P-ISSN: 2304-3075; E-ISSN: 2305-4360
International Journal of Veterinary Science
www.ijvets.com; editor@ijvets.com
Research Article https://doi.org/10.47278/journal.ijvs/2023.104

Decreasing Cyanide Acid Content Through Soaking in Betel Lime: Effect on Chemical Composition and Nutrient Digestibility of Cassava Peel

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Article History: 23-279 Received: 14-Sep-23 Revised: 03-Oct-23 Accepted: 07-Oct-23

ABSTRACT

The use of cassava peel in rations can be increased by reducing cyanide acid levels so that it is not dangerous for ruminants. The purpose of this study was to evaluate the nutrients with the optimum chemical composition and nutrient digestibility of cassava peels treated by soaking in calcium hydroxide or $Ca(OH)_2$. In the first step, cassava peels were soaked in $Ca(OH)_2$ using various dosages (0, 0.25, and 0.50%) and soaking time (1, 2, and 3h). The factorial 3x3 randomized block design with three replications was used to perform this investigation. The second stage was an in vitro investigation to find out the nutrients' digestibility. The results showed that there was a significant decrease ($P<0.05$) in the crude fiber content and a significant increase ($P<0.05$) in the nitrogen-free extract (NFE) content of cassava peel with increasing soaking time from 1-3h. There was a significantly different interaction ($P<0.05$) between calcium hydroxide dose and soaking time on the digestibility of nutrients: dry matter, organic matter, NFE, crude protein and crude fiber. In conclusion, the best soaking time to reduce crude fiber and increase nitrogen-free extract content was 3 hours, and the best nutrients digestibility was found in the treatment combination of 0.50% betel lime and 2h soaking time.

Key words: Betel lime, Cassava peel, Chemical composition, Digestibility, HCN.

INTRODUCTION

Hydrogen cyanide is an inorganic compound with the molecular formula HCN. HCN at certain doses can cause poisoning in animal. This compound is very toxic. HCN toxicity is determined by the HCN content in the feed consumed and the balance between HCN absorption and its detoxification by livestock (Hahn et al. 1988). HCN content varies between plants, between species, and between tissues in same plants. One of the plants containing HCN is cassava.

The by-product of processing cassava is cassava peel. Cassava peel makes up around 10% of the processed cassava (Aro et al. 2010). Cassava peel can be used as a mixture of feed ingredients for dairy cattle, especially as an energy source (Agustin et al. 2020; Suyitman et al. 2020), for goat (Ajagbe et al. 2020; Aribido et al. 2021; Arief et al. 2023) and for sheep (Niayale et al. 2020). Its nutritional content as a source of energy is quite good, with a nitrogen free extract content of 75.40% and Total digestible nutrients (TDN) of 68.86% (Agustin et al. 2021). However, cassava peel contains anti nutrient in the form of HCN which in high doses can be toxic (Kutay et al. 2017; Gensa 2019). It must be treated in order to lower the HCN concentration, which may be carried out by soaking cassava peel in calcium hydroxide. (Agustin et al. 2021). The nutritional content of cassava peel includes 94.35% dry matter, 97.68% organic matter, 7.20% crude protein, 19.51% crude fiber, 56.06% nitrogen-free extract, 56.91% TDN, 7.20% lignin, 13.8% cellulose, and 11% hemicellulose (Aregheore 2000). Dairy cows' diets can contain up to 9% cassava peel. The use of cassava peel as animal feed is complicated by the peel's high content of dangerous HCN. Fresh cassava peel can contain up to 120ppm HCN (Agustin et al. 2020). On the basis of this, it is predicted that reducing the HCN level of cassava peel will enable greater usage of the peel as animal feed.

Cite This Article as: Agustin F, Jamarun N, Ningrat RWS, Pazla R and Suryadi H, 2024. Decreasing cyanide acid content through soaking in betel lime: Effect on chemical composition and nutrient digestibility of Cassava peel. International Journal of Veterinary Science 13(3): 349-356. https://doi.org/10.47278/journal.ijvs/2023.104

One of the most crucial poisonings in animals is cyanide poisoning (Kutay et al. 2017). The toxicity of cyanide is dependent on its concentration and is typically correlated with the disruption of the respiratory process due to the complex formation with metals involved in the respiratory process. An example of an enzyme involved in respiration that cyanide inhibits is Fe(III) cytochrome oxidase. If ingested, cyanide in the form of hydrogen cyanide can result in very quick death (Gensa 2019). The greatest HCN content reduction was seen in gadung tuber slices following soaking in a $Ca(OH)_2$ solution at doses of 0.3% for 2, 4, and 6h, with a drop in the percentage of HCN content as high as 89.00%. HCN content in cassava peel can be reduced by soaking in $Ca(OH)_2$, because calcium from $Ca(OH)_2$ can bind cyanide from HCN to form CaCN which is not toxic (Djaafar et al. 2009). $Ca(OH)_2$ usage is anticipated to decrease the HCN concentration in cassava peels as they keep the peels' nutritive value, increasing the use of cassava peels for ruminants.

Immersion process with $Ca(OH)_2$ can lower HCN levels. The amount of HCN in cassava peels was decreased by the soaking duration. The range of the average HCN concentration was 24.83 to 33.51ppm (Agustin et al. 2021). The results of an *in vitro* fermentability evaluation on the parameters of rumen fluid from cassava peel immersed in $Ca(OH)_2$ indicated that the rumen fluid's pH value, which ranged from 6.57 to 6.92, had no impact by the combination of $Ca(OH)_2$ treatment and soaking period. However, there was a highly significant improvement in NH_3 and VFA content. The average concentrations of NH_3 and VFA were respectively 9.92–11.76mg/100mL and 98.33–115mM (Agustin et al. 2021).

