The Use of Calcium Hydroxide with Different Soaking Time on Cassava Peel for Reducing HCN, and Its Effect on Rumen Fermentation

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ABSTRACT
The objective of this study is to determine the best dose of calcium hydroxide [$Ca(OH)_2$] and soaking time for reducing hydrogen cyanide (HCN) level of cassava peel and its effect on rumen fermentation. This study consists of two stages. The first stage was the treatment of soaking cassava peels in $Ca(OH)_2$ with different doses and soaking times. This study was conducted using the factorial 3x3 randomized block design (RBD) with three replications. The first factor was $Ca(OH)_2$ doses (0%, 0.25%, 0.5%) and the second factor was the soaking time (1, 2, 3 hours). The Parameter in the first stage was to determine the HCN content of cassava peel. The second stage of this study was an *in vitro* study to determine the characteristics of the rumen fluid: pH, ammonia (NH_3) and volatile fatty (VFA) conducted using in randomized block design. The result of the first stage showed that there is no interaction ($P>0.05$) between $Ca(OH)_2$ doses and soaking time on the content of hydrogen cyanide (HCN) content of cassava peels. A factor, $Ca(OH)_2$ dose had a significant effect ($P<0.05$) on HCN content with an average of 27.23 ppm - 32.31 ppm; while B factor, soaking time had a highly significant effect ($P<0.01$) on HCN level and nutrient content. The average HCN content ranged from 24.83 ppm - 33.51 ppm. The results of the second stage in the *in vitro* fermentability study on the characteristics of rumen fluid showed that the pH value of rumen fluid ranged from 6.57- 6.92, was not affected by a combination of treatment, but levels of NH_3 and VFA were highly significant different ($P<0.01$). The average NH_3 and VFA content ranged from 9.92 - 11.76 mg/100 ml, and 98.33 - 115 mM respectively. It can be concluded that the best dose of $Ca(OH)_2$ used for reducing the HCN level of cassava peels was 0.25% with a soaking time of 3 hours. The best combination was found in the A3B2 treatment. at a dose of 0.50% $Ca(OH)_2$ and 2 hours of soaking time. The best levels of VFA and NH3 were 115 mM VFA, 11.62 mg NH_3/100 ml respectively with rumen pH 6.88.

Keywords: *Cassava peel, HCN, Calcium hydroxide, Rumen characteristic.*

1. INTRODUCTION

Cassava peel is an agricultural waste that is available in abundance but has not been used optimally as animal feed. In 2018 Indonesia's cassava production was 19,341,233 tons [1]. There was an increase in cassava production in Indonesia by 1.51% from 2017 to 2018. In West Sumatra, cassava production in 2020 was 154,728.76 tons [2]. The amount of cassava peel produced is 15% of the weight of the cassava [3]. It is predicted that the availability of cassava peel in West Sumatra in 2020 is 23,209 tons. This indicates that the more cassava production is produced, the more waste cassava peels can be used for animal feed. In addition to its sufficient availability potential, cassava peel also has a fairly good nutritional content. [4, 5, 6].

Cassava peel is one of the feed ingredients that can be used as an energy source, because it has good nutritional content, namely it contains high nitrogen free extract (75.40%) and high Total Digestible Nutrients (68.86%) and it contains 5.88% crude protein. However, the use of cassava peel as cattle feed has a limiting factor, namely the presence of anti-nutrient HCN compounds which can be toxic to livestock [7].

For this reason, a method is used to reduce HCN levels from cassava peels, so that it does not interfere with cattle production, so that he use of cassava peels in the ration can be increased, and it will increase the value of the benefits of cassava peels as an energy source for cattle. One method that can reduce HCN levels from cassava peel is by soaking it using whiting water, $Ca(OH)_2$. Cassava peels can be given to livestock directly or by pre-processing, but there is not much data on how much of its use in cattle rations does not interfere with livestock health.

The use of cassava peel in dairy cow rations can be used as much as 9%. Cassava peel has a limiting factor for its use as animal feed, namely the presence of a high content of HCN (cyanide acid) which is toxic. Giving cassava peels more than 9% can cause respiratory problems in dairy cattle [5]. The HCN content of fresh cassava peel is as much as 120 ppm [5]. Soaking cassava peels for 1 hour can reduce the HCN content to 59 ppm from its initial content of 120 ppm. Based on this, it is hoped that decreasing HCN level of cassava peel, it can increase the use of cassava peel as animal feed.

