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Evaluation of Dry Matter, Organic Matter and Energy Content of Tamarind Seed Affected by Soaking and Fermentation

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Abstract: The hard seed husk and the presence of tannin anti-nutrient in tamarind seeds are an obstacle to its use as animal feed. Soaking as a pretreatment and fermentation can lower the limitations. The aim of this study was to evaluate of dry matter, organic matter and energy content of tamarind seeds using processing method of soaking and fermentation using palm juice. Tamarind seeds were collected then sorted using floating test. The sorted seeds then soaked in water, drained, mixed with palm juice then fermented. The study used a randomized complete design with a 3×3 factorial patern and 3 replications. First factor was length of soaking (W0 = 0 days, W1 = 2 days, and W2 = 4 days), second factor was level of palm juice (L0 = 0% of palm juice, L1 = 20% of palm juice, and L2 = 40% of palm juice), and third factor was length of fermentation (F0 = 0 hours, F1 = 36 hours, F2 = 72 hours, and F3 = 108 hours). Parameters observed were dry matter, organic matter, and energy content. The results showed that there was interaction between soaking time, palm juice level, and fermentation time to dry matter, organic matter, and whole tamarind seed as well as best combination for treatment with 2 days soaking time, 20% palm juice level and fermentation time 72 hours. It is recommended to perform the digestibility test to be applied to pigs.

Key words: Soaking, palm juice, fermentation.

1. Introduction

Increasing the population density of Indonesia by 1.54% i.e. 130 people/km² (2013) to 132 souls/km² (2014) [1] encourages the increasing need of animal protein that can be fulfilled one of them from pigs. This can be seen from the increase of pig population of Indonesia by 3.92% i.e. 7,808,087 tail (2015) to 8,114,488 tails (2016) and East Nusa Tenggara province (NTT) has the highest population of 1,871,717 head or 23.07% of the total pig population throughout Indonesia [2]. Maintenance of pigs depends on the given feed that competes with human needs. The solution is to find conventional feed ingredients one of the tamarind seeds.

The tamarind seed is a waste from the processing industry of tamarind fruit content containing 131.3 gkg^{-1} crude protein, 67.1 gkg^{-1} crude fiber, and 48.2 gkg^{-1} crude fat [3] as well as many contains fatty acids [4]. However, having a hard grain structure and not easily destroyed and to produce 1 kg of skinless tamarind seeds takes ±2 hours in the process of grinding and removal of the seed shell. In addition, tamarind seeds have anti-nutrient substances in the form of tannin 56.2 gkg^{-1} DM (dry matter) and antitrypsin 10.8 gkg^{-1} DM most widely located in testa or seed shell [3]. These anti-nutrient substances tend to bind to other compounds such as proteins and carbohydrates to form complexes that are difficult to decide or digest. Therefore, practical processing technology of whole tamarinds is required so that there is no time, energy, cost and raw materials (30% of the tamarind seeds) and to soften and break the skin of the seeds and reduce the activity of anti-nutrient

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substances and increase the availability of tamarind seed nutrient.

One technique is soaking with water and fermentation [5]. The results of Ref. [6] showed that preliminary treatment of soaking, boiling, and stripping of the husk, and its combination can affect the chemical, physical, and functional properties of coral sword flour and significantly decrease the level of phytic acid, soluble food fiber, insoluble fiber, and total dietary fiber. Besides, fermentation is a process that occurs through the reaction of enzymes produced by microorganisms to convert both physical and chemical complex organic materials such as proteins, carbohydrates and fats into simpler molecules [7] so as to preserve, odor, increase digestibility, increase flavor, eliminate anti-nutrients and toxins that are usually present in raw foods [8].

One of the efforts to improve the quality of fermentation materials is by the addition of materials containing high soluble carbohydrates [9] and the addition of absorbants or water absorbers [10]. Local potential that can be used is sap that comes from palm tree (Borassus flabellifer, Linn). According to Cahyaningsih [11], when tapping occurs the formation of organic acids and spontaneous fermentation occurs which causes a decrease in pH to 3.28 on fermentation for 72 hours. Furthermore, it is stated that palm leaves contain amylolytic bacteria *Leuconoctoc mesenteroides*, *Leuconoctoc pseudonesenteroides*, *Lactobaccilus fermententum*, and *Lactobacillus frementum* and BAL are only found in the newly dug down sap up to 24 hours after spontaneous fermentation at room temperature with the highest total BAL (lactic acid bacteria) at 24 h are 7.1 × 103 CFU/mL, pH of 4.5 and the types of microorganisms available are BAL, yeast, and bacillus. Composition of palm juice after tapping is 1.04% protein, 0.19% fat, 76.86% sucrose, glucose 1.66%, minerals 3.15%, calcium 0.861%, phosphorus 0.052%, and iron 11.01 mg/100g [12].