The ability of a feed to be digested, absorbed, and processed by an animal's system, whether in terms of nutrients or energy, can be described as its nutritional digestibility. Digestibility in animal nutrition can vary according to the type of feed, and basic materials, even though the nutrients are presumably available in the nutritional table of the feed, low digestibility in animal nutrition can lead animals to lose weight and indicates nutritional gaps. The chemical component and digestion of nutrients from a feed component must thus be determined.

Many studies have shown the importance of the chemical composition of feed and the digestibility of nutrients for ruminants. The factors that influence the growth and activity of microbes in fermenting feed are feed processing and availability of nutrients. Consequently, it's important to establish the chemical composition and nutrient digestibility of cassava peel that has been processed by immersion with betel lime. The purpose of this study was to evaluate the nutrients with the optimum chemical composition and nutrient digestibility. of cassava peels treated by soaking in $Ca(OH)_2$ at different doses and soaking times, in relation to the decrease in HCN levels.

MATERIALS AND METHODS

Ethical Approval: Since live animals were not used in this research, ethical approval is not thought to be required.

Study Period and Experimental Site: This research was carried out at Andalas University's Ruminant Laboratory of Animal Science Faculty from July to November 2022.

Cassava Peel Source: The cassava peel was obtained from a cassava processing factory in Padang, West Sumatra, Indonesia. The nutritional content of cassava peel before soaking treatment is presented in Table 1.

Soaking Process of Cassava Peel: 5 and 10g of betel lime were weighed and then dissolved into 2L of water. Then mixed with a spoon to homogenize. After being sliced into pieces, the cassava peel was then drenched in betel lime solution at doses of 0, 0.25, and 0.50% for 1, 2, and 3h, respectively. Cassava peel and betel lime solution were mixed in a 1:2 ratio, resulting in 1kg of soaked cassava peel and 2L of betel lime water. The cassava peels were mixed and rolled over during soaking to ensure equal soaking. Cassava peel that was soaked, washed under running water and then drained. The cassava peel was then dried for 48h either outside in the sun or inside a 60°C oven. then the cassava peel was ground and ready to be analyzed.

Experimental Design: There are two stages to this investigation. In the first step, cassava peels were treated by being soaked in betel lime for various amounts of time. The factorial 3x3 and randomized block design with triple replications was used to perform this investigation (Gomez and Gomez 1995). Factor A was made up of betel lime doses of 0, 0.25, and 50%, and component B was made up of immersion times of 1, 2, and 3h. The study's chemical composition was initially examined. The study's initial step was to find out the chemical components. In order to estimate the digestibility of nutrients, the second step of this study used an in vitro approach (Tilley and Terry 1963) to assess the rumen's digestive system.

Samples Analysis

Determination of Chemical Composition: Sampling for analysis was carried out to determine the dry matter (DM) content and proximate analysis. The dried samples were weighed and ground to pass through a 2mm filter size. The milled samples were subjected to proximate analysis. Nutrient contents (Table 1) were also determined, namely the content of dry matter, organic matter, crude protein, crude fat, crude fiber, and nitrogen-free extracts using the proximate analysis method (AOAC 2016).

Table 1: Nutrients content of Cassava peel before soaking with calcium hydroxide

Nutrient (% dry matter)	Content (%)
Organic matter	96.56
Crude proteins	5.88
Crude fat	1.29
Crude fiber	13.99
Ash	3.44
Nitrogen free extract	75.40
Total Digestible Nutrients (TDN)	68.86
HCN (ppm)	120

It is analyzed at the Nutrition Laboratory of Animal Science Faculty, Andalas University.

***In Vitro* Method:** The *in vitro* study was used to determine the digestibility of nutrients. The method used in determining digestibility was the modified Tilley and Terry method (1963). The sample was put into the fermenter tube and added rumen fluid as a source of microbes and McDoughal's solution as artificial saliva. Fermentation was carried out for 48h at 39°C and anaerobic conditions. The fermentation process in the rumen was stopped after 48h. Rumen fluid pH was determined using a pH meter. Then centrifugation was carried out to separate the supernatant and residue. The residue is used to determine the nutrient's digestibility value.

Determination of Nutrients Digestibility: The residue was dried in an oven at 60°C after being filtered through Whatman No. 41 filter paper. Then, the residue was analyzed to determine nutrient digestibility following the proximate analysis method.

Implementation of Sample Analysis in the Laboratory: Measurement of *In Vitro* Dry Matter Digestibility (IDMD)

The value of *In Vitro* Dry Matter Digestibility

$$= \frac{sampel\ weight\ x\ sampel\ dry\ matter - (residu\ weight - blank\ weight) x\ residual\ dry\ matter}{sampel\ weight\ x\ dry\ matter\ sampel}\ x\ 100\%$$

Measuring the digestibility of other nutrients digestibility (digestibility of organic matter, crude fiber, nitrogen-free extract, crude lipid, and crude protein digestibility) has the same principle of calculation as measuring the dry matter digestibility.

Data Analysis

The data were statistically processed by using Analysis of Variance (ANOVA). The difference effect between treatments was tested by orthogonal contrasts (Gomez and Gomez 1995).

RESULTS

HCN Content of Cassava Peel: Table 2 provides information on the HCN content of cassava peel after soaking in betel lime water at different immersion times. The HCN level obtained decreases with the longer soaking of the cassava peel in betel lime water. HCN levels at 3h of immersion are 24.83ppm. This is the lowest HCN level when compared to 1 or 2h of immersion time. When it was soaked for 1h, the HCN content was 33.5ppm.

