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Research Article
Nutrient Evaluation of Fermented *Amorphophallus campanulatus* as Poultry Feed

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Abstract

Background and Objective: *Amorphophallus campanulatus* (AC) can be used for food and as animal feed but its utilization is limited because of the high content of oxalate and low crude protein it contains. Fermentation by oxalolytic bacteria such as *Bacillus subtilis* (*B. subtilis*), which produce the oxalate decarboxylase enzyme, has been used to improve the nutritive value of AC. The present study was conducted to improve the nutritive quality of AC through fermentation using *Bacillus subtilis*. **Materials and Methods:** AC was incubated for three different lengths of time 7, 14 and 21 days with 3 replicates of each treatment. The parameters observed included the dry matter, crude protein, fat and fiber contents, as well as Ca, P and oxalate contents. Data obtained were subjected to analysis of variance using a completely randomized design. **Results:** Results showed that the length of the incubation period significantly affected oxalate content ($p<0.05$). Fermentation with *Bacillus subtilis* decreased the oxalate content of *Amorphophallus* by 53.2, 50.4 and 41.2% at 7, 14 and 21 days of incubation time, respectively, when compared with raw *Amorphophallus* (315.8 mg/100 g) and increased crude fat content ($p<0.05$). Additionally, the lowest crude fat was found in AC with 14 days of incubation time. However, there were no significant effects on dry matter, crude protein, crude fiber, Ca and P ($p>0.05$). **Conclusion:** Fermentation using *Bacillus subtilis* with an incubation length of 7 days was the best treatment for improving the nutrient value of AC.

Key words: *Amorphophallus campanulatus*, fermentation, *Bacillus subtilis*, nutrient content, oxalate, poultry feed

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Amorphophallus campanulatus (AC) is a crop that originates in South Asia[1]. This crop is commonly known as elephant foot yam and in Timor, it is called Maek[2]. AC is cultivated as an intercrop plant along with ginger under coconut or banana trees in India, where Ravi *et al.*[1] reported that the production of AC is 50-80 t ha^{-1}. Conversely, in Indonesia, AC has low production and is an underutilized crop[3]. The nutrient content of AC consists of 2.14-7.56% crude protein[2,4-5], 1.04% crude fat, 9.43% crude fiber[2], Ca 50 mg/100 g, phosphorus[5] 34 mg/100 g and 0.78-6.24% oxalic acid[6-7].

Research concerning the utilization of AC fermented by *Rhizopus oligosporus* has previously been reported. Koni *et al.*[2] indicated that AC fermented by *Rhizopus oligosporus* can comprise 5% of broiler rations. Feed stuff containing high oxalate has negative effects such as reduced calcium absorption and reduced growth rate[8-10]. Therefore, AC is not widely utilized in poultry diets. To increase the AC proportion in poultry feed, it is necessary to improve the nutrient content and reduce the oxalate content. Fermentation by *Bacillus subtilis* can eliminate oxalate from feed stuff, as this microorganism can produce the oxalate decarboxylase enzyme[11].

Adegbehingbe *et al.*[12] reported that the oxalic acid content of *Phaseolus lunatus* beans fermented with *Bacillus subtilis* was reduced by 70.81%, from 1.61 mg g^{-1} before fermentation to 0.47 mg g^{-1} after fermentation. The objective of the present study was to determine the effect of the length of fermentation with *B. subtilis* on the nutrient and oxalate content of AC.

MATERIALS AND METHODS

AC tubers were collected from East Amarasi village, East Amarasi sub-district, Kupang, East Nusa Tenggara. The tubers were cleaned with tap water to remove the soil on the peeled tuber. The tubers 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* stock culture was grown on 10 mL de Man, Rogosa and Sharpe (MRS) and incubated at 37°C for 24 h. *Bacillus subtilis* derived from stock culture was used to create a 10% culture in semi-solid medium, which was prepared by adding 10% AC tuber and incubated at pH 5.5 and temperature 37°C for 4 days. This semi-solid medium was used as an inoculant source of solid fermentation. Solid fermentation was performed on AC tuber meal with added aquadest and 20% *B. subtilis* from semi solid fermentation (moisture content 40%), with the mixture then placed on a plastic bag and incubated at room temperature with different incubation periods as treatments.

The research was arranged in a completely randomized design with 3 incubation period treatments 7, 14 and 21 days with 4 replicates of each treatment. The parameters measured were dry matter (DM), crude protein, fiber and fat, as well as Ca, P (measured according to AOAC[13] and oxalate[14]).

Statistical analysis: All of the data obtained were analyzed by one-way analysis of variance and significant differences of the means were determined using Duncan's multiple range test at the level of $p<0.05$[15].

