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In Vitro Evaluation of Soaked Cassava Peels Using Whiting Solution in Ruminant Ration on Volatile Fatty Acids, Ammonia (NH_3) and Nutrients Digestibility

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Abstract
The purpose of this study was to evaluate the effect of using cassava peels which have been treated with whiting solution into ruminant rations in vitro so that the utilization of cassava peels in ruminant rations can be increased. This study used a randomized block design with a 3x3 factorial and 3 replications. Factor A is soaked cassava peel using whiting solution consisting of A1: cassava peel with 0% whiting dose soaked for 3 hours, A2: cassava peel with 0,25% whiting dose soaked for 3 hours, and A3: cassava peel with 0,50% whiting dose soaked for 2 hours. Factor B is the utilization of treated cassava peels in the ration consisting of B1: 10% of treated cassava peels, B2: 20% treated of cassava peels, B3: 30% of treated cassava peels in the ration. The results showed that there was an interaction (P<0,05) between factor A and factor B on digestibility of dry matter and organic matter, however there was no interaction (P>0,05) between factor A and factor B on rumen fluid characteristics (pH, VFA, ammonia NH3) and crude protein digestibility. Although each single factor, factor A and factor B of VFA, NH3, and crude protein digestibility, had significantly different effects. The results showed that the rumen pH, VFA and NH3 was still within the normal range to support microbial activity in the rumen. Digestibility of nutrients increased with increasing percentage of cassava peels in the ration. Based on the research results, it can be concluded that the treatment that gave optimal results was the A2B3 treatment (cassava peel with 0,25% whiting dose and 3 hours of soaking; 30% treated cassava peel in the ration). The utilization of soaked cassava peels in whiting solution can be increased up to 30% into the ruminant ration as an energy source which also can increase the digestibility of nutrients and does not interfere with microbial activity in the rumen.

Keywords*: In vitro, cassava peel, ruminant ration,rumen characteristics, nutrients digestibility*

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I. Introduction

Cassava peel is an agricultural waste which is obtained from cassava industries. Indonesia is one of the largest cassava producing countries. In 2015 Cassava production in Indonesia reached 21.801.415 tons/year [1]. West Sumatera is one of the cassava producing province in Indonesia that reached 153.412 tons/ year [2]. Each weight of cassava produces ± 10% cassava peel [3]. Based on this percentage, it is predicted that the availability of cassava peel in West Sumatra in 2021 is 15.341 ton/year. Its abundant availability can be used as an alternative feed for ruminants. Cassava peel is one of alternative feed ingredients that can be used as energy source because it contains high nitrogen free extract (75.40%) and high total digestible nutrient (68.86%) and it also contains 5.88% crude protein [4]. Cassava peel contains high nitrogen free extract so that it can be used as a potential energy source for the development of rumen microbes [5]. However, the use of cassava peel as ruminant feed has a limiting factor due to the presence of anti-nutrient HCN compounds which can be toxic to the livestock.

HCN (Cyanide acid) is an anti-nutritional substance that can toxic to the livestock. Cyanide compounds will decompose into HCN which can inhibit the absorption of oxygen in the respiratory system and in certain amounts can cause death [6]. The results of previous studies showed that the use of cassava peel in dairy cow rations could be used as much as 9% as a substitute for rice bran. Giving more than 9% can cause respiratory problems to the livestock [7]. Therefore, cassava peels need to be processed first to reduce the HCN content so that the utilization of cassava peels in the ration can be increased. HCN is soluble in water. One method that can reduce the HCN level from cassava peel is by soaking it in whiting solution(Ca(OH)2). The HCN formed will bind to Ca in a solution of whiting (Ca(OH)2) to form Ca(CN2) which is easily soluble in water [8].

The previous research showed that HCN levels in cassava peels decreased with increasing duration of soaking time up to 3 hours in 0,50% dose of whiting [4]. To find out which treatment of the 3 results of soaking

cassava peels using whiting that gives the best value when it substituted in ruminant rations for the fermentation process in the rumen, especially the characteristics of rumen fermentation and nutrient digestibility, Therefore is necessary to conduct research on the addition of using soaked cassava peels in whiting solution in rations which evaluated in vitro. The use of soaked cassava peel in whiting solution is intended to increase its use in the ration, so that it can overcome the lack of energy source feed in ruminants and cassava peel can be a good source of energy feed, with the result that it can be produced as rations based on cassava peel.