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

$Ca(OH)_2$ dose	Soaking time			Average
	B1 (1h)	B2 (2h)	B3(3h)	
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]	

Different superscripts in the same row and column show significant (P<0.05) effect.

Crude Fiber Content of Cassava Peel after Soaked in Calcium Hydroxide: Table 3 gives information that the crude fiber content of cassava peel soaked in calcium hydroxide was only affected by the soaking time with a very significant effect (P<0.01), while the dose of whiting 0-0.5% did not affect the crude fiber content cassava skin (P>0.05). In Table 3, it can also be seen that there is no interaction between the dose of calcium hydroxide and the soaking time (1-3h) on the crude fiber content and the dose of calcium hydroxide 0-0.5% has no significant (P>0.05) different effect.

Table 3: The average crude fiber content (%) of cassava peel treated by soaking in $Ca(OH)_2$ for 1-3 hours

Ca(OH)2 dose	Soaking time			Average
	B1 (1h)	B2 (2h)	B3 (3h)	
A1 (0%)	12.80	14.01	12.46	13.09
A2 (0.5%)	13.72	14.89	12.07	13.56
A3 (0.0%)	13.10	14.32	11.28	12.90
Average	13.21[b]	14.41[a]	11.94[c]	

Different superscripts in the same row show a significant (P<0.05) effect.

The lowest crude fiber content of cassava peel was found in cassava immersion time of 3 hours, with a crude fiber content of 11.94%. The crude fiber content for 3h of soaking indicated significantly different results (P<0.01) with 1h of immersion time with 13.21% crude fiber content and 2 hours immersion time with 14.41% crude fiber content.

Based on Table 2, information was obtained that soaking with betel lime did not affect the crude fiber content of cassava peel. The crude fiber content began to decrease at a dose of 0.50%, but this was not statistically significant.

Nitrogen Free Extract (NFE) Content of Cassava Peel after Soaked in Calcium Hydroxide: Table 4 provides information that there is no interaction between the dose of calcium hydroxide and soaking time on the nitrogen-free extract content of cassava peels. Factor A, the dose of calcium hydroxide did not have a significant (P>0.05) effect, while factor B, soaking time had a very significantly different (P<0.01) effect on the NFE content of cassava peel. The highest NFE content was found in 3h of the immersion, namely 77.70%, followed by 1h and 2h of the soaking time with successive values of 76.38 and 74.77% respectively. There was an increase in the NFE content of cassava peels from 74.77 to 77.70% which also coincided with a decrease in the crude fiber content during the soaking time. The longer the soaking time, the higher the nitrogen-free extract content.

Table 4: The average NFE (%) of cassava peel treated by immersion in lime water for 1-3 hours

$Ca(OH)_2$ dose	Soaking time			Average
	B1 (1h)	B2 (2h)	B3 (3h)	
A1 (0%)	76.87	76.01	77.75	76.87
A2 (0.25%)	76.40	73.73	75.75	75.29
A3 (0.50%)	75.88	74.57	79.61	76.69
Average	76.38[a]	74.77[b]	77.70[a]	

Different superscripts in the same row show a significant (P<0.05) effect.

Nutriens Digestibiliy of Cassava peel

In vitro Dry Matter Digestibility (IDMD) of Cassava Peel after Soaked in Betel Lime Water: Table 5 provides information on dry matter digestibility using *in vitro* method. *In vitro* dry matter digestibility value ranged from 51.41 to 55.10%.

Table 5: The average *in vitro* dry matter digestibility (IDMD) (%) of cassava peel treated by soaking in $Ca(OH)_2$ for 1-3 hours

$Ca(OH)_2$ dose	Soaking time			Average
	B1 (1 hour)	B2 (2 hours)	B3 (3 hours)	
A1 (0%)	51.43[d]	52.38[cd]	53.87[abc]	52.56
A2 (0.25%)	52.33[cd]	53.59[abc]	54.57[ab]	53.50
A3 (0.50%)	53.94[abc]	54.56[ab]	55.10[a]	54.53
Average	52.57	53.51	54.51	

Different superscripts in row or column show significant ($P<0.01$) effect.

The different soaking time and calcium hydroxide level resulted in highly significant ($P<0.01$) difference in IDMD. The highest dry matter digestibility value was obtained at 3h of immersion time with a dose of 0.50% calcium hydroxide with a digestibility value of 55.10%. The longer the immersion, the more soluble carbohydrates that are counted as dissolved nitrogen-free extract.

In vitro Organic Matter Digestibility of Cassava Peel after Soaked in Calcium Hydroxide: The *in vitro* organic matter digestibility (IOMD) values obtained are shown in Table 6. Each treatment combination had a significantly different ($P<0.01$) effect on organic matter digestibility. It is known that if the cassava peel is soaked in 0% betel lime water for 3h, it will give the same digestibility value as the 0.25% betel lime treatment, but the immersion time is reduced to 2h. When the dose of betel lime was increased again up to 0.50%, so the same digestibility value could be achieved at only 1h of immersion time. The highest organic matter digestibility value was found in the combination of immersion time of 3h and a dose of 0.5% betel lime, with the value of 56.35%. The same results were also obtained at a dose of betel lime 0.25% and a long immersion time of 3h, with the value of 55.99%. At a dose of 0.5% betel lime with 2 hours of soaking, it gives the same results as 3 hours of immersion with the value of 55.60%.

Table 6: The average *in vitro* organic matter digestibility (IOMD) (%) of cassava peel treated by soaking in $Ca(OH)_2$ for 1-3 hours.

