The Effect of Fermentation on the Nutritional Content of *Amorphophallus sp*. as Poultry Feed

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ABSTRACT

Amorphophallus is one of the underutilized aroid of Araceae family, which is not a cultivated plant, but high in productivity. The tuber contains a large amount of starch, but has antinutritional factors like oxalic acid leading on problems in palatability. This experiment was conducted to evaluate the effect of fermentation by *Bacillus subtilis* on nutritive values and oxalate content of *Amorphophallus* tuber. The *Amorphophallus* tubers were cleaned, sliced, dried, milled and then fermented with different level of *Bacillus subtilis*. This experiment used Completely Randomized Design with four treatments and three replicates. The treatments were 0, 10, 20, and 30% level of *Bacillus subtilis* inoculum. The parameters observed were dry matter, crude protein, crude fiber and oxalate content of *Amorphophallus* tuber. Results showed that dry matter, crude protein, crude fiber were not affected by the treatments but oxalate content of *Amorphophallus* were decreased along with increasing level of *Bacillus subtilis*. The lowest oxalate content of *Amorphophallus* was found on treatment with 20% of *Bacillus subtilis*.

Keywords: Fermentation, *Amorphophallus*, Nutritive value, Oxalate

INTRODUCTION

Feeding is important factor in poultry production, which achieve 70 to 85% of total expenditure in broiler production (Abdulrashid and Agwunobi, 2009; Maidala *et al.,* 2016). The major source of energy in poultry feeds is maize and it constitutes about 50 to 70% in broilers ration (Mathius and Sinurat, 2001; Ojowola and Olugbemi, 2011). Maize is also foodstuff and still imported for domestic demand resulting the price of ration (Mathius and Sinurat, 2001). The increasing of maize price considered an evaluation of utilization other feedstuff as alternative energy source from agricultural product and waste.

Amorphophallus sp. tubers can be used as feedstuff. This plant is wilding in the forest and has not been cultivated in Indonesia (Santosa *et al.,* 2013). *Amorphophallus* sp. production is of 3 to 5 kg/tree, contains 7.33% crude protein and 3570.60 kcal/kg gross energy, and also anti-nutrient component like oxalate (Koni *et al.,* 2015). Oxalate content of *Amorphophallus* tubers are about 0,45-0,78% (Ambazaghan *et al.,* 2007), and *Amorphophallus mueleri* are 6.24% calcium oxalate (Wijanarko *et al*., 2011)

Ingestion of high oxalate containing feedstuff by animals can cause severe effect such as hypocalcemia and hyperoksaluria. The effects are associated to minerals binding such as calcium and magnesium in metabolic processes. Hypocalcemia may caused by reducing calcium absorption as calcium binding by oxalic acid induced calcium deficiency (Cheeke, 1995). Oxalic acid may bind calcium in the blood so calcium feed is not available for livestock production. Low blood calcium stimulate the secretion of parathyroid hormone that

regulates the balance of blood calcium levels. In order to maintain the balance of blood calcium, calcium may be reabsorbed from bone (Rahman *et al.,* 2012). High consumption of oxalate caused hyperoksaluria and calcium oxalate deposits occur in the kidney commonly known as kidney stones (Makkar *et al.,* 2007).

Oxalates can be eliminated from the feed material by fermentation. Fermentation process using microbial services, convert feed ingredients into specific products. Bacillus subtilis is one of microbes that can degrade oxalate. Adegbegingbe *et al.* (2014) reported increasing of crude protein for 25.54% and crude fat for 2,63%, but crude fiber decreased 5,23% on fifth days fermentation on *Phaseolus lunatus* bean fermented with *Bacillus subtili*s. Furthermore, oxalate content decreased 70.81% from 1.61 mg/g before fermentation to 0.47 mg/g after fermentation. The objective of this study is to determine the effect of inoculum dose of *Bacillus subtilis* used in fermentation on nutrient and oxalate content of *Amorphophallus sp* tuber.

MATERIALS AND METHODS

Amorphophallus sp. tubers were taken from East Amarasi village, East Amarasi sub-district, Kupang, East Nusa Tenggara. Tubers cleaned with tap water to remove the soil on the skin tuber. Tuber were sliced to ± 7 cm length and ± 3 cm thickness, sun dried for ± 2 days, and milled.

