butyrate</th>
<th>Total VFA</th>
<th>Ratio Acetate to propionate</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>41.19</td>
<td>19.00°</td>
<td>9.43</td>
<td>2.89°</td>
<td>72.00°</td>
<td>2.11°</td>
</tr>
<tr>
<td>B</td>
<td>40.50</td>
<td>25.50°</td>
<td>9.75</td>
<td>3.57°</td>
<td>79.25°</td>
<td>1.60°</td>
</tr>
<tr>
<td>C</td>
<td>37.00</td>
<td>23.75°</td>
<td>9.30</td>
<td>3.76°</td>
<td>71.45°</td>
<td>1.35°</td>
</tr>
<tr>
<td>SE</td>
<td>1.25</td>
<td>0.34</td>
<td>0.4</td>
<td>0.45</td>
<td>1.3</td>
<td>0.3</td>
</tr>
</tbody>
</table>

Description: Means within row bearing different superscripts differ significantly (p <0.05).

The LSD test results showed that the addition of L. leucochepala to the TMR-based on ammoniated rice straw was able to increase VFA production to a level of 10% but a decrease in VFA production at the level of 20%. VFA is the main energy source for ruminants and also the final product of rumen microbial fermentation [15]. The composition of feed ration may affect the partial concentration of VFA. Production of acetic acid (C2), propionic (C3) and butyrate
C. Rumen pH, Protozoa population, NH3 and Methane Production

The results of research on rumen pH can be seen in Fig 1. The addition of L. leucochepala does not affect the pH of rumen fluid. Relatively stable rumen pH with L. leucochepala supplementation occurs because the concentration of lactic acid in the rumen is low so that L. leucochepala can stimulate the growth of users of lactic acid bacteria. The value of pH produced in this study ranged from 6.82 to 6.97. The value obtained sufficient to support optimal microbial rumen growth, where the rumen of the normal pH for microbial activity is from 6.0-7.0 [24].

![Fig. 1 Effect of treatments on rumen pH](image)

The concentration of rumen NH3 varied depending on the amount of feed protein, the rate of protein degradation and the time after feeding [25]. Feed proteins that enter the rumen were fermented by proteolytic microorganisms (bacteria and protozoa). Bacteria and protozoa produced proteolytic enzymes such as proteases, peptidases and deaminases to degrade proteins into amino acids, peptides and eventually become ammonia [26]. Ammonia is the main product of the process of deamination of amino acids and their adequacy in the rumen to supply most N for microbial growth and protein synthesis [27]. The average rumen NH3 concentration due to different feed treatments can be seen in Fig 2. The results of the analysis of variance showed that the treated feed and rumen fluid collection time had significant effect (P< 0.05) on rumen NH3 concentration.

![Fig. 2 Effect of treatments on NH3 Production](image)

The results of the study in Fig 2 showed that the concentration of NH3-N from the ammoniated rice straw without L. leucochepala supplement (A) had significant effect (P< 0.05) compared to the L. leucochepala supplement. Supplementation of L. Leucochepala tends to increase NH3-N concentration. The concentration NH3 indicated the degradative process of feed proteins in the rumen. It is also known that supplementation of L. leucochepala increased the protein content of diet but higher supplementation of L. leucochepala in treatment C reduced NH3 concentration. It due to the slight of tannin content in that treatment. It is also known that tannins bind proteins, thereby reducing their
degradation in the rumen. CP degradation decreased in all tannin treatments. This is in agreement with the low production of NH\textsubscript{3}-N in supplementation if more than 10% of L. leucocephala due to higher tannins in the ration. In this study, the NH\textsubscript{3}-N produced ranged from 8.50-10.93 mM and considered as normal. The optimum range of NH\textsubscript{3}-N in the rumen for maximum microbial growth was 3.75 mM - 15.00 mM[28].

The supplementation of L. leucocephala decreased the population of protozoa in incubation media significantly (P <0.05) Fig. 3. This result can be attributed to secondary metabolites present in forages that affect rumen microorganisms. The defaunation effect of L. leucocephala has been demonstrated in vitro and in vivo, and this has been associated with the presence of Tannin Condensation (CT) and saponins [29]. This coincides with the results where the effectiveness of L. leucocephala in removing protozoa can be attributed to the saponin content of forages that appears to be positive in the froth test analysis. Saponins high in molecular glycosides consisting of sugar units (s) associated with triterpene or steroid aglycones and widely distributed in higher plants. The sensitivity of protozoa ciliate to saponin can be attributed to sterols present in protozoa; it's different in bacterial membranes. Therefore, the ability of sterol-binding saponins is likely to result in damage to the protozoa cell membrane [30].