$Ca(OH)_2$ dose	Soaking time			Average
	B1 (1h)	B2 (2h)	B3 (3h)	
A1 (0%)	52.46[c]	53.85[bc]	55.28[ab]	53.86
A2 (0.25%)	53.83[bc]	54.89[ab]	55.99[a]	54.90
A3 (0.50%)	55.12[ab]	55.60[a]	56.35[a]	55.69
Average	53.80	54.78	55.37	

Different superscripts in row or column show significant ($P<0.01$) effect.

Crude Fiber Digestibility of Cassava Peel after Soaked in Calcium Hydroxide: Table 7 shows the digestibility of crude fiber. and the lowest value was found in the soaking time of 3h and the dose of calcium hydroxide 0.5% with a crude fiber digestibility value of 50.43%. The digestibility value of crude fiber in this study was influenced by the soaking process and the presence of rumen microbial activity. It was noted that the digestibility value of crude fiber decreased with increasing doses of betel lime from 0 to 0.5%, both for 1, 2 or 3h of the immersion.

Nitrogen Free Extract Digestibility of Cassava Peel after Soaked in Betel Lime Water: Table 8 provides information that the digestibility of nitrogen free extract (NFE) gives a good value of 68-69%. This value was found in 3h soaking time for all doses of calcium hydroxide, 0, 0.25, 0.50%. The same digestibility value was also obtained for 2h of immersion time at betel lime doses of 0.25 and 0.50%.

Table 7: The average crude fiber digestibility (%) of cassava peel treated by soaking in $Ca(OH)_2$ for 1-3 hours

$Ca(OH)_2$ Dose	Soaking Time			Average
	B1 (1 hour)	B2 (2 hour)	B3 (3 hour)	
A1 (0%)	52.32[a]	52.19[a]	51.92[ab]	52,14
A2 (0.25%)	51.83[ab]	51.87[ab]	51.32[abc]	51,67
A3 (0.50%)	50.97[bc]	50.54[c]	50.43[c]	50.65
Average	51.71	5.53	51.22	

Different superscripts in row or column show significant ($P<0.01$) effect.

Table 8: The average nitrogen free extract digestibility (%) of cassava peel treated by soaking in $Ca(OH)_2$ for 1-3 hours.

$Ca(OH)_2$ dose	Soaking Time			Average
	B1 (1h)	B2 (2h)	B3(3h)	
A1 (0%)	64.89[b]	66.81[b]	68.19[a]	66.63
A2 (0.25%)	66.68[b]	68.39[a]	69.50[a]	68.19
A3 (0.50%)	68.43[a]	69.28[a]	69.24[a]	68.98
Average	66.67	68.16	68.98	

Different superscripts in row or column show significant ($P<0.01$) effect.

Crude Protein Digestibility of Cassava Peel after Soaked in Betel Lime Water: The highest crude protein digestibility value in Table 9 was found in betel lime dose of 0.50% and 3h of immersion time with a crude protein digestibility value of 57.21%. However, this value was not significantly different from betel lime dose of 0.25% and immersion time of 2h, with a protein digestibility value of 56.11%.

Table 9: The average crude protein digestibility (%) of cassava peel treated by soaking in $Ca(OH)_2$ for 1-3 hours.

$Ca(OH)_2$ dose	Soaking Time			Average
	B1 (1h)	B2 (2h)	B3 (3h)	
A1 (0%)	53.24[d]	54.18[cd]	55.64[abc]	54.35
A2 (0.25%)	54.52[bcd]	56.11[a]	56.48[a]	55.70
A3 (0.50%)	55.91[ab]	56.82[a]	57.21[a]	56.65
Average	54.56	55.70	56.44	

Different superscripts in row or column show significant ($P<0.01$) effect.

DISCUSSION

Crude Fiber Content of Cassava Peel after Soaked in Betel Lime Water

Soaking cassava peel with whiting for 3 hours reduces HCN level and crude fiber content. HCN easily dissolves in water, so the longer the soaking time, the softer the structure of the cassava peel will be. This can make it easier for water to enter the cell structure of the cassava peel, so that the HCN in the cells comes out and dissolves in water. According to Suismono and Prawirautama (1998), lime water solution is alkaline and can damage the cell walls of cassava peel. This damaged cell wall results in a reaction to form HCN. The HCN formed binds to Ca in $Ca(OH)_2$, forming $Ca(CN)_2$ which are easily soluble in water. Djaafar et al. (2009) found that the longer the gadung tuber slices soaked in $Ca(OH)_2$ with a dose of 0.3%, more HCN is formed and the amount of HCN that dissolves is also increasing. In this study, at a soaking time of 3hours the crude fiber content decreased. After 3 hours of soaking, much of the starch was dissolved. This dissolved starch is counted as nitrogen free extract, so the crude fiber content drops to 11.94%.

In contrast to the immersion time, the dose of betel lime or $Ca(OH)_2$ from 0 to 0.50% had no significant effect on the crude fiber content of cassava peel. This is because the addition of betel lime up to 0.5% only has an effect on the fiber structure of the cassava peel, there is a loosening of the fiber bonds and has not reduced the fiber content and it is hoped that by violating the fiber structure this will later be more easily attacked by the digestive enzymes produced in the rumen by rumen microbes. This condition can increase the digestibility of fiber and the availability of energy for livestock will also increase (Pazla et al. 2020; Pazla et al. 2021). The soaking process with betel lime causes damage to the cell membrane, in addition to that soaking with betel lime loosens the pore network resulting in the transfer of material that is able to pass through the permeable membrane.

