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Value of pH, VFA and NH3 Rice Straw Silage Made with Additives Porang Flour (*Amorphophallus muelleri*) In Vitro

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ABSTRACT

This study aims to determine the pH, VFA and NH3 values of rice straw silage made with the addition of Porang flour additives. Research and silage analysis was conducted at the Faculty of Maritime Animal Husbandry and Fisheries, University of Nusa Cendana, Kupang. The design in this study was a Completely Randomized Design (CRD) with four treatments and four replications. Treatment P0 (rice straw silage without Porang flour additive) as control, P1 treatment (rice straw silage with 3% Porang flour additive), P2 (rice straw silage with 6% Porang flour additive) and P3 (rice straw silage with 9% Porang flour additive). The observed variables were pH, VFA and NH3. The data obtained were analyzed using analysis of variance and Duncan's multiple range test. The results showed that all treatments had a significant effect ($P<0.01$) on pH, VFA and NH3 values. The pH values ranged from 3.78 – 5.48, the total VFA values ranged from 61.13 mM – 86.06 mM, and the NH3 values ranged from 5.88 mM – 9.50 mM. As a result, adding Porang flour (*Amorphophallus muelleri*) as an additive in rice straw silage up to 9% significantly lowered the pH value and increased the VFA and NH3 values.

Keywords: fermentative quality, Porang flour, rice straw silage

INTRODUCTION

Even though its availability is relatively abundant, rice straw is an agricultural waste whose utilization is not optimal. So far, it is seen as not having sufficient nutritional content and is composed of cellulose, hemicellulose, silica and lignin, protein and low energy, resulting in low digestibility. Rice straw is a by-product of agricultural products produced during the rainy season and can be fed to livestock. To be utilized at the end of the dry season, it needs to be preserved by making it into silage.

It will ultimately affect the silage quality and the livestock's digestibility value. Sufficiently available additives are Porang. Porang is a biologically rich tuber, a plant that produces carbohydrates, fats, proteins, minerals, vitamins and food fiber found in Indonesia. Carbohydrates are an essential component of Porang tubers consisting of starch and glucomannan. According to Wang and Jhonson (2003) and Mulyono (2010), glucomannan has water-soluble properties, solid adhesive properties in the water, is stable and does not agglomerate in acidic conditions. The use of Porang flour functions as a source of carbohydrates in silage. It is expected that the ability of Porang, which can bind water as a growth medium for bacteria, can influence the growth of microorganisms in silage.

The provision of sustainable ruminant feed can be fulfilled, among other things, from the utilization of fibrous feed sources (Christiyanto and Subrata, 2005). Providing adequate feed with the nutrient content needed by livestock can produce high productivity (Purbowati et al., 2015). Ruminant livestock nutrient needs vary according to the level of productivity. The nutrient value of a feed ingredient can be determined based on the degree of degradation and digestibility. Nutrient degradation of feed ingredients in the rumen shows the quality of a feed ingredient. The protein contained in the feed ingredients is fermented into ammonia and then used as the main requirement for the growth of microorganisms. Carbohydrates in feed ingredients are fermented into *Volatile Fatty Acids* (VFA), which are then absorbed directly in the rumen wall and used as the primary energy source for basic life needs for growth. This study aims to evaluate the fermentative quality of rice straw silage added with Porang flour as an additive. The advantage of this research was to evaluate the fermentability quality of rice straw silage as feed for ruminants.

MATERIALS AND METHODS

This research lasted for approximately three months at the University of Nusa Cendana, which consisted of a preparatory stage, then continued with the silage-making stage, and the ensilage process and data analysis took place in the Lab. Feed Chemistry FPKP Undana.

The equipment used is pen and paper for the panellists, scales brand "Nankai" with a capacity of 50 kg to weigh the feed, machetes for chopping rice straws, clear plastic, label paper, basins, straps and data analysis equipment. The materials used when making silage are freshly harvested rice straw, EM-4, water and Porang flour.

