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Research Article

Using Molasses in a Rice Straw Urea Lime Molasses Mixture to Improve Digestibility and \textit{In vitro} Metabolite Fermentation

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Abstract

\textbf{Background and Objective:} The use of Rice Straw (RS) directly as a single feed does not meet the nutritional needs of livestock. Inhibiting factors of using rice straw as feed for ruminants include the low content of nutrients, problems with digestibility and problems with palatability. To determine the optimal use of molasses in a rice straw Urea Lime Molasses (ULM) mixture, the effects of molasses levels were studied with the aim of improving the nutrient value, rumen metabolite content, dry matter and organic matter digestibility \textit{in vitro}. 

\textbf{Materials and Methods:} The study was conducted using a completely randomized design with five treatments and four replications. The treatments were A (89% RS, 4% urea (U), 2% lime (L), 5% molasses (M)), B (84% RS, 4% U, 2% L, 10% M), C (79% RS, 4% U, 2% L, 15% M), D (74% RS, 4% U, 2% L, 20% M) and E (69% RS, 4% U, 2% L, 25% M). The variables observed were the nutrient value, fermentation metabolite level, Rumen Dry Matter Digestibility (RDMD), Rumen Organic Matter Digestibility (ROMD), \textit{In vitro} Dry Matter Digestibility (IDMD) and \textit{in vitro} Organic Matter Digestibility (IOMD). 

\textbf{Results:} Adding a urea lime molasses mixture increased the nutrient value of RS in proportion to the increase in molasses usage, especially considering Crude Protein (CP). The addition of the ULM mixture to RS could increase the CP content. Similarly, the positive effects of adding the ULM to RS can be seen in metabolite fermentation, based on NH\textsubscript{3} levels. Low levels of NH\textsubscript{3} are due to its high utilization by rumen microbes. The IDMD and IOMD were highest in treatment E. 

\textbf{Conclusion:} The use of molasses at a concentration of 25% is the best when considering the optimal nutrient content, rumen metabolites, IDMD and IOMD.

\textbf{Key words:} Rice straw, urea lime molasses, nutrient digestibility, livestock, crude protein

Received: January 09, 2018 \hspace{1cm} Accepted: August 24, 2018 \hspace{1cm} Published: November 15, 2018


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\textbf{Competing Interest:} The author has declared that no competing interest exists.

\textbf{Data Availability:} All relevant data are within the paper and its supporting information files.
INTRODUCTION

An important thing to consider in developing goat farms is the availability of feeds that meet the qualitative and quantitative needs of the goat. The availability of forage for cattle is increasingly limited by the narrowing of agricultural land and plantations. Rice straw (*Oryza sativa*) is an agricultural waste that is a potential alternative feed source instead of forage. Based on the total area of food crops in Indonesia, Indonesia's rice production in 2016 was 75,500,000 t. Each kilogram of harvested rice produces 0.8-1 kg of rice straw. In 2016, Indonesia produced approximately 60,400,000-75,500,000 t of rice straw based on dry matter. Assuming that one Animal Unit (AU) is equivalent to a cow weighing 325 kg and consuming 2% of its body weight, the available rice straw can supply 9,292,307-11,615,385 AU.

Utilization of rice straw directly as a single feed can not meet the nutritional needs of livestock. Inhibiting factors of using rice straw as ruminant feed include the low nutrient content of the straw and problems with digestibility and palatability. The protein content of rice straw varies from 3-5%. Rice crops harvested in late maturation have high cell wall content and high lignification levels, making the straw difficult for rumen microbes to degrade. Increasing the role of microbes in the provision of nutrients, especially ammonia, may improve the digestibility of rice straw. Supplementation of urea can be used as a source of ammonia (nitrogen); however, urea rapidly releases nitrogen (N) in to the rumen and rapidly produces ammonia thus excessive doses of urea would cause ammonia poisoning and even death in livestock.

Huntington *et al.* reported that urea was rapidly hydrolyzed in the rumen and that the peak production of ammonia was achieved at 1 h after offering urea. Techniques for slowing the release of ammonia from urea hydrolysis in the rumen are considered to be more efficient and safe because they can prevent ammonia toxicity.