Nitrogen Free Extract Content of Cassava Peel after Soaked in Calcium Hydroxide: The nitrogen free extract (NFE) content of a feedstuff is very dependent on other components, such as ash, crude protein, crude fiber and crude fat (McDonald et al. 2002). The longer the soaking time, the higher the nitrogen free extract content. This is presumably because at 3h of the immersion there are more easily dissolved substances, especially starch. Nitrogen free extract is part of easily digestible carbohydrates such as sugar, starch, organic acids which are included in non-structural carbohydrates that are easily digested. In accordance with the opinion of McDonald et al. (2002) which states that nitrogen free extract is a soluble carbohydrate which includes monosaccharide, disaccharides, trisaccharides and polysaccharides, especially starch. All of these components are soluble in acids and bases in the analysis of crude fiber and have high digestibility. Increasing the nitrogen free extract content can increase the amount of organic matter that can be easily digested (Cherney and Cherney 2000).

In this study, the NFE content before soaking was 75.40%, and after soaking for 3 hours, it increased to 77.70%. Nitrogen free extract describes the easily digestible carbohydrate fraction. Naturally, nitrogen free extract is easier for microbes to digest, so microbes tend to use it first. Decreasing the crude fiber content of a feed ingredient will increase its nitrogen free extract content.

In vitro Dry Matter Digestibility (IDMD) of Cassava Peel

The difference in the digestibility of the dry matter is due to the immersion process in betel lime water. There has been a change in the composition of the nutritional content of the cassava peel, especially a decrease in the content of crude fiber and an increase in the content of nitrogen free extract, which are components of the dry matter. This condition, namely the high levels of nitrogen free extract, makes cassava peel easier to digest by enzymes produced by rumen microbes and ultimately can increase the digestibility of dry matter. The digestibility of dry matter increased with increasing doses of betel lime and immersion time. This is because the digestibility values of the feed substances making up the dry matter also increased (Pazla et al. 2023). It can be seen in the digestibility values of nitrogen free extract, crude protein, and crude fat which determine the digestibility values of the dry matter.

Organic Matter Digestibility of Cassava Peel: The organic matter digestibility pattern is the same as the dry matter digestibility, where the longer the immersion time, the higher the organic matter digestibility. This has something to do with the process that occurs during soaking with betel lime. Betel lime or calcium hydroxide is a chemical compound with the chemical formula $Ca(OH)_2$. The betel lime can raise the pH because the betel lime is alkaline. Cassava peel that has been soaked in betel lime showed good rumen conditions for microbial growth, namely the ideal rumen fluid pH were 6.8 - 6.9, whereas cassava peel was soaked without using betel lime, then at 1h soaking the pH rumen fluid was only 6.57 (Agustin et al. 2021). Under these ideal rumen fluid pH conditions, rumen microbes can grow well and can increase their population so that with a large number of rumen microbes, there will be lots of enzymes produced by rumen microbes to digest and ferment food substances in the rumen which in turn increases the digestibility value.

In Table 6 it can be seen that the digestibility of organic matter increased with increasing doses of betel lime, either at 1, 2 or 3h of the immersion. The increased digestibility value of organic matter is due to the increased digestibility of the nutrients that make up the organic matter, namely the digestibility of mitrogen free extract, fat and protein except for crude fiber. Organic matter that are easily digested will provide energy for rumen microbial activity in degrading nutrients so that the digestibility of nutrients becomes better (Jamarun et al. 2017a; Zain et al. 2023).

Crude Fiber Digestibility of Cassava Peel: The digestibility value of crude fiber in this study was influenced by the immersion process, nutrients contents and the presence of rumen microbial activity. Based on the results of the first phase of the study, it was found that the crude fiber content decreased with increasing immersion time with betel lime. With lower levels of crude fiber, it will be more easily digested by enzymes produced by rumen microbes, so as to increase the digestibility value of fiber (Jamarun et al. 2017b; 2017c; Elihasridas et al. 2023). However, in this study the digestibility value of the fiber decreased with increasing betel lime dose. This could be as the more $Ca(OH)_2$ added, the more calcium binds to cyanide so that more and more cyanide is released from the cassava peel. However, the addition of $Ca(OH)_2$ that is too high will cause a saturation point of the binding of calcium to cyanide, causing slower or even no binding of calcium to cyanide of cassava peel. This condition can also cause an increase in the amount of easily soluble carbohydrates to be dissolved thereby increasing the starch content or nitrogen free extract. This can be explained that the cassava peel contains higher extracts without nitrogen, such as starch, so the microbes that grow and develop with this cassava peel substrate in the rumen are starch digesting microbes, so the high digestibility value of nitrogen free extract. so that the digestibility value of crude fiber was only about 50%.

Nitrogen Free Extract Digestibility of Cassava Peel after soaking in calcium hydroxide: Cassava peel is a feed ingredient that can be used as an energy source for dairy cows (Agustin et al. 2020). The nitrogen free extract (NFE) digestibility shown in Table 9 is digestibility value of single ingredient, namely cassava peel resulting from the soaking treatment. The increase in the NFE digestibility value of cassava peel after soaking for 1-3 hours was due to the increase in the NFE content. NFE is part of a carbohydrate that is easily soluble (McDonald et al. 2002). This study found that there was indeed an increase in nitrogen free exttract content with the longer soaking time and the higher calcium hydroxide dose. The fermentation process of structural and non-structural carbohydrates to volatile fatty acids (VFA) by microbial action in the rumen involves an interdependency of fermentable energy and microbial protein production (Dijkstra et al. 2005). Agustin et al. (2021) revealed that the best VFA concentration was 155 mM, which was found in a combination treatment of 0.50% $Ca(OH)_2$ and a soaking time of 2 h.