The toxic nature of cyanide is determined by its concentration and is generally associated with the formation of complexes with metals that play a role in the respiratory process so that the respiration process is disturbed. The enzyme Fe(III) cytochrome oxidase is an example of an enzyme in the respiration process that is inhibited by cyanide. Cyanide in the form of hydrogen cyanide (HCN) can cause very rapid death if inhaled in certain concentrations [7, 8]. The HCN content of gadung tuber slices after soaking in a $Ca(OH)_2$ solution at a dose of 0.3% for 2 hours, 4 hours, and 6 hours showed the best decrease in HCN content at a dose of 0.3% for 6 hours with a decrease in the percentage of HCN content as much as 89.00% [9]. The addition of soaking can reduce the HCN content due to the presence of calcium which binds cyanide so that cyanide is released from the material. The use of $Ca(OH)_2$ is expected to reduce the HCN content in cassava peels and can maintain the nutritional content in it, so that the utilization of cassava peels for ruminant animal feed can be increased.

Many studies have shown the importance of volatile fatty acids for ruminant nutrition [10]. VFA is a fermented product in the rumen which is the main energy source for ruminants and also functions as a carbon skeleton for microbial protein synthesis, while NH3 is a product of protein fermentation and non-protein nitrogen compounds in the rumen which are needed by rumen microbes as a nitrogen source for microbial protein synthesis in the rumen. One of the factors that influence the growth and activity of microbes in fermenting feed is rumen pH. Therefore is necessary to determine the characteristic of rumen fluid. The problem is how much cassava peel can be used in rations that do not interfere with production of ruminants.

2. MATERIALS AND METHODS

2.1. Cassava peel source

The cassava peel was obtained from a cassava processing factory in Padang. The nutritional content of cassava peel is presented in Table 1.

Table 1. Nutrient content of cassava peel

Nutrient Content (% Dry Matter)	Content (%)
Organic Ingredients (OM)	96.56
Crude Protein (CP)	5.88
Lipid (CL)	1.29
Crude Fiber (CF)	13.99
Ash	3.44
Nitrogrn-Free Extract	75.40
TDN	68.86

Source: Faculty of Animal Husbandry Nutrition Laboratory, Andalas University in 2020

2.2. Soaking process

$Ca(OH)_2$ was weighed as much as 2.5 grams and 5.0 grams, each dissolved in 1000 ml of water. The mixture wa stirred until homogeneous using a stir bar or spoon. Cassava peel that has been cut into pieces is then soaked using $Ca(OH)_2$ solution at a dose of 0%, 0.25%, and 0.5% for 1, 2, and 3 hours. The ratio between cassava peel and $Ca(OH)_2$ solution was 1:2, which is 1 kg of cassava peel soaked with 2 liters of $Ca(OH)_2$ solution. During soaking the cassava peels are turned over to mix and soak evenly.

Cassava peel that has been soaked, then rinsed with running water and drained first. Then the cassava peel is dried in the sun dry or in an oven at 60ºC for 48 hours. After that, the cassava peel is ground or mashed using a blender.

2.3. Experimental design stage

This study consists of two stages. The first stage was the treatment of soaking cassava peels in $Ca(OH)_2$ with different doses and soaking times. This study was conducted using the factoril 3x3 randomized block design with three replications [11]. The first factor (factor A) was $Ca(OH)_2$ doses (0%, 0.25%, 0.5%) and the second factor (factor B) was the soaking time (1, 2, 3 hours). The first stage of the study was to determine the HCN content. The second stage of this study was an invitro rumen fermentation study [12] to determine the

characteristic of the rumen fluid by using randomized block design.

2.4. Determination of hydrocyanic acid (HCN)

Hydrocyanic acid (HCN) is determined by the steam distillation method [13]. The 20 grams sample is put into an Erlenmeyer and added 100 ml of distilled water then the sample is soaked for 2 hours. The sample is distilled. The distillate was accommodated in an Erlenmeyer which had been filled with 20 ml of 2.5% NaOH. The distillation process is complete when it has reached 150 ml of the reservoir volume. Add 8 ml of NH_4OH, 5 ml of 5% Kl to the sample that has been distilled and titrated with a 0.02 N $AgNO_3$ solution until turbidity occurs (turbidity will be easily seen if under Erlenmeyer put black carbon paper).