This technology is expected to decrease the anti-nutrient substances so that nutrients become available for the growth and productivity of livestock. Wea et al. [5] showed that there was an increase of nutrient content from non-fermented and spontaneously fermented seeds with the best time duration of 72 hours and an increase in the digestibility of rations containing non-converted and bioconverted tamarind seeds with the best fermentation time of 72 hours [13].

Thus the results of Ue et al. [14] study showed that the use of palm juice after tapping for ±2 hours in fermentation of whole tamarinds by 30% for 21 days did not affect the nutrient quality of the whole tamarinds but had an effect on the reduction of the tannin anti-nutrient content by ±8.6% [15]. This is what encourages the implementation of whole tamarind seed processing technology by soaking and fermentation using soluble carbohydrates from palm juice.

2. Materials and Methods

Preparation of tamarind seed: Tamarind seeds were crop yielded in 2016 and collected from Soe Regency, East Nusa Tenggara Indonesia. The seeds were sorted using floating test. Selected seeds were then strained, dried, soaked in clean water with ratio 1:2 over period time according to soaking period treatment.

Preparation of palm juice: Palm juice was collected from Lasiana area in Kupang City. The juice was incubated in open container for ±24 hours at room temperature.

Fermentation of tamarind seed: The soaked tamarind seeds were drained then mixed with prepared palm juice. The amount of palm juice was added according to palm juice treatment. The mixture of tamarind seeds and palm juice was fermented in anaerobic condition with length of fermentation being according fermentation treatment. After the fermentation process, samples were sent to laboratory for further analysis of DM, crude protein, crude fat, crude fiber, ash, Ca, P, and tannin content. The proxymate analysis was determined according to Ref. [16]. The analysis was conducted at the Nutrition and Feeding Laboratory of

Kupang State Agricultural Polytechnic Laboratory.

Statistical Analysis: The study used a randomized complete design with a 3 × 3 factorial patern and 3 replications. First factor was length of soaking (W0 = 0 days, W1 = 2 days, and W2 = 4 days, second factor was level of palm juice (L0 = 0% of palm juice, L1 = 20% of palm juice, and L2 = 40% of palm juice), and third factor was length of fermentation (F0 = 0 hours, F1 = 36 hours, F2 = 72 hours, and F3 = 108 hours). Data analyses were conducted using SPSS software version 23.0. Differences between treatments were tested using DMRT (Duncan's Multiple Range Test).

3. Results and Discussions

The result of the study of intact tamarind seed treatment by soaking and fermentation using palm juice on DM is presented in Table 1. The analysis of variance shows that the lenght factor of soaking, palm juice level, fermentation length and interaction between soaking period with palm juice level, interaction between soaking time and fermentation length, the interaction between palm juice level with fermentation length and interaction between soaking time with palm juice level and with fermentation length significantly ($p < 0.01$) to the dry matter content of whole tamarinds.

3.1 Dry Matter

This is because soaking too long causes the seed husk to soften and water easily enters the tamarind cell structure and causes the water content increases while the dry matter decreases. In addition, soaking causes a decrease in pH due to the presence of lactic acid bacteria which will annihilate pathogenic bacteria in fermentation materials.

The mean content of dry matter of whole tamarinds in this research is 62.75% lower than result of Ref. [17] research that DM content of huskless tamarind seed is 89.14%, tamarind seed without husk soaking one day 89.89% and tamarind seed without spontaneous bioconversion 90.02% [5], and Ue Ref. [14] research results that the content of dry matter of whole-seeded tamarinds of fermented palm juice by 30% for 21 days was 72.89%. The low content of dry matter in this research is caused by the soaking process using water and fermentation using palm juice so that the high water content of the material causes the low content of DM.

3.2 Organic Matter

The content of organic tamarind seed processed material is presented in Table 2. Based on Table 2 it is

Tabel 1 Effect of treatments on dry matter of tamarind seed.