Cassava peel is rich in non-structural carbohydrates. This was proven in this study, namely the high NFE content of cassava peel (77.70%) after soaking with whiting for 3 hours. Zhao et al. (2015) state that non-fiber carbohydrate (NFC) type, rumen degradable protein (RDP) level and their interaction affected ruminal fermentation and microbial growth. The digestibility value of nutrients in the rumen is strongly influenced by the growth and activity of microbes in the rumen. The substrate that enters the rumen greatly determines the growth and the activities of microbes. Cassava peel is rich in nitrogen free extract.

The HCN content in cassava peel that has been treated with does not interfere with microbial activity in digesting nutrients. Cassava peel which has been treated with calcium hydroxide, has decreased HCN levels. The lowest HCN level was found at a dose of 0.25% betel lime and 3h of soaking time. The digestibility of NFE in cassava peel can represent the ability of feed to be utilized and converted by microorganisms. Increasing the availability of protein and energy has an effect on the absorption or utilization of food substances, so that NFE digestibility tends to increase (Budiman et al. 2006). In this study, NFE Carbohydrates in rumen will be hydrolyzed by the activity of microbial enzymes. The end product of this fermentation is volatile fatty acids (VFA) (Orskov and McDonald 1979).

Important influencing factors on the digestibility of feed ingredients is protein because it plays a role in maintaining the life of rumen microorganisms.

Crude Protein Digestibility of Cassava Peel after Soaking Betel Lime: The most crucial factors influencing the protein digestibility in rumen are type of protein, the interaction with other nutrients and microbial population, which depend on rumen pH (Bach et al. 2005). In this study, the digestibility value of this crude protein is influenced by the nutrient content of the cassava peel, including its anti-nutrients, HCN and rumen microbial activity in fermenting the protein contained in the cassava peel. Crude protein of cassava peel is 5.88% and this protein content is able to provide nitrogen for the growth of rumen microbes. Agustin et al. (2021) declared that the best amount of ammonia (NH_3) produced by soaking cassava peel with 0.5% $Ca(OH)_2$ for 2 hours was 11.62mg/100ml. This ammonia concentration is necessary for maximal growth of rumen microorganisms (Satter and Slyter 1974). Important factors that influence digestibility of feed ingredients is protein because it plays a role in maintaining the life of rumen microorganisms (Dijkstra et al. 2005). Microbial activity in rumen reaches optimum when ruminal pH is in normal range (Antonius et al. 2023). Agustin et al. (2021) reported that in this research series, the rumen fluid in all treatment combination had a normal pH, with pH value ranging from 6.57-6.92. Russell et al. (1992) stayed that rumen pH above 6.0 is required for microbial protein synthesis, and the optimal pH range of 6.7±0.5 required to maintain normal cellolysis (Van Soest 1994). A decline in rumen pH from 6.8 to 6.4 decreased feed digestibility, protein degradability, and the daily outputs of some fermentation end-products (gas, VFA, acetate, ammonia) but had no effect on the synthesis of microbial protein (Vargas et al. 2023), Santoso et al. (2017) found that ruminal pH is between 6.94-6.96.

The HCN level of cassava peel after 1-3 hours of soaking was 24.83-33.51ppm and did not interfere with the activity of rumen microbes in degrading nutrients in cassava peel. The nutrients can be digested well.

The results of the second stage showed that there was an interaction ($P<0.01$) between the dose of betel lime and the soaking time of the cassava peel on the digestibility of the nutrients. The nutrients digestibility of dry matter, organic matter, crude fiber, nitrogen free extract, fat and crude protein were 51.43-55.10%; 52.46-56.35%; 50.43-52.32%; 64.89-69.50%; 55.26-57.40% and 53.24-57-21% respectively. There was an increase in the digestibility of nutrients with increasing doses of betel lime and longer immersion time. Synchronization of nutrient supply in rumen is important to stimulate the microbial growth and maximize the rumen degradable protein binding into microbial cells (Valkeners et al. 2004).

Conclusion

There was a decrease in the crude fiber content and an increase in the NFE content of cassava peel with a soaking process of up to 3 hours. In this condition, crude fiber content was 11,94% and NFE content was 77.70%. The best soaking time to reduce crude fiber content and increase NFE content is 3hours of soaking time. In the second stage, there was an increase in the digestibility of nutrients with increasing doses of betel lime and longer immersion time. In conclusion, the best immersion time to reduce crude fiber and increase nitrogen free extract content is 3 hours, and the best nutrient digestibility is found in the treatment combination of 0.50% betel lime and 2 hours immersion time.

Author's Contribution: Fauzia Agustin designed the concept, searched for funding, conducted data analysis, drafted and reviewed the paper. Roni Pazla, Novirman Jamarun and Rusmana Wijaya Setya Ningrat supervised the field and laboratory work, drafted the paper. Hannisa Suryadi conducted field and laboratory work as well as data tabulation, prepared samples.

Competing Interest: All authors declare that they have no competing interests.