II. Material And Methods

Experimental site

The experiment was carried out at Laboratory of Ruminant Nutrition University of Andalas, Padang, West Sumatera, Indonesia.

Experimental materials and preparation

The cassava peel used in the study were consisted of 3 different treatment of cassava peels, namely: cassava peel soaked for 3 hours with 0% dose of whiting, cassava peel soaked for 3 hours with 0,25% dose of whiting, and cassava peel soaked for 2 hours with 0,50% dose of whiting. The ration was prepared with a ratio between forage and concentrate 50:50, with a content of crude protein ranging from 10-11% and a Total digestible nutrient ranging from 63-66%.The ration was composed of the following feed ingredients: soaked cassava peel in whiting solution, field grass, rice bran, dregs tofu and minerals. Materials used for in vitro are: rations with soaked cassava peels in whiting solution, rumen fluid, buffer fluid (McDougall's solution). This research used 9 combinations of treatment feed rations. Each treatment used 50% grass, 19% tofu dregs and 1% mineral mix. There are 3 different percentage of cassava peels that are used in the ration there are 10%, 20% and 30%.

Experimental Design

This study used a randomized block design with a 3x3 factorial and 3 replications. Factor A is soaked cassava peels using whiting solution consisting of A1: Cassava peel in 0% dose of whiting soaked for 3 hours, A2: cassava peels in 0,25% dose of whiting soaked for 3 hours, and A3: cassava peels in 0,50% dose of whiting soaked for 2 hours. Factor B is the addition of soaked cassava peels using whiting solution in the ration consisting of B1: 10% addition of cassava peels, B2: 20% addition of cassava peels, and B3: 30% addition of cassava peels into the ration. The combination of 3 x 3 treatments are shown in Table 1.

Table 1. The combination of treatments

Factor A Treated cassava peels	Factor B Utilization of treated cassava peel in the ration		
	B1: 10%	B2: 20%	B3: 30%
A1: 0% whiting dose, 3 hours	A1B1	A1B2	A1B3
A2: 0,25% whiting dose, 3 hours	A2B1	A2B2	A2B3
A3: 0,50% whiting dose, 2 hours	A3B1	A3B2	A3B3

Ration Nutrients Composition

This research used 9 combinations of treatment feed rations. The nutrient content of 9 treatments are shown in Table 2.

Table 2. Nutrient content of treatment ration

Nutrient content (% dry matter)	Treatments								
	A1B1	A1B2	A1B3	A2B1	A2B2	A2B3	A3B1	A3B2	A3B3
Dry matter	93,48	93,93	94,38	93,42	93,81	94,20	93,49	93,96	94,42
Organic matter	89,69	90,93	92,17	89,50	90,55	91,60	89,58	90,71	91,83
Crude protein	10,58	10,30	10,00	10,64	10,39	10,15	10,62	10,37	10,11
Crude fat	4,40	3,71	3,03	4,40	3,71	3,03	4,38	3,68	2,98
Crude fiber	21,11	20,53	19,95	21,08	20,45	19,83	21,30	30,90	20,51
NFE	53,59	56,39	59,19	53,40	55,99	58,59	53,28	55,76	58,23
Ash	10,31	9,07	7,83	10,50	9,45	8,40	10,42	9,29	8,17
TDN	64,57	65,47	66,38	64,47	65,28	66,10	63,14	64,11	65,07
NDF	59,10	57,04	54,99	59,12	57,10	55,08	59,35	57,55	55,75
ADF	30,95	29,41	27,87	31,01	29,53	28,05	31,38	30,27	29,16
Cellulose	23,13	21,82	20,52	23,22	22,01	20,79	23,43	22,43	21,43
Hemicelulose	28,81	27,96	27,12	28,77	27,90	27,03	28,63	27,61	26,59
Lignin	6,80	6,37	5,95	6,78	6,34	5,89	6,88	6,53	6,18
HCN (ppm)	2,69	3,38	8,07	2,06	4,13	6,19	2,60	5,20	7,80