Bacillus subtilis FNCC 0059 in solid form was obtained from Microbiology Laboratory Pusat Antar Universitas (PAU) Gadjah Mada University. *Bacillus subtilis* was grown on 10 ml de Man Rogosa Sharpe (MRS) incubated at 37ºC for 24 hours, used as a stock culture. A total of 10% of the stock culture is grown in medium containing oxalate (Campieri *et al.,* 2001).

Bacillus subtilis derived from oxalate medium was 10% reculture in semi solid medium were prepared as liquid medium, then 10% *Amorphophallus* tuber were added and incubated at pH 5.5 and temperature 37ºC for 4 days. This semi-solid medium is used as inoculant source of solid fermentation.

Solid fermentation were done on *Amorphophallus sp*. tuber with different dose of *Bacillus subtilis* at 0, 10, 20, 30% dry matter. Each treatment consisted of three replications. Moisture of *Amorphophallus sp*. tuber were 40%. *Amorphophallus sp*. were mixed with the inoculant according to the treatment then placed on plastic medium as silo at 1 kg capacity, then compacted and incubated at room temperature for 21 days. Harvesting after 21 days, oven dried at 50ºC for 48 hours and then analyzed for proximate, Ca, P and oxalate content.

Oxalates were measured using kinetic spectrophotometer catalyst method according to Jiang *et al.* (1996). Briefly, 1 g of sampel were mixed with 5 ml of HCl 2 M, centrifuged at 2500 rpm for 10 min, then the supernatant was filtered using Whatman no. 1, then dissolved with 10 ml of aquades. This solution was used for analysis of oxalate content using 0.5 ml of sample solution, 0.06 mol^{-1} potassium dichromate, 0.20 ml of sulfuric acid 2, 5 mol/l and 0.10 ml of rhodamine B 3.28 X 10^{-4} mol/l fortexed until homogenous and heated in water bath at 90 ºC for 15 minutes. The absorbance read at 555 nm wavelength. The oxalate standard values were measured on 10 points at different oxalic acid concentrations. Nutrient and oxalate content of *Amorphophallus* sp. tuber were analyzed by analysis of variance and Duncan's New Multiple Range Test (Gasperz, 1991).

RESULTS AND DISCUSSION

Proximat Analysis and Mineral Content of Fermented *Amorphophallus sp* tuber. Proximate analysis of fermented *Amorphophallus sp.* tubers inoculated with different doses

of *Bacillus subtilis* were presented in Table 1. Inoculum doses showed no significant effect (P>0.05) on dry matter, crude protein, crude fiber, crude fat, calcium and phosphorous.

The results showed that there was no effect of dose of inoculum on dry matter content of *Amorphophallus sp*. This is because the fermentation process is done anaerobically so there is no evaporation and no addition of moisture content during the fermentation process. In the process of fermentation, dry matter changes in fermented materials can occur due to the growth of microorganism, decomposition of substrate and presence of changes in water content (Nelson and Suparjo, 2011). Changes in moisture may occurred due to the evaporation process, substrate hydrolysis or metabolic water production.

Table 1. Nutrient content of fermented *Amorphophallus sp.* tuber with different doses of *Bacillus subtilis*

	Dose of *Bacillus subtilis* (% BK)			
Nutrien content (%)	0	10	20	30
Dry matter	81,463 ± 3,102	79,640 ± 1,114	80,580 ± 0,357	80,443 ± 2,319
Crude Fiber	7,060 ± 0,205	7,493 ± 0,575	7,333 ± 0,808	7,577 ± 0,035
Crude fiber	4,243 ± 0,021	4,390 ± 0,181	4,377 ± 0,145	4,270 ± 0,266
Crude fat	1,150 ± 0,231	0,993 ± 0,021	1,293 ± 0,215	1,383 ± 0,281
Ash	7,697 ± 0,119	7,637 ± 0,649	7,627 ± 0,612	7,870 ± 0,156
Phosporus	0,159 ± 0,03	0,178± 0,02	0,101± 0,05	0,144 ± 0,07
Calcium	0,159 ±0,003	0,178±0,016	0,102±0,047	0,147 ± 0,065

Based on analysis of variance, there was no effect of dose of inoculum on dry matter content of *Amorphophallus sp*. Ash content of fermented soybean by *Bacillus subtilis* increased 20% from 6 to 7.2 (Kim *et al.,* 2016). Changes in ash content in fermented materials were caused by changes in organic matter occurring in the bioconversion process (Haddadin *et al.,* 2009). Ash content levels describe mineral content in a material as minerals content reduction in fermentation processes caused by microbes utilization to grow (Oladonmoyo, 2007).