Acknowledgements: Acknowledgments to the Andalas University Research Institute for facilitating activities to prepare this published article through the Scopus Camp activity**.** We gratefully acknowledge the financial support from the Dean of Animal Science Faculty, Andalas University Dean. The funding for this study came from the Faculty of Animal Science, Andalas University, with contract agreement number: 001.05/UN16.6/PT.01/ SPP.RD/FATERNA-2022

REFERENCES

Agustin F, Erpomen and Ningrat RWS, 2020. The use of cassava peel as a source of energy for substituting rice bran in ration containing *Gliricidia maculata* leaves in dairy cows. IOP Conference Series: Earth and Environmental Science 478: 012077. https://doi.org/10.1088/1755-1315/478/1/012077

Agustin F, Erpomen, Suryadi H and Jamarun N, 2021. The used of calcium hydroxide with different soaking time on cassava peel for reducing HCN, and its effect on rumen fermentation. Proceedings of the International Conference on Improving Tropical Animal Production for Food Security (ITAPS 2021). Advance in Biological Science Research 20: 274-281. https://doi.org/10.2991/absr.k.220309.054

Ajagbe AD, Oyewole BO, Aribido SO and Sunday PA, 2020. Performance of west african dwarf goats fed cassava peels treated with nitrogen sources. GSC Biological and Pharmaceutical Sciences 10(1): 09-12. https://doi.org/10.30574/gscbps.2020.10.1.0247

Antonius A, Pazla R, Putri EM, Negara W, Laia N, Ridla M, Suharti S, Jayanegara A, Asmairicen S, Marlina L and Marta Y, 2023. Effectiveness of herbal plants on rumen fermentation, methane gas emissions, in vitro nutrient digestibility, and population of protozoa. Veterinary World 16(7): 1477–1488. https://doi.org/10.14202/vetworld.2023.1477-1488

AOAC, 2016. Official Methods of Analysis. 20th Edition. Association of Official Analytical Chemists, Washington, DC.

Aregheore EM, 2000. Chemical composition and nutritive value of some tropical by-product feedstuffs for small ruminants - *in vivo* and *in vitro* digestibility. Animal Feed Science and Technology 85 (1-2): 99-109. https://doi.org/10.1016/S0377-8401(00)00123-1

Aribido SO, Ajagbe AD and Oyewole BO, 2021. Growth performance of west african dwarf goats fed dietary models of urea treated cassava peel meal fortified with calcium-phosphorus salt-mix**.** Proceedings of 26th Annual Conference of ASAN-NIAS, Uyo, Nigeria. https://www.researchgate.net/publication/354596928

Arief, Pazla R, Jamarun N and Rizqan, 2023. Production performance, feed intake and nutrient digestibility of Etawa crossbreed dairy goats fed tithonia (Tithonia diversifolia), cassava leaves and palm kernel cake concentrate. International Journal of Veterinary Science 12(3): 428-435. https://doi.org/10.47278/journal.ijvs/2022.211

Aro S, Aletor V, Tewe OO and Agbede JO, 2010. Nutritional potentials of cassava tuber wastes: a case study of a cassava starch processing factory in south-western Nigeria. Livestock Research for Rural Development 22(11). https://www.feedipedia.orgnode

Bach A, Calsamiglia S and Stem MD, 2005. Nitrogen metabolism in the rumen. Journal of Dairy Science 88: E9-E21. https://doi.org/10.3168/jds.S0022-0302(05)73133-7

Budiman A, Dhalika T and Ayuningsih B, 2006. Uji kecernaan serat kasar dan bahan ekstrak tanpa nitrogen (BETN) dalam ransum lengkap berbasis hijauan daun pucuk tebu (Saccharum officanirum). Jurnal Ilmu Ternak 6(2): 132-135.

Cherney D and Cherney J, 2000. Characterization of Forage by Chemical Analysis. In book: Forage Evaluation in Ruminant Nutrition (pp.281-300). Chapter: Characterization of forages by chemical analys. CABI Publishing. Wollingford 281-300. http://dx.doi.org/10.1079/9780851993447.0281

Dijkstra J, Forbes JM and France J, 2005. Quantitative Aspects of Ruminant Digestion and Metabolism. Second Edition. CABI Publishing. CAB International, Wallingford, Oxfordshire OX10 SDE. UK.

Djaafar TFS, Rahayu M and Gardjito, 2009. Pengaruh *blancing* dan waktu perendaman dalam larutan kapur terhadap kandungan racun pada umbi dan ceriping gadung. Penelitian Pertanian Tanaman Pangan 28(3): 192-198.

Elihasridas, Zain M, Ningrat RWS, Erpomen, Putri EM and Makmur M, 2023. In vitro nutrient digestibility and ruminal fermentation characteristics of ammoniated and fermented treatment of soaked and unsoaked Cymbopogon nardus waste. International Journal of Veterinary Science 12(3): 395-400. https://doi.org/10.47278/journal.ijvs/2022.204

Gensa U, 2019. Review on cyanide poisoning in ruminants. Journal of Biology, Agriculture and Healthcare 9(6): 2224-3208. https://doi.org/10.7176/JBAH

Gomez KA and Gomez AA, 1984. Statistical Procedures for Agricultural Research. Second Edition. An International Rice Research Institute Book. A Willey-Inter science Publication. https://pdf.usaid.gov/pdf_docs/PNAAR208.pdf

Hahn SK, Reynolds L and Egbunike GN, 1988. Cassava as livestock feed in Africa: Proceedings of the IITA/ILCA/University of Ibadan workshop on the potential utilization of cassava as livestock feed in Africa. Ibadan, Nigeria: International Institute of Tropical Agriculture (IITA) and Addis Ababa, Ethiopia: ILCA. https://cgspace.cgiar.org/handle/10568/16475

Jamarun N, Zain M, Arief and Pazla R, 2017a. Populations of rumen microbes and the in vitro digestibility of fermented oil palm fronds in combination with Tithonia (Tithonia diversifolia) and elephant grass (Pennisetum purpureum). Pakistan Journal of Nutrition 17: 39-45. https://doi.org/10.3923/pjn.2018.39.45

Jamarun N, Zain M, Arief and Pazla R, 2017b. Effects of calcium (Ca), phosphorus (P) and manganese (Mn) supplementation during oil palm frond fermentation by Phanerochaete chrysosporium on rumen fluid characteristics and microbial protein synthesis. Pakistan Journal of Nutrition 16:393-399. https://doi.org/10.3923/pjn.2017.393.399

Jamarun N, Zain M, Arief and Pazla R, 2017c. Effects of calcium, phosphorus and manganese supplementation during oil palm frond fermentation by phanerochaete chrysosporium on laccase activity and in vitro digestibility. Pakistan Journal of Nutrition 16(3): 119-124. https://doi.org/10.3923/pjn.2017.119.124

Kutay Y, Banu D, Remzi G and Erman MO, 2017. Cyanide poisoning in cattle. Journal of Dairy and Veterinary Sciences Journal 1(4): 001-003. https://doi.org/10.19080/JDVS.2017.01.555567

McDonald P, Edwards RA and Greenhalgh JFD, 2002. *Animal Nutrition. 6th Edition.* Longman, London and New York. 543 p.