Palm juice	Fermentation	Soaking (days)		
(%)	(hours)	0	2	4
0	0	92.19 ^{aA}	43.49 ^{xB}	44.23 ^{xC}
	36	91.39 ^{bD}	59.31 ^{oE}	54.64 ^{qrF}
	72	90.64 ^{cG}	54.23 ^{rH}	56.96 ^{pI}
	108	87.82 ^{dJ}	63.37 ^{mK}	56.86 ^{pL}
20	0	83.92 ^{gM}	41.82 ^{yN}	41.25 ^{yO}
	36	85.02 ^{fP}	62.90 ^{mQ}	48.78 ^{uR}
	72	86.27 ^{eS}	52.12 ^{sT}	49.84 ^{tU}
	108	84.87 ^{fV}	50.32 ^{tW}	45.76 ^{wX}
40	0	79.96 ^{hM}	45.76 ^{wN}	43.53 ^{xO}
	36	78.86 ^{iP}	66.26 ^{kQ}	60.65 ^{nR}
	72	75.48 ^{jS}	50.36 ^{tT}	62.87 ^{mU}
	108	65.11 ^{lV}	55.35 ^{qW}	46.86 ^{vX}
SEM 0.042				

Different superscripted capital letter on the same row and different superscripted lower case letter on the same column indicated superscripted lower case letter on the same column indicated highly significant ($p < 0.01$).

Tabel 2 Effect of treatments on organic matter of tamarind seed.

Palm juice	Fermentation	Soaking (days)		
(%)	(hours)	0	2	4
0	0	89.80 aA	41.26 vB	41.79 vC
	36	89.04 abD	57.52 mE	52.52 pF
	72	88.40 bG	51.90 pH	54.69 nI
	108	85.41 cJ	61.17 kK	54.72 nL
20	0	81.77 eM	39.64 wN	39.28 wO
	36	82.59 eP	60.97 kQ	46.31 sR
	72	84.14 dS	50.47 qT	48.08 rU
	108	82.39 eV	48.74 rW	44.18 uX
40	0	77.77 fM	43.42 uN	41.43 vO
	36	76.41 gP	64.55 iQ	58.68 lR
	72	73.13 hS	48.89 rT	61.26 kU
	108	62.71 jV	53.70 oW	45.25 tX
SEM 0.047				

Different superscripted capital letter on the same row and different superscripted lower case letter on the same column indicated superscripted lower case letter on the same column indicated highly significant ($p < 0.01$).

known that the trend of increasing and decreasing organic matter is dried up. This is because the organic mater is part of the DM.

The analysis of variance showed that the soaking time, palm juice level and fermentation time had significant effect ($p < 0.01$) on organic matter content and there was very real interaction ($p < 0.01$) between soaking time and palm juice level, with long fermentation, between palm juice level with fermentation length, and between soaking time, palm juice level, and fermentation time.

As with DM content, DMRT test of organic matter content also showed that there was significant difference ($p < 0.05$) between soaking time of 0 days, 2 days and 4 days, there was significant difference ($p < 0.05$) between level palm juice 0% with 20% and 40%, and there was no significant difference ($p > 0.05$) between level palm juice 20% and 40%. The length of fermentation was significantly different ($p < 0.05$) between 0 hours, 36, 72, and 108 hours. Increasing the average content of organic matter is caused by the decrease in inorganic material content. This shows that the best combination is at the time of soaking 2 days, the use of palm juice 20%, and the duration of fermentation 108 hours.

This is in line with Despal et al. [18] statement that the processing of feed ingredients by spontaneous fermentation or using water-soluble carbohydrate will produce epiphytic BAL that ferments the water soluble carbohydrate in the plant to lactic acid and a small part converted to acetic acid which will lower the pH of the substrate and which will inhibit the development of harmful pathogenic microorganisms. The presence of acetic acid that is corrosive causes the texture of whole tamarind seeds to be soft with the release of complex compound bonds into simple compounds. This causes many nutrients to dissolve.

The time of soaking and addition of palm juice during the fermentation process also causes the dissolution of minerals so that the ingredients become reduced. The average content of inorganic acids of whole tamarind in this study is 2.08% lower than the result of Ref. [17] study that the content of inorganic acids without skins is 3.25% and whole fermented tamarinds are fermented palm juice up to 30% level 21 days ie 2.42% [14].

3.3 Energy Content

The result of statistical analysis shows that there is very significant interaction ($p < 0.01$) between soaking time, palm juice level and fermentation time to tamarind seed energy content. Based on Table 3 DMRT test

Table 3 Effect of treatments on energy content of tamarind seed.