The result of analysis statistical shows that fermentation using *Bacillus subtilis* up to 30% inoculum level based on dry matter has no effect on crude protein value of *Amorphophallus sp*. tuber. This was probably due to reshuffle of protein by bacteria did not occur in fermentation process, decreasing of crude fiber and addition of *Bacillus subtilis* as microbial protein source was not sufficient to increase tuber protein. Oboh *et al.* (2002) suggests that the protein in fermented material may increase due to single cell protein of microbes. Fermentation using Bacillus subtilis at 30% inoculum level based on dry matter had no significant difference in crude fat value of *Amorphophallus sp*.

There were no significance difference (P>0.05) in calcium and phosphorus content. Eka (1980), reported an increase in phosphorus and calcium content of 14.29 and 9.09% respectively in fermented Locust beans. The presence of oxalate decarboxylase enzyme activity of Bacillus subtilis bacteria causes the release of oxalate and calcium bonds raise the increasing of calcium in the tubers. Tanner and Nornemann (2000), find that *Bacillus subtilis* produces oxalate decarboxylase.

Oxalate Content in Fermented *Amorphophallus sp* Tuber. The study shown that 30% dose of *Bacillus subtilis* in 21-day incubation can decrease the oxalate content of *Amorphophallus sp.* This was probably due to the greater number of tuber inoculums, resulting on considerably enzyme that can degrade oxalate much lower. Fermentation may

decrease the antinutrient content due to the enzyme produced by the microorganisms used in the fermentation process.

Readdy and Pierson (1994) reported that foods such as tubers, beans, cereals contains antinutrients and toxins such as phytate, tannin, HCN, oxalate, saponins, lectins that can be reduced by fermentation. Ojokoh *et al.* (2013) states that the decrease of oxalate was due to the presence of enzymes produced by microorganisms. Adegbehingbe *et al.* (2014), finding was the decrease of oxalate in *Phaseolus lunatus* flour is 70,81% fermented with Bacillus subtilis and 72,05% with *Bacilluc pubmilus*. Ojokoh *et al.* (2013) states that fermentation with *Lactobacilluc plantarum* bacteria for 72 hours decrease the antinutrient content such as oxalate in *Treculia africana* and *Vigna unguiculata* by 25 to 65.12% and HCN content of 80 to 99%.

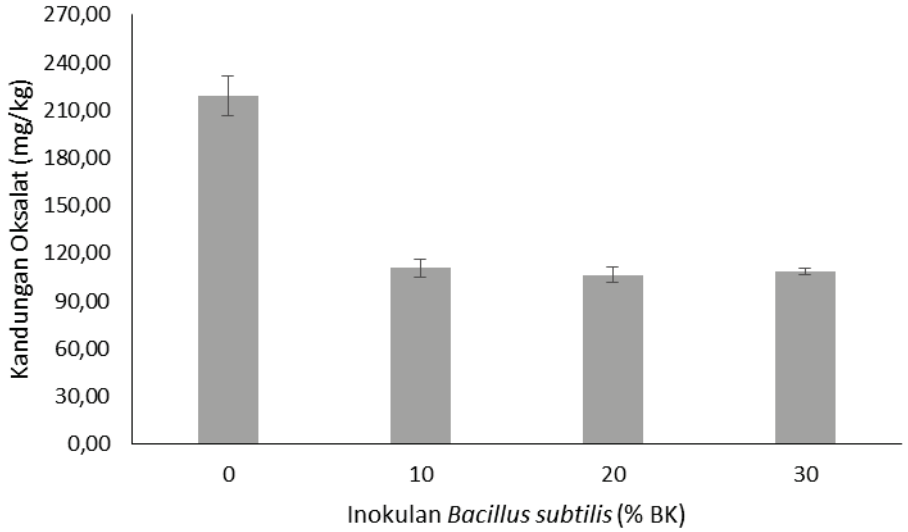

Figure 1. Effect of inoculum dose on tuber oxalate content

CONCLUSIONS

Fermentation of Amorphophallus sp. tuber using *Bacillus subtilis* bacteria can decrease 58% to 65% oxalate content without changing the nutrient content.

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