Niayale R, Addah W and Ayantunde AA, 2020. Effect of ensiling cassava peels on some fermentation characteristics and growth performance of sheep in-farm. Ghana Journal Agriculture Science 55(2): 107-121. https://doi.org/10.4314/gjas.v55i2.9

Orskov ER and McDonald, 1979. The estimation of protein degradability in the rumen from incubation measurements weighted according to rate of passage. Journal of Agriculture Science 92: 499–503**.** https://doi.org/10.1017/S0021859600063048

Pazla R, Jamarun N, Agustin F, Zain M, Arief and Cahyani NO, 2020. Effects of supplementation with phosphorus, calcium, and manganese during oil palm frond fermentation by Phanerochaete chrysosporium on ligninase enzyme activity. Biodiversitas 21(5): 1833–1838. https://doi.org/10.13057/biodiv/d210509

Pazla R, Jamarun N, Agustin F, Zain M, Arief and Cahyani N, 2021. In vitro nutrient digestibility, volatile fatty acids and gas production of fermented palm fronds combined with tithonia (Tithonia diversifolia) and elephant grass (Pennisetum purpureum). IOP Conference Series: Earth and Environmental Science 888: 012067. https://doi.org/10.1088/1755-1315/888/1/012067

Pazla R, Jamarun N, Arief, Elihasridas, Yanti G and Putri EM, 2023. In Vitro evaluation of feed quality of fermented Tithonia diversifolia with Lactobacillus bulgaricus and Persea americana miller Leaves as Forages for Goat. Tropical Animal Science Journal 46(1): 43-54. https://doi.org/10.5398/tasj.2023.46.1.43

Russell JB, O'Connor JD, Fox DG, Van Soest PJ and Sniffen CJ, 1992. A net carbohydrate and protein system for evaluating cattle diets: I. Ruminanl fermentation. Jornal Animal Science 70: 3551-3561. https://doi.org/10.2527/1992.70113551x

Santoso B, Widayati TW and Hariadi BT, 2017. Nutritive Value, in vitro fermentation characteristics and nutrient digestibility of agro-industrial byproducts-based complete feed block enriched with mixed microbe. Pakistan Journal Nutrition 16(6): 470–476. http://dx.doi.org/10.3923/pjn.2017.470.476

Satter LD and Slyter LL, 1974. Effect of ammonia concentration on ruminal microbial production in vitro. British Journal Nutrition 32(2): 199-208. https://doi.org/10.1079/BJN19740073

Suismono dan Prawirautama, 1998. Kajian teknologi pembuatan tepung gadung dan evaluasi sifat fisikokimianya. Prosiding Seminar Teknologi Pangan dan Gizi. PAU Pangan dan Gizi UGM, Yogyakarta.

Suyitman, Warly L, Rahmat A and Pazla R, 2020. Digestibility and performance of beef cattle fed ammoniated palm leaves, and fronds supplemented with minerals, cassava leaf meal and their combinations. Advances in Animal and Veterinary Sciences 8(9): 991-996. http://dx.doi.org/10.17582/journal.aavs/2020/8.9.991.996

Tilley JMA and Terry RA, 1963. A two stage technique for the in vitro digestion of forage crop. Journal of British Grassland 18: 104-11 https://onlinelibrary.wiley.com/doi/10.1111/j.1365-2494.1963.tb00335.x

Valkeners DA, Thewis F, Piron and Beckers Y, 2004. Effect of imbalance between energyand nitrogen supplies on microbial protein synthesis and nitrogen metabolism in growing double-muscled Belgian blue bulls. Journal of Animal Science 82(6): 1818-1825. https://doi.org/10.2527/2004.8261818x

Van Soest PJ, 1994. Nutritional Ecology of the Ruminant. Cornell University Press, Ithaca, USA, ISBN-13: 9780801427725, Pages: 476.

Vargas JE, Ferreras LL, Adres S, Mateos I, Horst EH and Lopez S, 2023. Differential doet and pH: Effect on ruminal microbiota, fermentation pattern and fatty acid htdrogenation in RUSITEC continous culture. Fermentation 9(4): 320. https://doi.org/10.3390/fermentation9040320

Zain M, Despal, Tanuwiria UH, Pazla R, Putri EM and Amanah U, 2023. Evaluation of legumes, roughages, and concentrates based on chemical composition, rumen degradable and undegradable proteins by in vitro method. International Journal of Veterinary Science 12(4): 528-538. https://doi.org/10.47278/journal.ijvs/2022.218

Zhao XH, Gong JM, Zhou S, Fu CB, Liu CJ, Xu LJ, Pan K and Qu MR, 2015. Effects of degradable protein and non-fibre carbohydrates on microbial growth and fermentation in the rumen simulating fermenter (rusitec**).** Italian Journal of Animal Science 14(2): l 220-224. https://doi.org/10.4081/ijas.2015.3771