Palm juice (%)	Fermentation (hours)	Soaking (days)		
		0	2	4
0	0	5,140.00 ± 18.52 ^{aA}	5,000.49 ± 13.50 ^{efghijklB}	5,039.33 ± 40.00 ^{cdefgC}
	36	5,044.33 ± 4.51 ^{cdefA}	4,905.33 ± 4.04 ^{opB}	5,075.67 ± 9.50 ^{bcdC}
	72	5,155.67 ± 22.01 ^{aA}	4,948.67 ± 2.52 ^{lmnoB}	4,972.67 ± 4.51 ^{hijklmnC}
	108	5,029.00 ± 23.0 ^{defgD}	5,000.33 ± 28.50 ^{efghijklE}	5,009.00 ± 28.00 ^{efghijkF}
20	0	5,109.33 ± 3.06 ^{abG}	4,902.67 ± 10.50 ^{opH}	5,009.00 ± 25.00 ^{efghijkI}
	36	5,045.33 ± 7.02 ^{cdeG}	4,997.33 ± 27.01 ^{efghijklH}	5,019.00 ± 8.00 ^{efghI}
	72	5,018.67 ± 21.03 ^{efghiG}	4,990.33 ± 63.50 ^{fghijklmH}	4,935.33 ± 2.08 ^{nopI}
	108	4,992.67 ± 10.50 ^{fghijklmJ}	4,997.33 ± 45.00 ^{efghijklK}	4,889.00 ± 1.00 ^{pL}
40	0	4,964.67 ± 9.50 ^{ijklmnM}	4,947.33 ± 23.01 ^{lmnoN}	5,012.67 ± 66.50 ^{efghijO}
	36	4,958.00 ± 6.56 ^{klmnM}	4,989.53 ± 33.50 ^{ghijklmN}	5,038.00 ± 49.00 ^{cdefgO}
	72	4,957.67 ± 12.50 ^{klmnM}	5,018.33 ± 53.00 ^{efghiN}	5,081.67 ± 36.50 ^{bcO}
	108	4,942.67 ± 15.01 ^{mnoP}	4,963.67 ± 24.50 ^{jklmQ}	4,971.33 ± 17.01 ^{hijklmnR}
SEM		2.621		

Different superscripted capital letter on the same row and different superscripted lower case letter on the same column indicated superscripted lower case letter on the same column indicated highly significant ($p < 0.01$).

showed that the best energy content was found in tamarind seed with 4 days soaking time, 20% palm juice level and 72 hours fermentation time.

This indicates that in the soaking for 2 days has happened softening of the seeds causing water imbibe into the seed content increases. This causes a reshuffle of the structure of the tamarind seed cell from the complex into a simpler structure and the activity of microorganisms began to occur. This is indicated by the change in color and aromatic acidity. In addition, the addition of palm juice as much as 20% which has experienced spontaneous fermentation caused organic microorganisms shrink becomes more available. Likewise long-term fermentation for 72 hours provides an opportunity for microorganisms to produce enzymes. The available enzymes are proteases, lipases, and amylases that will digest organic matter proteins, fats, and carbohydrates into simpler compounds that can be utilized by livestock.

This is in line with Ref. [19] statement that fermentation is closely related to dosage and time. The dose level is related to the size of the microbial population that is likely to determine whether or not microbial development is successful in generating enzymes to break down the substrate that will affect the outcome. Thus Fardiaz [6] states that the length of time associated with the growth of microorganisms is characterized by increasing the number of periods of the cell so that metabolic concentration increases until finally becomes limited. Complex recombination of carbohydrates, proteins, and fats into simple compounds by enzymes makes nutrients available including energy.

According to Cahyaningsih [3], palm juice during tapping occurs the formation of organic acids and spontaneous fermentation occurs which causes a decrease in pH to 3.28 on fermentation for 72 hours. Furthermore, it is stated that palm leaves contain amylolytic bacteria *Leuconoctoc mesenteroides*, *Leuconoctoc pseudonesenteroides*, *Lactobaccilus fermententum*, and *Lactobacillus frementum* and BAL. This is supported by Despal et al. [4] that spontaneous fermentation or water-soluble carbohydrate ingredients will produce epiphytic BAL that ferment the water-soluble carbohydrates in the plant to lactic acid and a small portion converted to acetic acid which will lower the pH of the substrate and which will inhibit the development of pathogenic microorganisms which is detrimental so nutrients especially energy become available.

4. Conclusions

Based on the discussion, it can be concluded that there is interaction between soaking time, palm juice level, and fermentation time to DM, organic matter, and whole tamarind seed as well as best combination for treatment with 2 days soaking time, 20% palm juice level and fermentation time 72 hours. It is recommended to perform the digestibility test to be applied to pigs.

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