Note: NFE (nitrogen free extract); TDN (total digestible nutrient); NDF (neutral detergent fiber); ADF (acid detergent fiber)

Source: Faculty of Animal Science Ruminant Nutrition Laboratory, Andalas University in 2022

Experimental procedures

The study was conducted in vitro followed the procedure of Tilley and Terry's [9] using rumen fluid taken at animal slaughter-house and taken immediately to the laboratory and filtered before mixing with buffer solution. Buffer solution was mix with rumen fluid at a ratio 4:1 then filled to Erlenmeyer flask 250 ml that has contained 2.5 gram samples of each treatment ration. Then flowed CO2 gas for 30 seconds and incubated in shaker water bath at 39^{o}C for 48 hours. Blanko is provided without sample and each treatment unit is repeated 3 times. Fermentation stopped by soaking the Erlenmeyer flask in cool or ice water for 30 minutes.

Parameters evaluated

After incubation completed then the contents of Erlenmeyer flask measured with pH meters to determine ruminal pH. The rumen fluid was centrifuged at 3000 rpm for 5 minutes. The supernatant was used to determine Volatile Fatty Acids (VFA) and Ammonia (NH_3) concentrations. VFA determination was carried out by steam distillation technique [10]. The determination of NH3 levels was determined by the Conway micro diffusion technique [10]. The residue was filtered with a Wathman 41 filter paper, then the residues were oven-dried at 105^{0}C for up to 8 hours, then weighed for analysis of dry matter, organic matter, crude protein.

Data analysis

The data were statistically processed by using Analysis of Variance (ANOVA) in factorial randomized block design according to Steel and Torrie (1993) [11]. The significant differences are continued by using the Duncan's Multiple Range Test (DMRT) test at significance level of 5%.

III. Resultand Discussion

Rumen pH

The results of the analysis of variance of the samples are shown that there was no interaction (P>0,05) between Factor A (soaked cassava peels in whiting solution) and factor B (the addition of soaked cassava peels in whiting solution in the ration) on rumen pH. Factor A (soaked cassava peel in whiting solution) showed no significant difference (P>0,05) on rumen pH. Factor B (the addition of soaked cassava peels in whiting solution in the ration) also showed that were not significantly different (P>0,05) on rumen pH. The rumen pH of samples with different compostion of cassava peels in ration are shown in Table 3.

Table 3. The results of rumen pH

Factor A Treated cassava peels	Factor B Utilization of cassava peels in the ration			Average
	B1 (10%)	B2 (20%)	B3 (30%)	
A1 (0%; 3 hours)	6,69± 0,161	6,62± 0,243	6,56± 0,083	6,62
A2 (0,25%; 3 hours)	6,71± 0,159	6,85± 0,090	6,87± 0,075	6,81
A3 (0,50%; 2 hours)	6,83± 0,245	6,79± 0,170	6,79± 0,102	6,80
Average	6,74	6,75	6,74	

Note: treatment shows no significant different effect (P>0,05)

The pH value obtained in this study was still in the normal range, which ranged from 6.56-6.87, these values was still in optimal conditions to support microbial activity in the rumen, which means that whiting solution treatment on cassava peels in the ration does not interfere with microbial activity in the rumen. This is in accordance with the opinion [12] that the optimal pH value to ensure microbial growth nd activity is in the range of 6.3-7.0. The pH value of the rumen fluid is an interaction between the balance of the buffer capacity with the acidity and alkalinity of the fermentation product. McDougall solution was used as a buffer solution in in vitro testing to maintain the stability of rumen pH. The rumen pH value obtained in each treatment showed a relatively similar value so that statistically different results were not significantly different (P>0,05).

. The rumen pH value in this study was influenced by microbial activity in the rumen. Cassava peel contained in the ration which easily produces VFA will quickly lower rumen pH levels, but the presence of artificial saliva helps in maintaining pH stability in the rumen. According to Sugoro et al., [13] the concentration of VFA and NH3 also affects the pH value of the rumen fluid. This is influenced by the use of McDougall's solution in in vitro testing, where the solution is used as a buffer solution to replace saliva which functions to maintain rumen pH to remain in normal conditions. Rumen pH values that are not at normal susceptibility will interfere with rumen microbial activity. Rumen pH values below 6.2 will interfere with the work of cellulolytic microbes in the rumen which can reduce fiber digestibility [14].