Treatments

This research was conducted experimentally using a Completely Randomized Design (CRD) with four treatments, each repeated four times. The treatment given is:

P_0 = Rice straw 2.5 kg + EM4 solution (control)
P_1 = Rice straw 2.5 kg + EM4 solution + 3% of Porang flour
P_2 = Rice straw 2.5 kg + EM4 solution + 6% of Porang flour
P_3 = Rice straw 2.5 kg + EM4 solution + 9% of Porang flour

The portion of Porang flour is based on the amount of chopped rice straw.

Preparing Rice Straw Silage

Rice straw that has been dried in the sun is laid out on a clean floor and then chopped into 3-5 cm sizes. Weigh rice straws as much as 2.5 kg. Sprinkle the Porang flour according to the treatment on the straw evenly. Mix EM-4 with water and sprinkle it on the chopped rice straw evenly. Mix/stir all the ingredients evenly by turning the rice straw back and forth. Enter the mixture into the silo (plastic fermenter) little by little while condensing it so that air can be reduced or eliminated. After all the mixed ingredients are added, close the silo tightly so that no air enters and the anaerobic process runs well. The ensilage process was carried out for 21 days. Open the silo cover after the ensilage process is complete.

NH3 Concentration Measurement (Conway Microdiffusion Method)

Cover the lips of Conway's cup and cover with Vaseline. The supernatant derived from the fermentation process was taken 1.0 mL and then positioned in the Conway cup's groove at one end; 1.0 mL of saturated Na_2CO_3 solution was added at one end of the Conway cup, next to the supernatant (*should not mix*); 1.0 mL of indicated boric acid solution was placed in a little cup in the centre of the Conway cup; A Vaseline-smeared Conway cup is securely closed to create an airtight seal, the Na_2CO_3 solution is assorted with the supernatant until it is constantly deal out by waggling and bending the cup; Then, leave it room temperature for 24 hours; After 24 hours at room temperature, the indicator boric acid was titrated with 0.005 N H_2SO_4 till the colour switched from blue to red.

VFA Concentration Measurement (Steam Distillation Method)

Fill the press cooker with distilled water up to the MAX mark. Then make sure the water from the faucet flows as a cooler. Turn on the gas stove so that the aqua dest in the press cooker pot boils and produces steam which will enter the distillation tubes, which indicates that we can start the VFA analysis; NH_3 analysis is taken as much as 5 ml, then put into the distillation tube. Place the Erlenmeyer containing 5 mL of 0.5 N NaOH under the reservoir hose; 1 mL H_2SO_4 15% is added to the distillation tube with the sample solution, then immediately close the glass cover; Rinse with sufficient aqua dest. Hot water vapour will crowd the VFA and will condense in the cooler. The water formed in the Erlenmeyer flask contains 5 mL of 0.5N NaOH until it reaches 300 mL; PP indicator (Phenol Pthalin) is added as much as 2 or 3 drops and titrated with 0.5 N HCl until the colour of the titration changes from red to light pink; Note: HCl 0.5 N as a titrant must be standardized so that a concentration with four decimal places is obtained.

Data Analysis

The data obtained were analyzed using Analysis of Variety (ANOVA). If the treatment significantly affects the parameters observed, then Duncan's further test is carried out. The data analysis process was carried out using SPSS Software Version 26.

RESULTS AND DISCUSSION

pH Value

Based on the data in Table 1, the pH value ranges from 3.78 to 5.48. The analysis of variance showed that the treatment had a very significant effect ($P<0.01$) on the pH value of rice straw silage. Further test results showed that the pH value of the P_0 treatment was significantly different ($P< 0.05$) to P_1 and P_3. Meanwhile, the P_2 treatment was not significantly different ($P> 0.05$) from P_1 and P_3. Low (1984) states that good silage must have a pH value < 4.5. The addition of Porang flour additive to rice straw silage showed a value below 4.5. It indicates that the curing process occurred under anaerobic conditions, and acidity was formed in the silage.