HCN calculation formula:

HCN (ppm) =(x ml $AgNO_3$ 0.02 N x 54X 1000)/Y
Information :
X = Volume of titration result
Y = sample weigh

2.5. Determination of Rumen Characteristic (Volatile Fatty Acids /VFA, Ammonia /NH3) and pH in Rumen Fluid

Determination of VFA levels was carried out by steam distillation technique [14]. The supernatant was pipetted as much as 5 ml and put into an Erlemeyer glass, added 1 ml of 15% H_2SO_4 and closed. The results of the distillation were accommodated in an erlemeyer containing 5 ml of 0.5 N NaOH. The distillation process ends until the volume is ± 250 ml. Then 3 drops of phenolphthalein indicator were added and titrated with 0.5 N HCl until the pink color changed to clear. The total VFA level can be calculated by the formula:

Total VFA (mM) = (a-b) × N HCl × 1000 / 5 (mM)

Description: a = ml HCl for blank titration

b = ml HCl for sample titration

The determination of NH3 levels was determined by the Conway micro diffusion technique [14]. A total of 1 ml of the supernatant were placed on the left of Conway's bulkhead and 1 ml of saturated Na_2CO_3 solution was placed in the partition on the right. In a small cup in the middle filled with boric acid with a methyl red indicator and 1 ml of bromine cresol green.

Then the Conway cup was tightly closed with a vaseline lid and then shaken so that the supernatant mixed with Na_2CO_3. The cup was left for 24 hours at room temperature. Ammonia bound with boric acid is titrated with 0.005 N H_2SO_4 until the color changes to pink.

NH_3 levels can be calculated by the formula:

NH_3 level (mg/100 ml) = (N H_2SO_4 x ml H_2SO_4 titrant x 100

2.6. Data analysis

The data were statistically processed by using Analysis of Variance (ANOVA). The difference in effect between treatments was tested by orthogonal contrasts [11].

3. RESULTS AND DISCUSSION

3.1. HCN Content

HCN is an anti-nutritional factor found in cassava peel, which can cause poisoning in livestock if it is present in excessive amounts. The result shows that HCN content of cassava peel was influenced by each factor ($P<0.05$), namely $Ca(OH)_2$ dose and soaking time. However there was no interaction between these two factors on the HCN content of treated cassava peel. The average HCN content of cassava peel treated by soaking in $Ca(OH)_2$ for 1-3 hours is shown in Table 2.

The HCN level obtained decreases with the longer soaking of the cassava peel in $Ca(OH)_2$. HCN levels at 3 hours of immersion are 24.83 ppm. This is the lowest HCN level when compared to 1 or 2 hours of soaking time. When it is soaked for 1 hour, the HCN content is 33.51 ppm. Of course, the expected result is that the HCN content of cassava peels is as low as possible, so that the use of cassava peels can be increased without disturbing the health of livestock.

Based on soaking time (factor B), the highest percentage of HCN reduction occurred at 3 hours of soaking time, with a percentage decrease in HCN was 25.90%. While, at 2 hours of soaking time, the percentage decrease in HCN content was only 9.82%. The longer the immersion of the cassava peel in $Ca(OH)_2$, the greater the percentage decrease in HCN levels. The best effect of treatment on HCN levels was found at a soaking time of 3 hours.

Based on $Ca(OH)_2$ dose (factor A), the lowest HCN level occurred at a dose of $Ca(OH)_2$ of 0.25%, with a decrease in the percentage of HCN of 15.72%. While the highest HCN levels were found at a dose of 0% $Ca(OH)_2$. There is no decrease in HCN content in the treatment from 0.25% to 0.50% $Ca(OH)_2$. The more the dose of $Ca(OH)_2$ was increased from 0.25% to 0.50%, the HCN level did not decrease with a decreasing percentage value which also decreased to 53.15%. This is because with increasing doses of $Ca(OH)_2$ from 0.25% to 0.5%, there can be saturation between the binding of Ca from $Ca(OH)_2$ and HCN in cassava peel so that the binding process of calcium with cyanide is getting slower. It is known [17] that the decrease in cyanide content will stop; there will be no further decrease in HCN levels if it reaches a certain equilibrium point so that there is no more binding of

Table 2. The average HCN content (ppm) of cassava peel treated by soaking in $Ca(OH)_2$ solution for 1-3 hours

$Ca(OH)_2$	Soaking Time			Average
	B1 (1 hour)	B2 (2 hours)	B3 (3 hours)	
A1 (0%)	34.11	35.90	26.92	32.31^{a}
A2 (0.25%)	32.31	28.72	20.64	27.23^{b}
A3 (0.50%)	34.11	26.03	26.92	29.02^{ab}
Average	33.51^{a}	30.22^{a}	24.83^{b}	