The use of cassava peels which have been soaked with water solution in the ration does not interfere with microbial activity. This is proven by research results which show a stable pH value of 6.56-6.87. Processing cassava peels by soaking using Ca(OH)2 does not interfere with rumen microbial activity and can provide an ideal pH for rumen microbial growth, with the result that under normal pH conditions rumen microbes can digest food substances by producing enzymes to digest nutrient substances [4].

Volatile Fatty Acid (VFA)

The results of analysis of variance showed that there was no interaction (P>0,05) between factor A (soaked cassava peels in whiting solution) and factor B (the addition of soaked cassava peels in whiting solution in the ration) on total VFA production.The resultsof volatile fatty acids (VFA) of rumen fluid are shown inFigure 1.

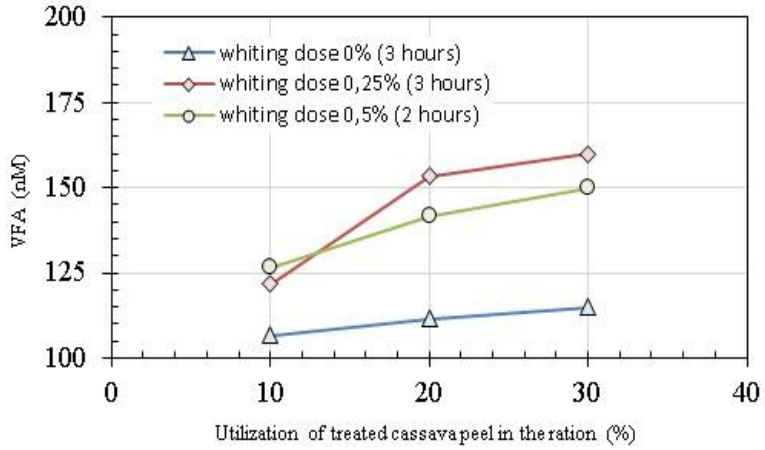

Figure 1. Theresult of volatile fatty acids of rumen

Every single factor had a significant effect on VFA production. Factor A showed a very significant different effect on the production of rumen VFA (P<0,01). Factor A obtained VFA production ranging from 111.11 to 145.00 mM, where the highest VFA production was in treatment A2 (3 hours of soaked cassava peel in 0,25% whiting solution) which was 145.00 mM. While factor B showed a significantly different effect (P<0,05) on the production of rumen VFA. Factor B obtained VFA production in the range of 118.33 – 141.67 mM, where the highest VFA production was in treatment B3 (30% addition of soaked cassava peels in whiting solution in the ration) which was 141.67 mM.

VFA values in factor A obtained ranged from 111.11 to 145.00 mM. This range is quite normal to support rumen microbial activity because VFA value that required to support rumen microbial growth and activity ranges from 80-160 mM[15]. The increase in VFA value in factor A was thought to be due to the effect of soaking treatment with whiting on the cassava peel. The role of whiting solution besides being able to reduce the HCN content in cassava peels, can also loosen the fiber network bonds in cassava peels so there were more nutrients are easily soluble in the ration which are potential to be used as energy sources to support microbial activity in the rumen. This is in accordance with the opinion of Djafaar et al., [8] that soaking with whiting solution is able to dissolve HCN, by stretching the tuber tissue, the toxic compounds (HCN) contained in the cells will come out. This whiting solution is alkaline and can damage cell walls. The more carbohydrates dissolved in it, so that can increase the digestibility and the final product of carbohydrate fermentation by the rumen will also increase [14]. Damage to the cell wall will cause the formation of HCN, but with whiting solution, the HCN formed will bind to Ca in whiting solution, and formed Ca(CN)2 which is easily soluble in water [16] .The HCN content in each ration (table 2) is relatively low and still within safe limits (2.02 ppm - 8.07 ppm). Processing by soaking cassava peels with whiting can reduce the HCN content in cassava peels. Cyanide acid in small amounts in the ration will be metabolized by microbes. The detoxification process in the rumen involves reduction reactions and hydrolysis by enzymes of microbial origin [5]. Abrar [17] stated that there are rumen bacteria that can degrade acid after adaptation to feed ingredients that contain lots of cyanide.

