Effect of urea in steamed sago waste on rumen fermentation parameters in vitro tested

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Abstract. This study aims to determine urea’s effect in steamed sago waste on rumen fermentation parameters in vitro testing. Sago waste was dried for two days, discarded fibre sticks, steamed for 30 minutes, cooled and dried. Weighed 250 g of steamed sago waste, added urea with levels of 0%, 2%, 4% and 6%. Weighed 0.5 g of samples per treatments, inserted in fermentor tubes, added 10 ml buffer and 10 ml of rumen fluid (1:1). Fermentor tubes are inserted in waterbath with a temperature of 39°C, flowed with CO2 gas and covered with a valved rubber cover. For NH3 and VFA testing, incubation was carried out for 4 hours, while for DMD and OMD testing, incubation was carried out for 48 hours. The data obtained were analyzed using a completely random design with four urea level treatments (0%, 2%, 4%, 6%), with five replications. The results showed that increase of urea level up to 6%, increasing (P<0.01) NH3. The increase of urea level 2%, increasing (P<0.01) VFA, DMD and OMD. The increase of urea levels 4% and 6%, not significant effect on DMD and OMD, while at urea level 6%, decreasing (P<0.01) VFA. It can be concluded that urea was added in steamed sago waste, have an optimal effect on the rumen fermentation parameters at level 2–4%.

Keywords: Urea, Steamed sago waste, Fermentation parameters.

1. Introduction
The feed is an important factor in the maintenance and productivity of ruminants [1], so that feed must be available in quantity, quality and continuity. One way to solve this problem is by utilizing industrial waste and processing agricultural products.

One of the byproducts of agricultural product processing that is quite available in Maluku is sago waste, which are the residual products of sago flour processing. In the processing of sago flour, sago waste is usually produced six times that of sago flour, so that sago waste can be obtained as much as 2.7 million tons/year [2]. The limiting factor for the use of sago waste in livestock rations is the high content of crude fibre (especially lignin) which reduces digestibility, due to the presence of lignocellulose bonds that inhibit the penetration of fibre digesting enzymes from rumen microbes. Sago waste contains residues of lignin and cellulose, respectively 21% and 20% [3], containing NDF (Neutral Detergent Fiber) and ADF (Acid Detergent Fiber), respectively 57.27% and 49.54% [4]. In addition, the crude protein content in sago waste is also very low. Crude protein in Tuni sago was 0.92%, while in Ihur sago was 1.01% [5].

To improve the digestibility of the sago waste, try to do the steaming with the aim of loosening the lignocellulose bonds so as to facilitate the penetration of the fibre digestive enzymes from rumen microbial, so that digestibility can be increased. To increase the protein content of sago waste, try to...
do the adding urea because urea contains 46.7% Nitrogen [6], which is equivalent to 291.88% crude protein. Urea is a source of non-protein nitrogen (NPN) which is most often used as a substitute for true protein feed because it can reduce the cost of animal feed [7].

Urease enzymes will convert urea in the rumen from rumen microbes to CO$_2$ and NH$_3$, which are very important for the forming microbial protein if a carbon framework is available from carbohydrate sources. Excessive addition of urea in the ration can cause poisoning in the absence of a source of carbohydrates as the carbon framework. Steaming sago waste is expected to increase the release of the carbon framework, so that urea can be given in large quantities without causing poisoning because the production of NH$_3$ will be in line with the availability of the carbon skeleton so that protein synthesis and rumen microbial growth are run well so that ration digestibility and protein formation microbes can be increased.

This study aims to determine the effect of urea level, which added in steamed sago waste on rumen fermentation parameters in vitro tested. Through the results of this study, will be obtained a good balance between urea and steamed sago waste so that it becomes the basis for making concentrate feed based on steamed sago waste, which was applied in vivo.