The use of urea in the ration needs to be accompanied by the use of an energy source (carbohydrate source) that is easily soluble or available in the rumen, because synthesizing the optimal microbial protein requires a balance of energy (volatile fatty acids) and nitrogen in N-NH₂ form. Molasses is a feedstuff that is commonly used as a carbohydrate source. A study of the effect of molasses levels in a rice straw urea lime molasses mixture was conducted to determine the optimal level of molasses with respect to nutrient value, rumen metabolite content and dry matter and organic matter digestibility in vitro.

MATERIALS AND METHODS

**Material:** The materials needed for making the rice straw urea lime molasses mixture are sun-dried rice straw, urea, lime and molasses. The equipment used was a chopper, a tarpaulin and a sprayer for spraying the urea lime molasses mixture above the sun-dried rice straw.

**Research location:** This study was conducted over a 3 month period at the Nutrition and Feed Laboratory, Faculty of Animal Husbandry, Udayana University and Research Center of Livestock Ciawi Bogor, West Java.

**Preparation of rice straw urea lime molasses:** The mixture of urea lime molasses consists of 4% urea, 2% lime and molasses one of the following levels 5, 10, 15, 20, or 25%. The mixture of urea lime molasses, was prepared according to the treatment, splashed on the dried rice straw, was cut into pieces at a length of approximately 3 cm and spread to 5 cm thickness.

**In vitro fermentation:** In vitro fermentation was performed according to the methods of Minson and McLeod. The steps are as follows; the milled sample of 0.25 g is put into an in vitro tube and supplemented with 25 mL of rumen fluid and McDougall’s buffer before being incubated in a shaker bath at 40°C for four h. After four hours of incubation, the substrate was centrifuged at 3500 rpm for 10 min. The substrate was separated into precipitate at the bottom and a clear supernatant at the top. This supernatant was sampled for total pH, NH₃, and Volatile Fatty Acid (VFA) measurements. The precipitate was used for the analysis of the digestibility of dry matter and organic matter. The residue of the precipitate was transferred into a vessel with known empty weight, evaporated and dried in a forced-drought oven at 70°C for approximately 12 h and then at 105°C for 9 h before being cooled in a desiccator and then weighed. The mixture was burned into the furnace until the weight of ash was obtained. The blanks were fermented to obtain residues without substrate.

**Measurement of (DMD) and (OMD):** Coefficient of DMD and OMD was calculated as follow:

\[
\text{DMD} (\%) = \frac{DM_{\text{sample}} (g) - DM_{\text{residue}} (g) - DM_{\text{blanko}} (g)}{DM_{\text{sample}} (g)} \times 100
\]

\[
\text{OMD} (\%) = \frac{OM_{\text{sample}} (g) - OM_{\text{residue}} (g) - OM_{\text{blanko}} (g)}{OM_{\text{sample}} (g)} \times 100
\]
Measurement of NH₃ concentration (phenol hypochlorite method): N-NH₃ levels were determined by the phenol hypochlorite method using a spectrophotometer reading. A total of 15 mL of supernatant was put into a bottle containing 5 drops of concentrated sulfuric acid, which was then diluted 100 times. A total of 5 mL of the diluted supernatant was inserted into a spectro-filled tube with standard solution which was supplemented with 0.2 mL of the phenol solution, 0.2 mL of the nitroprusside solution and 0.5 mL of the oxidizing solution. Color reaction readings with the spectrophotometer were performed 5 min after the addition of the oxidizing solution.

Measurement of VFA total concentration: (steam distillation method): A total of 5 mL of supernatant is fed into a heated vapor distillation tube. The tube was immediately closed after adding 1 mL of 15% H₂SO₄. The distillation tube is connected to a flask containing boiling water and is heated continuously during the distillation process. The hot vapor will push the VFA through a condensed cooling condenser and accommodated with an erlenmeyer containing 5 mL of NaOH 0.5 N to a volume of about 300 mL, plus a 2-3 drop of phenolphthalein and titrated with HCl 0.5 N. The test is reached the color change from pink to clear or colorless. There was also a blank titration of 5 mL H₂SO₄.