The total production of VFA obtained in factor B ranged from 118.33 to 141.67 mM. VFA value that obtained was still in the optimal range to support microbial growth and activity in the rumen. Factor B indicates that the more use of cassava peel in the ration, the higher the production of VFA obtained. The highest total VFA value of factor B was in treatment B3 which obtained a total VFA of 141.67 mM. Then followed by treatment B2 which is 135.56 mM and B1 which is 118.33 mM. The use of cassava peel in the ration up to 30% is able to provide a supply of nutrients, especially the optimal carbohydrate content for the fermentation process by rumen microbes, resulting in the highest VFA production. Davies [18] stated that increased VFA levels

reflected a diet with high carbohydrate solubility. It is known that cassava peel is a feed that is high in BETN content which is a source of energy for microbes in the rumen. VFA is a dynamic element which its amount depends on the fementability of the feed ingredients used, its absorption on the rumen wall and its utilization by rumen microbes [19]. VFA production depends on the diversity of carbohydrates contained in the ingredients or rations used. In this study, the more use of cassava peel in the ration up to 30%, the more BETN contained in it, which means that the more soluble carbohydrates that microbes can use to produce VFA. As stated by Nurhaita et al., [14], namely by increasing the total production of VFA, it will also increase carbohydrate fermentation in the rumen.

In treatment B1 and B2 the VFA level was lower than treatment B3, namely 118.33 mM and 135.56 mM, respectively. However, the value that obtained is still in the optimal amount for the needs of microbial activity in the rumen. The lower VFA level in treatments B1 and B2 compared to treatment B3 was due to the higher crude fiber content in the rations in treatments B1 and B2 so that the nitrogen free extract content of the rations decreased. This is in accordance with the opinion of Hernaman et al.,[20] which states that the low production of VFA is influenced by the high crude fiber and low nitrogen free extract, because it is part of carbohydrates in the form of starch, sugar and non-fiber parts which are easier to digest in the rumen. Usman [21] also added that the difference in fermentation conditions in the rumen is strongly influenced by differences in the carbohydrate sources of the feed, especially the crude fiber content.

NH_3 (Ammonia)

The results of the analysis of variance showed that there was no interaction (P>0,05) between factor A (soaked cassava peels in whiting solution) and factor B (the addition of soaked cassava peels in whiting solution in the ration) (P>0 0,05) on the of NH3 level. Factor A (soaked cassava peels in whiting solution) showed no significant effect (P>0,05) on NH3. However, factor B showed a very significant effect (P<0,01) on NH3.The resultsof ammonia (NH_3) of rumen fluid are shown in Table 4.

Table 4. The results of ammonia (NH_3) of rumen (mg/100ml)

Factor A Treated cassava peel	Factor B Utilization of cassava peels in the ration			Average
	B1 (10%)	B2 (20%)	B3 (30%)	
A1 (0%; 3 hours)	22,38± 4,067	21,82± 3,148	19,69± 2,805	21,30
A2 (0,25%; 3 hours)	23,09± 3,280	20,26± 2,509	16,29± 5,313	19,88
A3 (0,50%; 2 hours)	25,22± 2,950	18,56± 1,780	19,84± 2,559	21,20
Average	23,56[a]	20,21[b]	18,61[b]	

Note: different superscripts in the same row and column show a significant effect (P<0,05) and highly significant effect (P<0,01)