Table 1. The average value of pH, NH_3 and VFA of rice straw silage

Variable	Treatment				P-values
	P0	P_1	P_2	P_3	
pH	5.48 ± 0.10^{c}	4.33 ± 0.13^{b}	3.95 ± 0.10^{ab}	3.78 ± 0.45^{a}	0.000
NH_3 (mM)	5.88 ± 0.32^{a}	7.75 ± 0.46^{b}	8.63 ± 0.95^{b}	9.50 ± 2.03^{b}	0.005
VFA (mM)	61.13 ± 6.31^{a}	70.42 ± 4.66^{ab}	78.24 ± 3.99^{bc}	86.06 ± 8.64^{c}	0.001

Note: Different superscripts in the same row are highly significant (P<0.01).

The acids formed are organic acids such as lactic, acetic, and butyric acids as a result of dissolved carbohydrate fermentation by bacteria which causes the pH value to decrease. In this study, the pH value decreased in all treatments that were given the addition of Porang flour additives; in an acidic environment, this could prevent protein degradation by microbes. It shows that the higher the percentage of Porang flour, the higher it inhibits protein degradation in silage. Homofermentative lactic acid bacteria can reduce silage proteolysis through a faster decrease in pH. When viewed from the physical quality of the aroma of silage, the addition of the additive of Porang flour produces a characteristic sour aroma of silage. The pH value indicates good-quality silage.

Silage was made with the hope of producing a low pH value so that the clostridium bacteria cannot grow and develop in the preservation medium. According to Kung and Nylon (2001), pH is one of the determining factors for preservation success. The pH value in the P_3 treatment was 3.78, which can be categorized as good silage. It is under the opinion of Hermanto (2011), which states that a silage pH of 4.3 – 4.5 is quite good, and a pH of 3.8 – 4.2 is ideal.

The pH value of silage is also affected by microbial activity during the silage process, where lactic acid bacteria utilize simple sugars in forages and additives as an energy source and break down complex compounds into simple substances; this microbial activity will lower the pH value (Wakano et al., 2019). The results showed the same thing that the addition of Porang flour additive reduced the pH value of rice straw silage; this indicated that lactic acid bacteria make good use of Porang flour sugar.

NH_3 (Ammonia)

The data in Table 1 shows that the highest to the lowest NH_3 (Ammonia) is P_3 of 9.50 mM; P_2 (8.63 mM); P_1 (7.75 mM) and P_0 (5.88 mM). The analysis of variance showed that treatment had a very significant effect (P<0.01) on NH_3 levels. Further test results showed that the P_1, P_2 and P_3 treatments were significantly different from P_0 but not significantly different between the three treatments with the addition of Porang flour. It can be said that an increase in the percentage of Porang flour causes an increase in ammonia or the breakdown of protein.

When viewed from the pH, the higher the percentage of Porang flour, the more acidic the atmosphere. This acidic atmosphere is expected to suppress the growth of *Clostridium bacteria* (bacteria that break down proteins), according to Telleng (2017). The acidic atmosphere formed is not yet able to suppress the breakdown of feed protein, so the NH_3 content increases with increasing Porang flour percentage.

Kurnani (1995) stated that good silage has an ammonia content of <11% of total nitrogen, and according to McDonald *et al*. (2002), good silage NH_3 value < 10 mM. The NH_3 value of all treatments in this study was still below this value, so the silage in this study was included in good silage. Dryden (2021) states that the high NH_3 content of silage can be caused by using materials that are high in protein. In this study, with an increase in the percentage of Porang flour, the protein content also increased; this might cause an increase in NH_3 with an increase in the percentage of Porang flour. According to Riswandi et al. (2015), an increase in NH_3 content causes an increase in the microbial population for rumen fermentation. NH_3 production is reflected in crude protein because ammonia is the result of nitrogen fermentation by microbes in the rumen. NH_3 levels will increase in line with increasing CP levels.

VFA (Volatile Fatty Acids) Total

The highest VFA concentration was in the P_3 treatment, namely 86.06 mM, then P_2 (78.24 mM), P_1 (70.42 mM) and P_0 (61.13 mM), respectively. The results of the analysis of variance showed that the treatment had a very significant effect on the total silage VFA. Further test results showed that the concentration of VFA between the P_0-P_3 and P_0-P_2 treatments was significantly different (P < 0.05), while between the P_0-P_1 and P_1-P_2 and P_2-P_3 treatments, it was not significantly different (P>0.05). It can be stated that increasing the percentage of Porang flour increased the total VFA concentration of silage.