RESULT AND DISCUSSION

The effects of incubation time on dry matter, crude protein, crude fiber, crude fat, Ca and P are shown in Table 1. It is observed that incubation time had no significant ($p>0.05$) effects on dry matter, crude protein, crude fiber or Ca of AC. The lack of significant effects on some of the dry matter content was probably due to the anaerobic fermentation process, with no evaporation and no addition of moisture content during the process. This finding is supported by Hardini[16], who stated that in aerobic fermentation longer fermentation times cause an increase in evaporation resulting in an increase in dry matter in the fermented bran using *Aspergillus*. Furthermore, this finding is also supported by Nelson and Suparjo[17], who found that dry matter changes in fermented materials can occur due to the presence of changes in water content. Changes in moisture may occur due to the evaporation process, substrate hydrolysis or metabolic water production. The results of our analyses showed that fermentation using *B. subtilis* up to 21 days of incubation time had no effect on the crude protein value of AC. This was likely because a reshuffle of protein by the bacteria did not occur in the fermentation process and the decreasing of crude fiber and addition of *Bacillus subtilis* as a microbial protein source was not sufficient to increase tuber protein. The crude protein content of AC over all incubation times may have been caused by the low crude protein content of AC. According to Nuraini *et al.*[18] the protein content of raw material will be affected by the protein content substrate in fermentation. Oboh *et al.*[19] suggested that the protein in fermented material may increase due to the single cell protein of microbes.

In the present study, there was no effect of incubation time on P and Ca content, which was likely due to the low Ca and P tuber AC and oxalate contained in AC in the form of soluble oxalate. Results of the present study contradict with

Table 1: Effect of incubation time on dry matter, crude protein, crude fiber, ether extract, Ca and P *Amorphophallus campanulatus* (%)

	Incubation time (days)		
Nutrient contents (%)	7	14	21
Dry matter	80.380±0.527	80.603±1.510	80.580±0.357
Crude protein	7.593±0.309	7.573±0.412	7.333±0.808
Crude fiber	4.737±0.085	4.267±0.508	4.377±0.147
Crude fat	1.087±0.098[b]	0.627±0.156[a]	1.293±0.215[b]
Phosphorus	0.209±0.037	0.272±0.211	0.102±0.047
Calcium	1.007±0.052	1.062±0.175	1.187±0.125

[a,b]In the same row with different superscripts are significantly different (p<0.05)

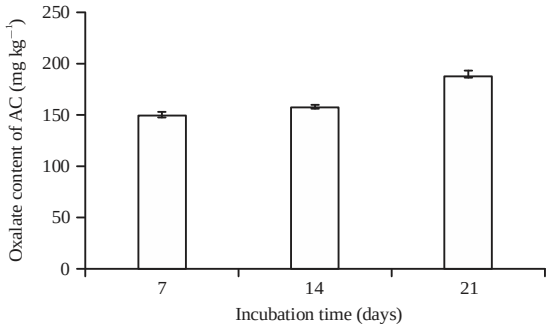

Fig. 1: Effect of incubation time on oxalate content of *Amorphophallus campanulatus*

the findings of Eka[20], who reported increases in phosphorus and calcium content of 14.29 and 9.09%, respectively, in fermented locust beans. The presence of oxalate decarboxylase enzyme activity in *Bacillus subtilis* bacteria causes the release of oxalate and calcium bonds to rise, leading to increased calcium in the tubers.

It is observed that incubation time had a significant effect on the crude fat of AC (p<0.05). An increase in crude fat content of AC when the incubation time was 7 days was caused by lipase enzymes produced by *B. subtilis*. Ghori *et al*.[21] found that *Bacillus* sp. can produce extracellular lipase. The effect of incubation time on oxalate content of AC is shown in Fig. 1. It was found that incubation time had a significant effect on the oxalate content of AC (p<0.05). The lowest oxalate levels in AC were observed after fermentation with *Bacillus subtilis* for 7 days. Oxalate reduction was likely facilitated by the oxalate decarboxylase enzyme, which was produced during fermentation. Adegbehingbe *et al*.[12] also found that oxalate on *Phaseolus lunatus* flour fermented by *Bacillus subtilis* and *Bacillus pumilus* decreased by 70.81 and 72.05%, respectively. Reddy and Pierson[22] reported that foods such as tubers, beans, cereals contain anti-nutrients and toxins such as phytate, tannin, HCN, oxalate, saponins, lectins that can be reduced by the fermentation process. Ojokoh *et al*.[23] stated that the decrease in oxalate was due to the presence of enzymes produced by microorganisms. Additionally, Tanner *et al*.[24] found that *Bacillus subtilis* produces oxalate decarboxylase. Commercial *Bacillus subtilis* is available in the market and it is a common bacteria used as probiotic[25], villagers can use it for *Amorphophallus campanulatus* tuber fermentation. Consequently, fermentation technology can be used to improve nutrients in feed ingredients. Incubation time in the fermentation process will also affect the nutrients of the fermented feed material.

CONCLUSION

Fermentation by *Bacillus subtilis* with 7 days of incubation time was selected as the best incubation time for decreasing oxalate.

SIGNIFICANCE STATEMENT

The present study was conducted to increase the use of *Amorphophallus campanulatus* through fermentation by *Bacillus subtilis* with different incubation times. The use of AC as poultry feed is still limited due to oxalate content. Findings of the present study indicated that fermented AC using different incubation times significantly reduced the oxalate content.

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