Calculation:
- Total VFA production was calculated as follows:

\[
VFA \text{ total (mM)} = \frac{(a - b) \times N \text{HCl} \times 1000 / 5 \text{ mM}}{\text{Sample (g) } \times \text{ DM sample (%)}}
\]

Where
- \( a \) = volume of sample titration
- \( b \) = volume of blank titration

- A partial VFA Level measurement is determined using Gas Chromatography

Experimental design: In this study, a completely randomized design with five treatment levels of molasses (5, 10, 15, 20 and 25%) was tested and repeated 5 times. The five treatment rations were A (rice straw 89, urea 4, lime 2 and molasses 5%), B (rice straw 84, urea 4, lime 2 and molasses 10%), C (rice straw 79, urea 4, lime 2 and molasses 15%), D (rice straw 74, urea 4, lime 2 and molasses 20%) and E (rice straw 69, urea 4, lime 2 and molasses 25%).

Variables observed: A chemical analysis of the nutrient content from the mixture of rice straw urea lime molasses was conducted. In vitro studies observed pH, NH₃ level, total VFA and partial VFA (acetic, propionate and butyric), dry matter digestibility and organic matter digestibility.

Data analysis: The data obtained were analyzed by one-way analysis of variance. If there was a significant difference between treatment (p<0.05), Duncan’s multiple range test was conducted.

RESULTS AND DISCUSSION

The results showed that the nutrient value of rice straw increased proportionally to the level of molasses in the mixture of urea lime molasses added to the rice straw, especially the protein content. The protein content in treatments A, B, C, D and E were 12.01, 12.45, 12.60, 12.69 and 12.81% DM, respectively (Table 1). These findings suggest that the addition of a mixture of urea lime molasses to the rice straw may increase the crude protein content. This indicates that urea when mixed with lime and molasses is still attached to the straw after drying. The protein content in this study was higher than those studies which obtained protein content (8.11%) of dry matter from a urea molasses mixture. This difference in this study is due to adding lime to a mixture of rice straw urea lime molasses to increase the total urea bound to lime.

Table 3 reveals that the decreasing of NH₃ levels in treatment A were due to high utilization of molasses by rumen microbes which can be used as an energy source for microbial growth. These results indicated, the effect of interaction between protein sources and easily fermentable carbohydrate sources on the NH₃ levels.

The high growth of the microbes can be seen from the increasing levels of rumen and in vitro digestibility, where the rumen dry matter and organic matter digestibility was significantly higher in treatment E than in treatment A.

Statistical analyses revealed that the average Dry Matter Rumen Digestibility (DMRD) in treatment A was significantly lower (p<0.05) than those of the treatments C, D and E but was not significantly different from treatment B (Table 2). These findings indicated that the addition of urea lime molasses could increase the rumen microbial population, thereby producing the enzymes needed in the fermentation of rumen feed in vitro. The DMRD in treatments A, B, C and D increased, which shows that the increase of molasses from 5-20% could increase the energy readily available for microbial
growth to increase the rumen fermentation in vitro. The Organic Matter Rumen Digestibility (OMRD) values in treatments A, B, C, D and E were 32.52, 34.35, 39.49, 51.06 and 50.92%, respectively. Based on an analysis of variance, the OMRD in treatments A, B and C were not significantly different (p<0.05) and the OMRD in treatment D was not significantly different from the OMRD in treatment E. However, the OMRD in treatments A, B and C was significantly lower (p<0.05) than the OMRD in treatments D and E. This difference is due to the higher molasses use in treatments D and E.

The average in vitro Dry Matter Digestibility (IDMD) in treatments A, B, C, D and E was increase. An analysis of variance found that the IDMD in treatment E (p<0.05) was highest. The addition of molasses may be able to increase the rumen DMD so that when followed by digestion of the enzyme in vitro, the IDMD will also increase. Similarly, in vitro OMD followed the same pattern as DMD because the organic matter is part of the dry matter.

