

## CHARACTERISTICS OF RUMEN FERMENTATION WITH FEED FORMULATION USING MOLASSES IN-VITRO

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### ABSTRACT

This study aims to determine the effect of molasses use in ruminant livestock rations on rumen fluid characteristics in vitro. This study was conducted using a Completely Randomized Design experimental method with 3 treatments and 5 replications, the treatments consisted of: P0 = Ration formulation without molasses use, P1 = Ration formulation with 3% molasses use, P2 = Ration formulation with 6% molasses use. The variables observed were pH value, NH<sub>3</sub> production and rumen fluid VFA production. The results of the study for pH value obtained P0 = 6.98; P1 = 6.94; P2 = 6.89. NH<sub>3</sub> production P0 = 10.02 mg / 100ml; P1 = 10.89 mg / 100ml; P2 = 11.42 mg / 100ml and VFA production P0 = 104.00 mM; P1 = 120.00 mM; P2 = 130.00 mM. Based on the results of the study, it was shown that the use of molasses in the ruminant livestock ration formula had no significant effect ( $P>0.05$ ) on the pH, and had a significant effect ( $P<0.05$ ) on the production of NH<sub>3</sub> and VFA in the rumen in vitro. Where the use of molasses up to 6% in the ration formulation can increase NH<sub>3</sub> production, VFA production and maintain the pH value of the rumen fluid.

**Keyword** : molasses, In vitro, feed formulation, pH, NH<sub>3</sub>, VFA.

### INTRODUCTION

Highly productive livestock such as during pregnancy and lactation, require a balance between protein and carbohydrates in feed. Fermentation in the rumen can run smoothly if the feed given contains balanced and continuous nitrogen and carbohydrates. Readily available carbohydrates (RAC) are carbohydrates that are easily available or degraded in the rumen, so that they quickly provide products in the form of  $\alpha$  keto acids from carbohydrate fermentation which are used

for microbial protein synthesis. The speed of availability of carbon chain sources must be balanced with the speed of availability of ammonia as a source of N. The addition of carbohydrates to feed can increase microbial activity, microbial growth rate, and the rate of substrate degradation by rumen microbes. Therefore, the balance of nitrogen and carbohydrates in ruminant livestock feed rations needs to be considered to optimize the rations eaten so as to produce high production in livestock.

One of the available RAC is molasses, Molasses is a simple carbohydrate that is easily digested in the rumen. Molasses is widely used as an additional ration, and in fermentation in the feed industry.

Molasses is a by-product of the sugar processing industry in liquid form. Molasses produced by the sugar cane industry in Indonesia is known as molasses. Molasses is an essential source of energy with a sugar content in it, therefore molasses is widely used as one of the ingredients in rations with a fairly good nutritional content. According to Yudith (2010) molasses is a by-product of sugar cane processing waste which is brownish black in color with a fairly good nutritional content in it so that it is good for use and is liked by livestock. According to Wirihadinata (2010) molasses has a nutritional content of dry matter (DM) 67.5%; crude protein (CP) 4%; crude fat (LK) 0.08%; crude fiber (SK) 0.38%; TDN 81%; P 0.02% and Ca 1.5%.

Fermentative digestion of carbohydrates will produce VFA or volatile fatty acids. Volatile Fatty Acids (VFA) are the main source of energy for ruminant livestock which comes from the end product of carbohydrate fermentation found in the rumen. VFA acts as a carbon framework for the formation of microbial

proteins. Ammonia (NH<sub>3</sub>) concentration is the result of the degradation process of protein and non-protein nitrogen that enters the rumen of ruminant livestock. Ammonia concentration is related to microbial protein synthesis, because microbes in the rumen utilize ammonia as the main source of nitrogen for microbial protein synthesis. NH<sub>3</sub> concentration is one of the indicators to determine the fermentability of feed protein, microbial activity and rumen microbial population. This study aims to determine the effect of the use of molasses in ruminant livestock rations on the characteristics of rumen fluid in vitro.

## MATERIALS AND METHOD

**Materials.** The materials of this study include green fodder consisting of field grass, titonia, and indigofera and concentrate consisting of bran, tofu dregs, palm kernel cake, corn and minerals, and molasses as a source of non-structural carbohydrates, Mc. Daugall Solution, Rumen fluid taken at the UPTD Air Pacah Slaughterhouse, Padang City.