NH_3 that obtained in Factor A ranged from 19,88 – 21,30mg/100ml. The value of A1 is 21,30mg/100ml; A2 is 19,88 mg/100ml; A3 is 21,20 mg/100ml. The value obtained from factor A is in the normal range. The increased NH_3concentration was thought to be due to the crude protein content contained in each treatment ration (Table 2), because cassava peel contains low crude protein, so the more cassava peel contained in the ration, the lower the crude protein content contained in the ration.Amalia [22] stated that an increase in rumen NH_3 concentration could occur because the feed ingredients contain crude protein that is easily digested by rumen microbes.Factor B gave significantly different effect (P<0,01) on NH_3 value. NH_3 that obtained from factor B ranged from 18,61-23,56mg/100ml. The highest NH_3 value in factor B was in treatment B1 which was 23,56 mg/100ml. The concentration of ammonia (NH_3) is the result of the breakdown of feed protein into peptides and amino acids by rumen microbes [23]. McDonald et al., [24] stated that ration protein in the rumen is broken down by microbes into peptides and amino acids, some amino acids are further broken down into ammonia. The results in this research showed that the higher of cassava peel in the ration, the lower the concentration of NH_3 produced. The highest NH_3 concentration was in treatment B1 and followed by treatments B2 and B3. It was suspected that this was due to differences in the crude protein content of the rations for each treatment. The value of crude protein ration in treatment B1 was higher than treatment B2 and B3 (Table 2). Crude protein content of the ration decreased with the increase in the percentage of cassava peel use in the ration. However, the range of NH_3 concentrations obtained in factor B was still in the normal range and could still meet the needs of rumen microbial activity. The minimum concentration of ammonia that required for microbial protein synthesis is 5 mg/ 100 ml of rumen fluid [25].The main factor that affects the utilization of rumen NH_3 by microbes is the source of energy available in the rumen, namely easily digestible carbohydrates [26]. Ammonia is the largest nitrogen source for microbial protein synthesis. Overall, the NH_3

concentration obtained in this study has met the sufficient NH_3 requirement for microbial growth and activity in the rumen.

Dry Matter Digestibility

The results of the analysis of variance showed, there was an interaction (P<0,05) between factor A (soaked cassava peels in whiting solution) and factor B (the addition of soaked cassava in whiting solution in the ration) on dry matter digestibility. The interaction of the two factors gave a significantly different effect (P<0,05) on dry matter digestibility in vitro. The highest dry matter digestibility obtained in this study was in the A2B3 treatment with the dry matter digestibility value reaching 72.19%. Followed by A3B3 treatment with a digestibility value of 71.78% and so on.The resultsof dry matter digestibility are shown in Table 5.

Table 5. The results of dry matter digestibility (%)

Factor A Treated cassava peel	Factor B Utilization of cassava peels in the ration			Average
	B1 (10%)	B2 (20%)	B3 (30%)	
A1 (0%; 3 hours)	$68,31^{bc}$± 1,649	$69,99^{ab}$± 1,843	$70,35^{ab}$± 3,803	69,55
A2 (0,25%; 3 hours)	$71,56^{a}$±2,141	$69,52^{ab}$± 0,998	$72,19^{a}$± 1,396	71,09
A3 (0,50%; 2 hours)	$65,75^{c}$±1,309	$69,47^{ab}$± 1,411	$71,78^{a}$± 2,532	69,00
Average	68,54	69,66	71,44	

Note: different superscripts in the same row and column show a significant effect (P<0,05) and highly significant effect (P<0,01)

The results showed that the higher the percentage of cassava peels in the ration, the digestibility of dry matter increased. The highest dry matter digestibility was in the A2B3 treatment (3 hours soaked cassava peels in 0,25% whiting solution; 30% addition in the ration) followed by the A3B3 treatment (2 hours soaked cassava peel in 0,50% whiting solution; 30% addition in the ration). The higher dry matter digestibility in the A2B3 treatment than the A3B3 treatment was influenced by the composition of the nutrients contained in the ration (Table 2). The difference in dry matter digestibility occurs due to differences in the composition of the nutrients [20]. Based on the table 2 of ration nutritional composition it showed that the A2B3 treatment contained higher nitrogen free extract than the composition of the rations (Table 2) in the A3B3 treatment, thus obtaining higher digestibility results. Mc Donald et al., [24] stated that feed digestibility is influenced by the chemical composition of the feed and the fibrous feed fraction has a large effect on digestibility. The dry matter content in a feed is very necessary to be able to determine the food substances contained in a feed ingredient [27].