Note: Different superscripts in the same row and column showed a significant effect ($P<0.05$)

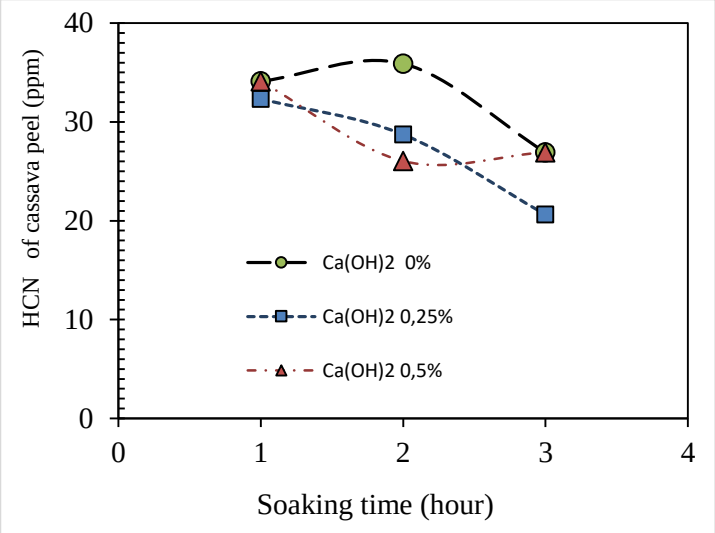

Figure 1. The HCN content of cassava peel

calcium to cyanide. The more $Ca(OH)_2$ is added, the more calcium will bind cyanide. However, if the addition is too high, there will be a saturation point for the binding of calcium to cyanide so that it becomes slower and there is even no more CN^- that binds to Ca^{2+}. Giving $Ca(OH)_2$ is too high causing cyanide that should be absorbed by lime water cannot be absorbed [18] The best effect of treatment on HCN levels was found at 0.25% $Ca(OH)_2$ dose. The HCN content of cassava peel that has been treated by soaking in $Ca(OH)_2$ is shown in Figure 1.

In Figure 1, it can be seen that the HCN content of cassava peels as a result of soaking in $Ca(OH)_2$ decreased with the soaking time from 1 hour to 3 hours, especially at a dose of 0.25% $Ca(OH)_2$. The decrease in HCN levels was because HCN was easily soluble in water. The longer the soaking time in 0.25% $Ca(OH)_2$, the cassava peel became softer and the HCN in the cassava peel cells would out and dissolve in water. The HCN formed will bind to Ca from $Ca(OH)_2$ to form $Ca(CN)_2$. The $Ca(OH)_2$ dissolved in water will decompose into Ca^{2+} and OH^- ions while HCN in water will decompose into H^+ and CN^-. In the form of these ions, there is an attraction to form a bond, ie negatively charged ions will attract each other with positively charged ions. H^+ will bind OH^- to form H_2O while Ca^{2+} binds CN^- to form a white precipitate of calcium cyanide which is soluble in water. Therefore the HCN content of cassava peel decreases. According to the statement [16] that the betel lime ($Ca(OH)_2$ is hygroscopic or attracts water and can raise the pH and damage cell walls until the HCN dissolves. This causes HCN which can bind to Ca from $Ca(OH)_2$ which dissolves immediately with positively charged ions. H^+ will bind OH^- to form H_2O while Ca^{2+} binds CN^- to form a white precipitate of calcium cyanide which is soluble in water so that the HCN content of cassava peel decreases. Betel lime is hygroscopic or attracts water and can raise the pH and damage cell walls until the HCN dissolves. This causes HCN which can bind to Ca from $Ca(OH)_2$ which dissolves immediately [19].

The insignificant effect ($P>0.05$) on the interaction between $Ca(OH)_2$ dose and soaking time was thought to be due to a change in response caused by the effect of error or residue. So the cooperation between the two combined factors is said to be independent of each other. However, in the results of the study, it can be seen that the single factor of giving the dose of whiting and soaking time affects the HCN content.