Carbohydrate as an additive in the ensiling process, greatly influences microbial activity; this can be seen from the resulting products such as VFA. In this study, with the increase in the percentage of additives, the VFA concentration also increased because more carbohydrate sources were available. According to Ohshima & McDonald (1978), good silage is characterized by a high concentration of lactic acid, whereas a low concentration of acetic, propionic and butyric acids. Santoso et al. (2009) reported that adding lactic acid bacteria to ensilages increased acetic acid and decreased VFA. Furthermore, Filasari *et al.* (2019) explained that the high carbohydrate content in the feed ingredients also supports the high production of VFA in forage feed ingredients.

In this study, although the percentage of Porang flour increased, the atmosphere became more acidic (indicating good silage or it is estimated that more lactic acid was formed), but VFA increased. Ohshima & McDonald (1978) stated that acetic acid is produced by heterofermentative lactic acid bacteria (heterotactic) ferment hexoses into lactic acid and other products such as ethanol and acetic acid. In addition, acetic acid can be formed from amino acid carbon chains during secondary fermentation. In contrast, Clostridia saccharolytic bacteria produce butyric acid from glucose and lactic acid breakdown. The concentration of lactic acid was not calculated in this study. Still, the percentage of lactic acid was possibly greater than that of VFA because the atmosphere became more acidic with increased Porang flour. VFA could be produced during the formation of lactic acid.

Physical Quality of Rice Straw Silage

The results of the physical quality test were carried out by 9 panellists using the silage assessment criteria table according to the Ministry of Agriculture (1980). The analysis of variance showed that the treatment had a significant (P<0.01) effect on texture and aroma. The average physical quality, including the texture and aroma of rice straw silage added with Porang flour, can be seen in the following table.

Table 2. The average value of texture and scent of rice straw silage

Variables	Treatment				P-value
	P_0	P_1	P_2	P_3	
Texture	3.72 ± 0.19^b	3.92 ± 0.17^b	3.94 ± 0.11^b	3.08 ± 0.17^a	0.001
Aroma	2.97 ± 0.56^a	3.97 ± 0.06^b	3.92 ± 0.17^b	3.83 ± 0.21^b	0.002

Note: Different superscripts in the same row are highly significant (P<0.01).

The results of the analysis of variance showed that the addition of Porang flour additive affected aroma; other test results showed that treatments P_1, P_2 and P_3 were very significantly different (P<0.01) from P_0 (without Porang flour additive), whereas between treatments P_1, P_2 and P_3 different is not significant. The score displayed in the P_0 treatment was 2.97, meaning that the aroma was less sour, while the other treatments ranged from 3.83 to 3.97 producing a fragrant aroma of acidity. The average score for the aroma of rice straw silage with the addition of Porang flour additives showed that each treatment had an aroma that tended to smell sour, like the typical aroma of silage. The results of this study are almost similar to those of Infitria et al. (2022), who reported that fermenting rice straw with the addition of various types of sugar produced a sour aroma. The addition of Porang flour to manufacture agricultural waste silage causes change in the aroma to become much sour.

A good texture is the same as the original material because the ensilage process is a preservation process (Marawali et al., 2022). The texture of silage is affected by the water content in forage, so in this study, it is suspected that the water content of silage is standard so that the silage texture is at a reasonable/average level. In this assessment, all panellists stated that the texture of the silage in each treatment had a clear texture like the basic ingredients of silage feed with a dense texture and easy to separate. In his research, Suadnyana et al. (2019) found similar results in rice straw silage with the addition of rumen fluid, that the silage has a relatively fine texture, which indicates that the silage is solid and does not clump. Aglazziyah et al. (2020) stated that the denser the texture produced or the texture of the silage, the more like the essential ingredients, indicating good quality silage.

CONCLUSION

The study results show that adding Porang flour (*Amorphophallus muelleri*) as an additive in rice straw silage up to 9% significantly lowers the pH value and increases the VFA and NH_3 values. In addition, it has a good effect on physical quality, which includes texture and aroma.

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