2. Methods
Sago waste was obtained from a processing place for sago flour, which was dried in the sun for two days, then removed the fibre sticks. The sago waste is steamed for 30 minutes, then cooled and dried in the sun. Weighed as much as 250 g of sago waste then added urea according to the treatment level, namely 0%, 2%, 4% and 6%. The rumen fluid was taken from 2 bulls slaughtered at the Slaughterhouse, which was filtered through the cloth into a hot water flask, then added solid CO$_2$ so that the atmosphere becomes anaerobic.

2.1. Incubation 4 hours
Weighed as much as 0.5 g of the sample, put it in the fermenter tube, then added 10 mL of rumen fluid and 10 mL of Mc buffer. Dougall solution with a pH of 6.9 (1:1). The fermenter tube was supplied with CO$_2$ gas and covered with a valved rubber cover, and then it was put into a waterbath at 39 °C and incubated for 4 hours. The fermentation process was stopped by adding 0.2 mL of saturated HgCl$_2$ to kill microbes. This fermentative product was centrifuged at 6,000 rpm for 30 minutes to separate the precipitate and the supernatant. The Supernatant was taken to be tested for NH$_3$ concentration and total VFA. The NH$_3$ concentration was tested using the micro diffuse Conway technique, while the total VFA was tested by the steam distillation technique [8]. The concentration of NH$_3$ can be calculated by the formula:

$$\text{mM N-NH}_3 : \frac{\text{vol. titrant (mL) } \times N \text{H}_2\text{SO}_4 \times 1000}{\text{vol. supernatant (mL)}}$$

The concentration of total VFA can be calculated by the formula:

$$\text{mM T-VFA : } \frac{[\text{vol. titer blanco (mL)} - \text{vol. titer sample (mL)}] \times N \text{HCl} \times 1000}{\text{vol. supernatant (mL)}}$$

2.2. Incubation 48 hours
Weighed as much as 0.5 g of the sample, put it in the fermenter tube, then added 8 mL of rumen fluid and 12 mL off buffer Mc. Dougall solution with pH of 6.9 (1:1.5). The fermenter tube was supplied with CO$_2$ gas and covered with a valved rubber cover, and then it was put into a waterbath at 39°C and incubated for 24 hours. The fermentation process was stopped by adding 0.2 mL of saturated HgCl$_2$ to kill microbes. This fermentative product was centrifuged at 6.000 rpm for 30 minutes to separate the precipitate and the supernatant. The precipitate was taken and incubated with 20 mL of pepsin 0.2 percent solution for 24 hours aerobically. The fermentation product was filtered with Whatman filter paper number 41 with the aid of a vacuum pump and washed with 25 mL hot water. The filter and
filter paper was put into a porcelain dish to be analyzed for dry matter digestibility (DMD) and organic matter digestibility (OMD) [8].

For the DMD test, the residue in a porcelain cup was evaporated in an electric oven at 105°C for 24 hours until its weight was constant. The percentage of DMD can be calculated using the formula:

\[
\% \text{ DMD} = \frac{\text{DM sample} - (\text{DM residue} - \text{DM Blanco})}{\text{DM sample}} \times 100
\]

For the (OMD) test, the residue in the porcelain cup was ashed in an electric furnace at 650°C for 8 hours. The percentage of OMD can be calculated using the formula:

\[
\% \text{ OMD} = \frac{\text{OM sample} - (\text{OM residue} - \text{OM Blanco})}{\text{OM sample}} \times 100
\]

2.3. Statistic Analysis

The data obtained were analyzed variance with a completely randomized design, 4 treatments, namely 0% (steamed sago waste + 0% urea); 2% (steamed sago waste + 2% urea); 4% (steamed sago waste + 4% urea); and 6% (steamed sago waste + 6% urea), each with 5 replications. Significant treatment was tested further by Duncan's New Multiple Range Test [9].

3. Results and discussion

3.1. Effect of urea levels in feed with steamed sago waste on NH\textsubscript{3} concentration

The effect of urea level in feed with steamed sago waste on the NH\textsubscript{3} concentration can be seen in Figure 1.