3.2. Rumen Volatile Fatty Acids

Fermentative digestion of carbohydrate in the rumen produces fermentation products, namely volatile fatty acids (VFA). The average VFA values produced with

Table 3. Rumen Volatile Fatty Acids (mM) of Cassava peel

$Ca(OH)_2$ Dose	Soaking Time			Average
	B1 (1 hour)	B2 (2 hours)	B3 (3 hours)	
A1 (0%)	98.33^{d}	98.33^{d}	100^{cd}	98.89
A2 (0.25%)	103.33^{bcd}	108.33^{abc}	106.67^{bcd}	106.11
A3 (0.50%)	111.67^{ab}	115^{a}	115^{a}	113.89
Average	104.44	107.22	107.22	

Note: Different superscripts in the same row and column showed a significant effect ($P<0.05$)

Table 4. Average NH_3 levels of rumen fluid of cassava peel (mg/100 ml)

$Ca(OH)_2$ Dose	Soaking Time			Average
	B1 (1 hour)	B2 (2 hours)	B3 (3 hours)	
A1 (0%)	10.77^{c}	10.34^{de}	10.49^{cd}	10.53
A2 (0.25%)	10.34^{de}	10.14^{de}	11.10^{bc}	10.56
A3 (0.50%)	11.76^{a}	11.62^{ab}	9.92^{de}	11.10
Average	10.96	10.70	10.50	

Note: Different superscripts in the same row and column showed a highly significant effect ($P<0.05$)

different $Ca(OH)_2$ doses and soaking times are listed in Table 3.

Analysis of variance showed that each treatment combination had a significantly different effect ($P<0.05$) on the mean VFA of the rumen fluid. The range of VFA levels produced is between 93.33 mM to 115 mM. The total production of VFA produced can meet the needs of rumen microbes for optimal development and growth. The previous study stated that the concentration of VFA required for microbial growth and activity is 80-160 mM [18].

An increase in the amount of VFA in the rumen fluid indicates whether or not the feed ingredients are easily degraded by microbes in the rumen. The best VFA value in this study was shown by the interaction of treatment A3B2 and A3B3. This is because at a dose of 0.50% $Ca(OH)_2$ and soaking time of 2 hours and 3 hours there is a change in the texture and composition of nutrients that produce VFA in the fermentation process. Changes in the texture of crude fiber will be easier for microbes to degrade nutrients. It increases its digestibility. Therefore, the VFA level increased. This is in accordance with the results of the study [19] which states that $Ca(OH)_2$ decreased the cell wall composition of soybean straw. High VFA production can provide sufficient energy for ruminants [20]. Energy is conserved in the form of adenosine triphosphate, during the fermentation process. The growth of the microbial population depends on this energy [21].

For the measurement of fermentation activity in the rumen, it can be predicted from the data on the rate of VFA formation. Based on the levels of VFA produced from cassava peel that has been treated by immersion in $Ca(OH)_2$, with different doses and soaking times, it can be seen that cassava peel is an energy source feed ingredient that is easily fermentable.

The best VFA levels obtained in this study can be used by rumen microbes to increase their population and activity. by producing enzymes to digest nutrients. According to the statement [18] that the VFA has two functions. The first is the main energy source for ruminants and secondly is a carbon skeleton for microbial protein synthesis.

3.3. Rumen Ammonia (NH_3)

Fermentative digestion of protein in the rumen produces fermentation products, namely ammonia. The average rumen NH_3 is presented in Table 4.

The result showed that each treatment combination had a very significant ($P<0.01$) effect on NH_3 levels. The resulting rumen fluid NH_3 levels ranged from 9.92 mg/100 ml to 11.76 mg NH_3/100 ml rumen fluid. This level of NH_3 meets the minimum requirement of ammonia for microbial growth. Meanwhile, the minimum concentration of ammonia required for microbial protein synthesis is 5 mg/100 ml of rumen fluid [22].

The highest NH_3 level was found in the combination of 0.5% $Ca(OH)_2$ dose treatment and 1 hour soaking time with rumen fluid NH_3 content of 11.76 mg/100 ml rumen fluid (A3B1), while the lowest NH_3 level also occurred at 0.5% $Ca(OH)_2$ dose, but the soaking time is 3 hours (A3B3). Because NH_3 is a fermentation product of protein in the rumen, so the level of NH_3 is influenced by the crude protein content of the feed ingredients. After analyzing the crude protein content of cassava peel, it was found that the

Table 5. Rumen pH of Cassava peel

$Ca(OH)_2$ Dose	Soaking Time			Average
	B1 (1 hour)	B2 (2 hours)	B3 (3 hours)	
A1 (0%)	6.57	6.85	6.83	6.75
A2 (0.25%)	6.88	6.85	6.85	6.86
A3 (0.50%)	6.83	6.88	6.92	6.88
Average	6.76	6.86	6.87	

Note: there is no significant difference in the effect on the pH value of the rumen fluid

crude protein content of A3B1 was 5.65%, while that of A3B3 was 4.57%. In the treatment combination 0.50% $Ca(OH)_2$, the longer the soaking time, which is up to 3 hours, it causes a lot of dissolved nutrients. This affects the composition of nutrients. The difference in crude protein content of 1.08% has given significantly different results to NH_3 levels of rumen fluid.