**Methods.** This study was conducted using an in vitro experimental method with the design used being a Completely Randomized Design with 3 treatments and 5 replications. Where the treatment is the

ration formula added with molasses as a source of Non-Structural Carbohydrates with different percentages, namely:

P0 = Ration formulation without the use of molasses

P1 = Ration formulation with the use of 3% molasses

P2 = Ration formulation with the use of 6% molasses

The ration is formulated based on the needs of lactating goats, with TDN 67% and PK around 18%, with a ratio of greens and concentrates of 70:30.

The chemical composition of each diet can be seen in Table 1..

Tabel 1. Chemical composition diet

Component	P0	P1	P2
<b>Ingredient composition (%)</b>			
Native grass	20	20	20
<i>Tithonia diversifolia</i>	32	32	32
<i>Indigofera zollingeriana</i>	18	18	18
Palm kernel cake ( <i>Elaeis guineensis</i> )	10	7	7
Tofu Waste	5	5	5
Rice Bran ( <i>Oroza sativa</i> )	6	6	6
Mayze ( <i>Zea mays</i> )	8	8	5
Molasses	0	3	6
Mineral	1	1	1
	100	100	100
<b>Chemical Composition (%)</b>			
Organic matter	89.18	88.65	88.37
crude protein	18.73	18.41	18.20
Crude fiber	18.01	17.59	17.02
Crude fat	4.49	4.25	3.98
Nitrogen Free Extract	43.78	43.69	43.87
TDN	67.18	67.22	67.34

**Statistical Analysis.** f the treatment shows a highly significant effect ( $P < 0.05$ ), it will be further tested by Duncan's New Multiple Range Test (DNMRT) ( Steel and Torrie, 1993).

**In vitro Method** .Samples of diets were incubated with buffered rumen according to Tilley and Terry method (Tilley and Terry 1963). Rumen liquor was obtained from a

slaughterhouse in the city of Padang from one simmental cow which was fed ad libitum coarse and concentrate with a weight of  $\pm 350$  kg.. The rumen content was filtered with four layers of nylon (100m strainer size) and then placed into thermos flasks after the slaughter ( $39^{\circ}\text{C}$ ). As suggested by McDougall (1947), filtered rumen liquor was diluted with buffer

solution at a ratio of 1:4 (rumen fluid:buffer solution). An amount of 2.5g sample was mixed with 250mL of mixed solution (rumen liquor and buffer) in each Erlenmeyer flask and incubated anaerobically by injecting CO<sub>2</sub> gas into the Erlenmeyer, which was then immediately sealed with a rubber lid. Each sample was replicated three times. Each Erlenmeyer was kept in an orbital shaking incubator at 39°C and 100 rpm for 48 hours. The pH of the Erlenmeyer was tested using a pH meter after it was immersed in ice water for 48 hours to cease microbial activity.

The supernatant and residue were then separated using a centrifuge at 3000rpm for 5min at 4°C. The NH<sub>3</sub>, total VFA, supernatant was stored in bottles in a -18°C freezer. The Conway and O'Malley (Conway and O'Malley 1942) method was used to determine the NH<sub>3</sub> concentration. Steam distillation method was used

according to Abdurachman and Askar (2000) to determine total VFA concentration.

## RESULT AND DISCUSION

Increased adding molasses to ruminant feed using *Indigofera zollingeriana* and *Tithonia diversifolia* did not affect the rumen fluid pH value ( $P>0.05$ ), increasing the percentage of added molasses tended to increase total VFA production ( $P<0.05$ ). It can be seen that the highest VFA and NH<sub>3</sub> is found in addition of 6% molasses. This shows that addition of 6% molasses as a source of Readily available carbohydrate of fermented in the rumen (RAC) has high fermentable carbohydrates. The characteristics of rumen fermentation in this study can be seen in Table 2. Table 2. Rumen fermentation characteristic of experimental diets.