The results of statistical analysis showed that the addition of urea with different levels in steamed sago waste feed had a significant effect (P<0.01) on the NH\textsubscript{3} concentration. These results indicate that changes in the concentration of NH\textsubscript{3} follow the urea level added in steamed sago waste, meaning that the higher the added of urea in the steamed sago waste will increase the NH\textsubscript{3} concentration. This is caused by urea is very easily broken down in the rumen by urease enzyme produced by rumen microbes which convert the available urea to NH\textsubscript{3} continuously in the rumen. According to Kardaya et al. [10], urea in the rumen was rapidly hydrolyzed into NH\textsubscript{3} by rumen microbes, then most of it was absorbed rapidly in the bloodstream system, which can have a negative impact on livestock, including decreased consumption and production performance, and death due to poisoning of urea. This shows that the change of N sources (including urea) to NH\textsubscript{3} in the rumen takes place continuously, even though NH\textsubscript{3} has been sufficient or excessive so that the NH\textsubscript{3} concentration continues to increase.
Excess of NH$_3$ will be absorbed in the rumen, then converted into urea by the liver, which is finally filtered out by the kidneys and excreted in the urine, so urea breaking down rapidly [11].

3.2. Effect of urea levels in feed with steamed sago waste on T-VFA concentration
The effect of urea level in feed with steamed sago waste on the T-VFA concentration can be seen in Figure 2.

![Figure 2]

Figure 2. The average of T-VFA concentration (mM) due to the use of urea with different levels in feed with steamed sago waste, in vitro tested.

The results of statistical analysis showed that the addition of urea with different levels in steamed sago waste feed had a significant effect (P<0.01) on the T-VFA concentration. When compared with the un-addition of urea (0%), the addition of urea 2–4% in the steamed sago waste, increased (P<0.01) the T-VFA concentration, but the addition of urea by 6% decreased (P<0.01) of T-VFA concentration. The increase in urea level up to 6% may negatively affect rumen microbes, which reduces rumen microbes ability to degrade carbohydrates in steamed sago waste feed, thereby reducing T-VFA concentration. According to Sharma et al [12], the addition of urea in feed, if not done properly, will cause poisoning and interfere with the rumen’s fermentation process. In addition, the decrease in T-VFA concentration may also be caused by the VFA was used for microbial protein synthesis by utilizing the availability of N sources from urea. According to Tiven et al [13], the concentration of VFA tends to decrease, it may be used as a source of C framework for microbial protein synthesis when N sources are available, so that microbial protein increases, but the T-VFA concentration decreases. According to McDonald et al [14], the optimum VFA content in the rumen is between 10 to 70 mM. It can be said that the decrease in T-VFA is still within the normal range.

3.3. Effect of urea levels in feed with steamed sago waste on dry matter digestibility (DMD) and organic matter digestibility (OMD)
The effect of urea level in feed with steamed sago waste on the DMD and OMD, can be seen in Figure 3 and 4.
The results of statistical analysis showed that the addition of urea with different levels in steamed sago waste feed had a significant effect (P<0.01) on the DMD and OMD. The addition of urea to a level of 2% in the steamed sago waste feed, increased (P<0.01) the percentage of DMD and OMD. The addition of urea at the 4% and 6% levels had no significant effect on the percentage of DMD and OMD, even at the 6% level, it tended to reduce DMD. This is probably caused by at the urea level of 6%, the concentration of NH₃ produced is very high so that it has a negative effect on DMD and OMD. The high NH₃ concentration is not in line with the low T-VFA concentration so that the released NH₃ cannot be synthesized by rumen microbes into microbial protein, so the percentage of DMD and OMD tend to decrease. This means that the percentage of DMD and OMD was influenced by rumen microbial activity due to the availability of energy, through the high concentration of T-VFA, but with an increase in urea at the level of 6%, decreases T-VFA (Figure 2), which shows a decrease in microbial activity, so that DMD and OMD also decreased. This shows that the high percentage of DMD and OMD was caused by rumen microbial activity due to the availability of energy.
4. Conclusion
It can be concluded that urea was added in steamed sago waste, have an optimal effect on the rumen fermentation parameters at level 2–4%.

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