NH_3 levels were determined not only by the protein content in each treatment combination, but also by the availability of energy for microbes to degrade the protein of the cassava peel. With different amounts of protein digested, the NH_3 produced is also different because NH_3 protein is the result of protein fermentation of feed ingredients in the rumen. This is in accordance with the opinion [23] that the NH_3 produced in the rumen is the result of protein fermentation in the rumen. The best NH_3 levels were found in the combination treatment of 0.50% $Ca(OH)_2$ and immersion time of 1 hour and 2 hours.

The results of this study found that the use of $Ca(OH)_2$ to reduce HCN levels in cassava peels *in vitro* produced sufficient NH_3 to support rumen microbial growth. The degradation of protein in the rumen can continue even though the ammonia produced is more than sufficient to meet microbial needs. Excess ammonia in the rumen will not increase microbial growth rumen. Ammonia or NH_3 produced will be utilized to form microbial protein if it is supported by the availability of energy from easily digestible carbohydrates. The best NH_3 levels found in this study were shown by the interaction of treatment A3B1 and A3B2. The NH_3 produced is used by rumen microbes as a nitrogen source to synthesize microbial protein. This condition will increase the ability of microbes to ferment feed protein so that the NH_3 produced increases. Ammonia produced will be used by rumen microbes with available energy. The previous study [24] that the addition of a rapidly degradable carbohydrate source may optimize the utilization of ammonia.

3.4. Rumen pH

The average value of the rumen fluid pH of cassava peel that has been treated by soaking in $Ca(OH)_2$ at a dose of 0-0.5% with a soaking time of 1-3 hours is presented in Table 5.

The rumen pH range did not rise much above 7. The results showed that each treatment with a combination of $Ca(OH)_2$ dose and soaking time had a non-significantly different effect ($P>0.05$) on the pH value of the rumen fluid. Based on the results the pH value of the rumen fluid in this treatment ranged from 6.57 to 6.92.

The results were not significantly different in the pH of the rumen fluid due to the balance of VFA production and NH_3 levels, as well as the presence of a buffer in the form of artificial saliva which was able to maintain optimal conditions of rumen fluid pH. The pH of the rumen fluid did not indicate that the treatment given did not interfere with microbial activity in rumen. The ideal rumen pH ranges from 6.50 to 7.00 where these pH conditions can support rumen microbial growth, Rumen pH less than 6.0 can inhibit the proteolysis and deamination processes so that digestion feed can be disturbed. The pH of the rumen fluid will be normal if there is a balance between VFA (acidic) and NH_3 (alkaline).

Rumen pH in this study is influenced by the amount of fermentation activity. Cassava peel that easily produces VFA will quickly lower the pH of the rumen fluid. Saliva acts as a buffering capacity helps maintain pH to maintain a stable pH of the rumen fluid [25]. The type of feed, incubation time can affect the pH of the rumen fluid

The treatment of soaking cassava peel in $Ca(OH)_2$ can provide an ideal pH for the growth of rumen microbes with a pH value of 6.76-6.87. In this pH condition, rumen microbes can digest feed substances by producing enzymes to digest feed substances that enter the rumen.

4. CONCLUSION

The best dose of $Ca(OH)_2$ to reduce the HCN content is at a dose of 0.25% with an HCN content of 27.03 ppm and the best soaking time to reduce the HCN content of cassava peels was 3 hours with an HCN content of 24.83 ppm.

The best combination was found in the A3B2 treatment at a dose of 0.50% $Ca(OH)_2$ and 2 hours of soaking time. The results for the levels of VFA, NH_3 and pH were 115 mM VFA, 11.62 mg NH_3/100 ml and 6.88 rumen pH, respectively.

AUTHORS' CONTRIBUTIONS

Fauzia Agustin designed and directed the project experiments. All authors contribute to the implementation of the study and the final results of the manuscript.

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