The increase in dry matter digestibility was also influenced by the treatment using a water solution of whiting on the cassava peel which was substituted into the ration. Besides being able to reduce the HCN content in cassava peels, whiting solution is also able to loosen the fibers in cassava peels. The stretchable fiber bonds will make it easier for microbes in the rumen to digest food substances. The content of HCN in the cassava peel has decreased due to the immersion treatment using whiting solution with different doses and soaking time. HCN in small amounts in the feed will be metabolized by rumen microbes. The detoxification process in the rumen involves reduction and hydrolysis by enzymes derived from microbes [5]. The treatment using whiting solution on cassava peel in the ration did not interfere with microbial activity in the rumen.

Organic Matter Digestibility

The results of analysis of variance showed that there was an interaction (P<0,05) between factor A (soaked cassava peels in whiting solution) and factor B (the addition of soaked cassava peels in whiting solution in the ration) on organic matter digestibility. The interaction of the two factors gave a significantly different effect (P<0,05) on the organic matter digestibility. The highest organic matter digestibility obtained in this study was in the A2B3 treatment with an organic matter digestibility value of 70.80%. The resultsof organic matter digestibility are shown inFigure 2.

The results showed that the higher the percentage of cassava peel use in the ration, the higher the organic matter digestibility, this was also in line with the dry matter digestibility which increased with the higher use of cassava peel in the ration. Most of the dry matter consists of organic matter, so the digestibility of organic matter is closely related to dry matter [28]. The increase in organic matter digestibility has a positive correlation with dry matter digestibility. Digestibility of organic matter and dry matter are interconnected, where dry matter is composed of two chemicals, namely organic matter and inorganic matter [29].

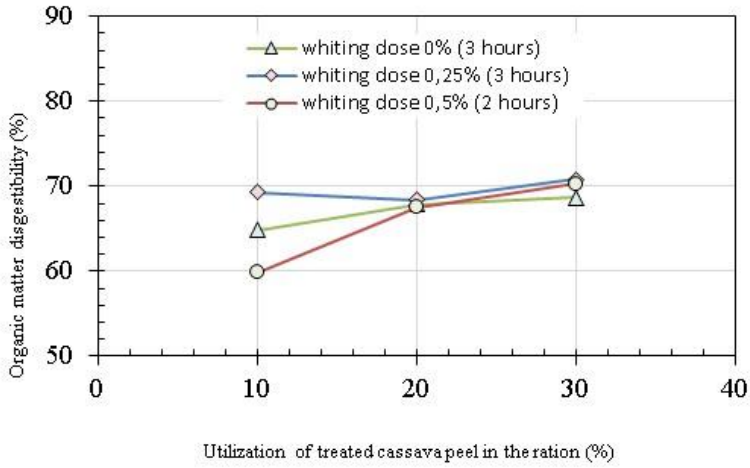

Figure 2. The result of organic matter digestibility

The results of this study indicate that by increasing the dose of whiting and the longer the soaking time, the lower the HCN content in the cassava peel obtained. In addition to reducing the HCN content, the immersion treatment using whiting solution also had an effect on the content of nutrients in the cassava peel. Soaking using whiting is an alkaline treatment that can increase the digestibility of organic matter because the water solution of whiting is considered capable of loosening the coarse fiber bonds in the cassava peel [8], thereby increasing the amount of easily digestible nutrient substances. The digestibility of organic matter obtained in each treatment was also influenced by the composition of the nutrients contained in each treatment ration.

Digestibility of organic matter is an important factor to determine the value of feed. The presence of microbial activity in the digestive tract greatly affects digestibility [30]. Feed composition is a factor that affects feed digestibility. Feeds with complete nutritional content will increase digestibility itself [29]. VFA and NH3 are fermentation products of carbohydrates and proteins for rumen microbial growth and activity, optimal microbial growth in the rumen can increase dry matter and organic matter digestibility [31]. In addition, crude fiber content also affects digestibility, where high crude fiber can cause low digestibility [20].