Some literature that suspects tannin is responsible for removing the protozoa population. Condensed tannin reduces the total population of protozoa in the rumen. L. leucocephala has tannin compounds and has anti-protozoa effects by damaging the protozoa cell membrane. The ability of tannins to bind to sterols on protozoan cell membranes or their ability to change the permeability of cell membranes can destroy protozoa cells. Therefore, the tannin function is similar to saponin which is a lipid compound that changes the structure of the protozoan cell membrane which has the potential to destroy protozoan growth and change the pattern of fermentation in the rumen system. The use of tannins in rations can reduce the population of ruminal protozoa. Protozoa population is proportional to production of methane, due to methanogenic bacteria symbiosis with protozoa in the rumen. The effect of 10% supplementation of L. leucocephala on protozoal population was significantly different (P> 0.05) compared to control (A). This shows that the presence of tannins in L. Leucocephala reduced the protozoal population. This is in line with the decrease in methane gas production (12.67mM) compared to other treatments. The decrease in the protozoan population has an effect on the increase in the bacterial population because protozoa are predators for bacteria to meet protein needs. Furthermore, partial defaunation in the rumen was able to increase the population of rumen bacteria [31].

Apart from the partial VFA production ratio (C2 / C3 ratio), feed efficiency in ruminants can be seen from CH\textsubscript{4} gas production. CH\textsubscript{4} is one of the final products of feed fermentation in the rumen where CH\textsubscript{4} gas is formed from H\textsubscript{2} and CO\textsubscript{2} by methanogenic bacteria. The amount of H\textsubscript{2} used in methanogenesis is 4 mol / mol CH\textsubscript{4} and the change in free energy from this reaction is -134 kJ / mol CH\textsubscript{4}. The reaction from the conversion of pyruvate to acetate when combined with ΔF = -55 kJ / mol of the substrate is 1 mole of ATP. Therefore, the estimation of methanogenesis with ΔF = -134 kJ / mol CH\textsubscript{4} will produce equivalent energy of at least 3 moles of ATP / mol CH\textsubscript{4}[32]. The higher the production of CH\textsubscript{4}, the more energy is released (wasted energy). [33] stated that the higher the CH\textsubscript{4}gas produced the more inefficient the feed was. The molar average of CH\textsubscript{4}and CO\textsubscript{2} can be seen in Table 3. The results obtained between treatments were the molar production of CO\textsubscript{2} and CH\textsubscript{4}gas, the lowest is produced by treatment B and C which means it is more efficient to use as feed. This is in accordance with the opinion of [33] that feed efficiency in ruminants can be seen from the production of CH\textsubscript{4}gas in the rumen, namely the higher the CH\textsubscript{4}gas produced, the more inefficient the feed is. Feed ingredients that contain high carbohydrates or high cell walls produce a lot of CO\textsubscript{2} gas which ultimately results in a large total gas when compared to feed containing high protein [34].

L. leucocephala supplementation was able to decrease methane gas production compared to controls (Fig. 4). Tannin is known to affect the protozoa population [35] since parts of methanogens attached to protozoa [19] and contribute to reduce methane emissions. The greater decrease in methane in this study due to higher tannins content compared to other studies [36]. Some literature reports the relationship of symbiosis of rumen protozoa and methanogens. It is evident that defaunation results in a decrease in CH\textsubscript{4} gas production.

![Fig. 3 Effect of treatments on protozoa population](image)

![Fig. 4 Effect of treatments on methane production](image)

Retnani et al [37] presented a study of the effectiveness of L. leucocephala in reducing methane gas production, which
gave 27% L. leucocephala in P. purpureum-based basal ration to reduce methane gas production by 15.6% in L/kg DM consumed without affecting digestibility nutrition in sheep. Therefore, this study provides the initial idea that reducing protozoan populations will reduce methanogenic bacteria, thereby reducing methane gas production. This can lead to developing further research to explore various sources and dosages of tannins to be used.

The inhibition of methanogenesis by the presence of tannins may also be the result of a reduction in fiber degradation. The mechanism of tannin ability to reduce methane gas emissions in the rumen, through reducing digestive fibers which in turn indirectly reduces H2 production as a methane precursor [38], and or directly through inhibition of growth or methanogen activity, thus also with the formation of complex compounds by tannins with proteins and carbohydrates in the rumen [39, 40 and 41].

IV. CONCLUSION

Supplementation of L. leucocephala on TMR based on ammoniated rice straw can increase fiber degradability, rumen fermentability, and reduce methane gas production in vitro. Supplementation of 10% and 20% L. leucocephala were suitable to be used for further studies, therefore in vivo experiment is required to study the effects on animal production.

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