Addition of Molasses (%)	Variable		
	pH	NH <sub>3</sub> (mg/100ml)	VFA (mM)
P0 (0%)	6,98	10,02 <sup>a</sup>	104,00 <sup>c</sup>
P1 (3%)	6,94	10,89 <sup>b</sup>	120,00 <sup>b</sup>
P2 (6%)	6,89	11,42 <sup>b</sup>	130,00 <sup>a</sup>
SEM	0.02	0,13	1,72

<sup>a,b,c</sup>Superscript means significantly different in a column ( $P<0.05$ ), SEM=Standard error of mean

Table 2, showed that the use of molasses had no significant effect ( $P>0.05$ )

on the acidity level (pH) of rumen fluid. This is because the provision of molasses

up to 6% does not disrupt the balance of microorganisms in digesting feed. A stable pH value indicates optimal rumen conditions for rumen microbial growth in the process of feed degradation and fermentation in the rumen. In accordance with the opinion of Perry et.al., (2003), which states that the use of carbohydrate-rich materials (molasses) can accelerate the decrease in pH, because carbohydrates are energy for the growth of lactic acid-forming bacteria. It can be seen that by increasing the percentage of molasses use, it can reduce the pH of the rumen fluid. This is in accordance with the results of Wanapat's (2009) study which stated that the addition of molasses to low-quality green fodder can reduce the pH of the rumen fluid in vitro at the level of adding 8% molasses, the pH of the rumen fluid dropped from 6.8 (without molasses) to 6.5 in line with the study of Suparjo et.al., (2011) the addition of 6% molasses to complete rice straw-based feed reduced the pH of the rumen fluid in vitro from 7.0 (without molasses) to 6.8. Khotijah et.al., (2016) added that the addition of molasses to ammoniated rice straw reduced the pH of the rumen fluid in vitro. At the level of adding 10% molasses, the pH of the rumen fluid dropped from 6.9 (without molasses) to 6.8.

Table 2, It can be seen that the use of molasses has a significantly different effect ( $P < 0.05$ ) on the production of  $\text{NH}_3$  in rumen fluid. This is because molasses provides an energy source that supports the growth and activity of rumen microbes, including proteolytic microbes that degrade protein into  $\text{NH}_3$ . In accordance with the opinion of Suwandiyastuti and Rimbawanto, (2012) who stated that molasses contains easily soluble carbohydrates such as sucrose, glucose, and fructose which can increase rumen microbial activity. Increasing rumen microbial activity can help the protein degradation process to be more optimal, so that crude protein digestibility increases. Molasses is a fermentable carbohydrate used as energy for the growth of lactic acid-forming bacteria and the lactic acid produced reacts with ammonia or  $\text{NH}_3$ . In addition, bacteria can also fix  $\text{NH}_3$  as a source of N for their reproduction (Migo et.al., 1993). The high concentration of  $\text{NH}_3$  in P1 and P2 indicates the availability of rumen degradable protein (RDP) and energy in the form of Readily available carbohydrate (RAC) which is sufficient for the growth of rumen microbes (Anggraeny et.al., 2015).

Table 2. showed that the treatment of molasses use had a

significantly different effect ( $P < 0.05$ ) on the production of rumen fluid VFA. This can be caused by the use of molasses in the ration can provide a source of easily soluble carbohydrates (simple sugars) that can be fermented quickly by rumen microbes to become VFA. VFA is the main source of energy for ruminant livestock produced from feed fermentation in the rumen. so that the increase in VFA shows that molasses can increase the availability of energy for livestock. In accordance with the opinion of Durago et.al., (2021) which states that the carbohydrate content in feed and its decomposition determine the amount of VFA produced in the rumen. The high production of VFA in treatments P1 and P2 can be influenced by the amount of non-structural carbohydrates added to the ration in the form of molasses, where non-structural carbohydrates have a higher level of fermentability and are easily dissolved in the rumen, thereby increasing the digestibility of feed ingredients and the number of bacteria in the rumen (Wijayanti, 2012). If the higher the concentration of VFA, the energy source in livestock is optimum, this indicates that the fermentation process is more effective, but if the VFA is too high it can have an impact on disrupting the balance of the rumen system (Sandi et.al., 2015).

## CONCLUSION

Based on the results of the study, it can be concluded that the use of molasses up to 6% has a significantly different effect ( $P < 0.05$ ) on the production of  $\text{NH}_3$  and VFA in vitro, and can maintain the pH of the rumen fluid. The higher the dose of molasses, the higher the production of  $\text{NH}_3$  and VFA in vitro, the characteristics of the rumen fluid in the study were in optimal conditions for the growth and activity of rumen microbes.

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