The average NH₃ levels in vitro in treatments A, B, C, D and E were 11.22, 10.92, 10.56, 9.99 and 7.92 mM, respectively. The NH₃ level, in treatment E (p<0.05) was lower than that of the treatments A, B, C and D, although the difference was not significant (p>0.05). Low NH₃ levels in treatment E showed an increased use of NH₃ by rumen microbes as a result of the availability of molasses as a soluble carbohydrates. Lee et al.¹⁷, reported that NH₃ decreased as a result of the addition of fermented carbohydrates. The addition of molasses aims to balance the provision of NH₃ for microbial protein synthesis so as to reduce the presence of NH₃ in the rumen.

The average pH value in treatment D was the highest and was related to the increased use of molasses, from 5-25%. This pH value follows the pattern of the low ratio of NH₃ to VFA, where there is an increase in pH according to the pattern of the addition of molasses. This fact is in accordance with the results of research demonstrating that the NH₃/VFA ratio
without the addition of molasses was higher than the NH₃- 
VFA ratio with the addition of a molasses treatment of 6 or 
12%¹⁰. 

Table 3 shows that VFA levels in treatments A, B, C, D and 
E increased and were highest in treatment E, which, may be 
due to the presence of 25% molasses in treatment E, because 
this molasses is an easily fermentable carbohydrate producing 
VFA. The VFA are the result of carbohydrate metabolism in the 
rumen. 

An analysis of variance revealed that the average 
concentration of acetic acid among treatments A, B and C was 
not significantly different but the acetic acid concentrations in 
treatments D and E are significantly higher than those in the 
other treatments (Table 3). The increased concentration of 
acetic acid showed that more fiber digestion occurred in 
treatments D and E. This dynamic was also indicated by the 
results of DMD and OMD in treatments D and E, which were 
significantly higher than those in other treatments. 

Table 3 shows that the concentrations of propionic acid 
in treatments A, B and C were not significantly different but 
the concentration of propionic acid in treatments D and E was 
significantly higher compared with that in treatments A and 
B. The addition of molasses would produce higher propionic 
acid because molasses is a soluble carbohydrate that can 
provide a source of energy for microbes and thus improve the 
digestibility of both dry matter and organic matter (Fig. 1). 

Table 3 reveals that the concentrations of butyric acid in 
treatments A, B and C were not significantly different but the 
concentrations of butyric acid in treatments E and D, although 
not significantly different from each other, were significantly 
higher from concentrations in treatments A, B and C. This 
dynamic indicates that the increased use of molasses in 
treatments D and E yields higher butyric acid as a result of 
fermentative digestion by microbes (Fig. 1). The low levels of 
NH₃ was due to the availability of soluble carbohydrates as a 
source of energy. Microbes consumed higher NH₃, therefore, 
the remaining NH₃ concentration becomes the least in 
treatment E (Table 3). 

The addition of molasses (20-25%) in the mixture of urea 
lime molasses can improve the digestibility of dry matter and 
organic matter compared with other treatments. The use of 
25% molasses in the mixture of urea lime molasses gives the 
highest total VFA fermentation product, acetic, propionic and 
butyric acid concentrations compared to the other molasses 
levels. 

CONCLUSION 

Based on the results of this study, the addition of 
molasses is optimal at a concentration of 25% when 
considering the nutrient content, rumen metabolite, dry 
matter and organic matter digestibility in vitro. 

SIGNIFICANCE STATEMENT 

This study found that the nutritional value of rice straw 
can be increased with the mixture of urea lime molasses 
consists of 20-25% molasses, 4% urea and 2% lime, through 
splashed on the dried rice straw. The optimal molasses 
concentration is 25% gives the highest nutrient content, 
rumen metabolite, dry matter and organic matter digestibility 
in vitro. 

ACKNOWLEDGMENTS 

This study was fully funded by the Institute of Research 
and Community Service Udayana University, an opportunity 
for which the authors express gratitude. 

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