Crude Protein Digestibility

The results of the analysis of variance showed that there was no interaction (P>0,05) between factor A (soaked cassava peels in whiting solution) and factor B (the addition of soaked cassava peels in whiting solution in the ration) on crude protein digestibility. However, each single factor had a significant effect on crude protein digestibility. Factor A (soaked cassava peels in whiting solution) had a significantly different effect (P<0,05) on crude protein digestibility. The results of crude protein digestibility obtained from factor A sequentially were A1 67.81%; A2 71.54%; A3 67.23%. Factor B (the addition of soaked cassava peel in whiting solution in the ration) had a very significant effect (P <0,01) on crude protein digestibility. The digestibility of crude protein obtained from factor B in sequence were B1 65%; B2 70.18%; B3 70.92%. The results of crude protein digestibility are shown in Table 6.

Table 6. The results of crude protein digestibility (%)

Factor A Treated cassava peel	Factor B Utilization of cassava peels to the ration			Average
	B1 (10%)	B2 (20%)	B3 (30%)	
A1 (0%; 3 hours)	64,13± 4,500	70,32± 3,753	68,99± 4,502	67,81[b]
A2 (0,25%; 3 hours)	70,73± 5,418	70,38± 6,028	73,51± 2,121	71,54[a]
A3 (0,50%; 2 hours)	61,59± 3,103	69,84± 3,726	70,27± 6,185	67,23[b]
Average	65,48[a]	70,18[b]	70,92[b]	

Note: different superscripts in the same row and column show a significant effect (P<0,05) and highly significant effect (P<0,01)

Factor A (soaked cassava peels in whiting solution) showed that as the dose of whiting increased and the longer duration of soaking the cassava peels, the digestibility of crude protein increased. The highest protein digestibility in Factor A was in treatment A2 (3 hours soaked cassava peels in 0,50% whiting solution) which

reached 71.54%. The treatment using whiting with and soaking for 3 hours can reduce HCN and also loosen the fiber bonds in the cassava peel so that there more soluble substances can be utilized by microbes for their growth and development. High VFA supply will provide opportunities for rumen microbes to grow more, especially proteolytic bacteria. Proteolytic bacteria increase with the availability of high VFA so that it can increase the ability to degrade protein components more optimally [20].The HCN content in each treatment ration (table 2) is relatively low, so it is not harmful to livestock and does not interfere with rumen microbial activity. [5] stated that small amounts of cyanide acid in feed will be metabolized by rumen microbes, the detoxification process in the rumen involves reactions by enzymes produced by rumen microbes.

There was a decrease in crude protein digestibility in A3 treatment. The decrease in crude protein digestibility in the A3 treatment (0,50% whiting; 2 hours of soaking) was thought to be due to the higher fiber content in the treatment. The use of a dose of whiting as much as 0,50% is thought to have not been able to optimally loosen the fiber bonds in the cassava peel within two hours. The fiber content in the feed will cause a low degradation value because it is difficult to break down by digestive enzymes [29]. High crude fiber in feed can reduce fermentability which causes decreased digestibility. As Despal [32] stated that the higher the fiber contained in the feed, the lower the digestibility.

Factor B (the addition of soaked cassava peels in water in the ration) showed an increase in crude protein digestibility along with the increase in the percentage of cassava peels substituted into the ration. The highest crude protein digestibility was in the B3 treatment, namely the use of cassava peel as much as 30% in the ration, which obtained a crude protein digestibility of up to 70.92%. The increase in crude protein digestibility in the B3 treatment was also related to the high production of VFA in that treatment. VFA is an energy source for rumen microbes, with the high production of VFA in this case provides an opportunity for proteolytic bacteria to grow more because of the sufficient energy supply. Russel at al., [33] stated that easily digestible carbohydrate sources cause microbial growth to be efficient in producing ATP and microbial protein synthesis. The optimal microbial growth in the rumen, will increasethe digestibility of dry matter and organic matter.

IV. Conclusion

The most optimal results were obtained in the combination of A2B3 treatment (cassava peel with 0,25% dose of whiting and 3 hours of soaking; 30% addition of cassava peels in the ration) where the results of rumen pH, VFA, NH_3, digestibility values of dry matter and organic matter, and crude protein were respectively 6,87 rumen pH, 160,00 mM VFA, 16,29 mg/100 ml NH_3, 72.19% DMD, 70.80% OMD, and 73,51% CPD.The soaked cassava peelsusing whiting solution into rations can be used up to 30% as energy source feed for ruminants, and is safe and does not interfere with microbial activity in the rumen.

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