Quality evaluation of moringa (Moringa oleifera) leaves fermented by various dosages of Aspergillus niger as ingredients for fish feeds

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Quality evaluation of moringa (*Moringa oleifera*) leaves fermented by various dosages of *Aspergillus niger* as ingredients for fish feeds

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Abstract. The *Moringa oleifera* leaves are a promising candidate plant protein source for fish feeds because of its high crude protein content, varying from 21% to 32%. However, because of high content of crude fiber in moringa, it can reduce the digestibiliy of the nutrients. Therefore, there is a need for intervention to improve the quality of *M. oleifera* leaves. The current research was carried out to assess the nutrient quality of *M. oleifera* leaf meal (MOLM) following fermentation with *Aspergillus niger* different dosages as alternative feed ingredients for fish. In this study, a completely randomized design with four treatments and three repetitions was used. The treatments were as folllows: F0 = Fermentation of MOLM without *A. niger* (control), F1 = Fermentation of MOLM with *A. niger* at 2% (w/w) of MOLM, F2 = Fermentation of MOLM with *A. niger* at 4% (w/w) of MOLM and F3 = Fermentation of MOLM with *A. niger* at 6% (w/w) of MOLM. Results showed that MOLM fermented with *A. niger* had a significant effect on crude fiber and crude protein. It can be concluded that MOLM supplemented with 6% *A. niger* could improve crude protein and crude fiber quality of *Moringa oleifera* leaves.

1. Introduction

The moringa (*Moringa oleifera*) leaves are one of the local raw materials that can be used as sources of plant protein in fish feed. Moringa leaves have high crude protein level ranging from 21% to 32% [1,2,3,4]. Aside from being rich in essential amino acids [5], the moringa leaf contains calcium, potassium and iron [6]. Previous studies have been conducted to use the moringa leaves as ingredient for animal feeds [7,8,9] including in some fish species [10,11,12,13].

The utilization of moringa leaf meal as a component in fish feed is not widely accepted because the leaves contain high amounts of crude fiber and anti-nutritional factors including tannins and saponins, resulting in poor digestibility in fish [14]. Using *Aspergillus niger* during fermentation is one method for reducing the content of crude fiber and neutralize anti-nutritional substances in moringa leaves. *A. niger* produces enzymes such as protease, amylase and cellulase that aid in improving the digestibility of feeds [15]. The addition of *A. niger* in the fermentation process is expected to enhance the nutritional value of moringa leave meal so that it can be utilized as an alternative raw material for the production of fish feed. This has the potential to replace soybean meal which is fairly expensive source of crude protein in commercial feeds for fish. Therefore, the current research was carried out to assess nutrient quality of moringa leaves following fermentation with *A. niger* at different doses as alternative feed ingredients for fish.

2. Materials and Methods

2.1 Preparation of moringa leaf meal

Moringa leaves were get from Cipta Karya Street, Pekanbaru, Riau Province, Indonesia. The moringa leaves were sorted from the stalks and washed thoroughly using running water to remove such a adhering dirt. Then, the leaves were sundried for five days to obtain a constant weight. The dried moringa leaf were blended become powder using an electric grinding machine obtaining *M. oleifera* leaf meal (MOLM). The leaf meal was placed in an airtight bag and kept for subsequent use.

2.2 Moringa leaf meal fermentation process

The completely randomized design was used in this study. The different dosages of *A. niger* were prepared to ferment MOLM at 0% (F0), 2% (F1), 4% (F2), and 6% (F3). Each treatment was carried in duplicate. Fermentation process was accomplished by carefully taking the weight of 10 g of MOLM in each treatments, wrapping it in tinfoil paper, steaming into hot water for 15 minutes, and cooling at ambient temperature. Following cooling, the starter of *A. niger* was combined with MOLM based on the types of treatment, initiated by distilled water (DW) addition with the ratio of 1:1 (1 g MOLM:1 mL DW). This MOLM mixtures were incubated at ambient temperature for 72 hours in a clear plastic container with small perforations. After this duration, the MOLM mixture was steamed for 5-10 minutes using boiling water. This step was done to deactivate *A. niger*. In addition, both the MOLM and fermented MOLM were analyzed for nutrient compositions using the of AOAC [16] standard

procedures. The Deng et al [17] method was used to determine the nitrogen free extract (NFE) of samples.

The data is shown as average ± standard deviation (SD). Duncan's multiple range test was used to identify the treatments means tested for significances ($P<0.05$) with analysis of variance (ANOVA).

3. Results and Discussion

Table 1 shows the nutrient composition of *Moringa oleifera* leave meal (MOLM) fermented by different dosages of *A. niger* starter. The content of crude fiber (CF) of MOLM fermentation decreased as the dosage of *A. niger* addition increased, whereas the content of crude protein (CP) was increased. Furthermore, the moisture and ash contents increased as the *A. niger* supplementation dosage increased.

Table 1. Nutrient composition of MOLM by various dosages of *A. niger* addition

Dosage of *A. niger*	Proximate composition (%)					
	Moisture	Ash	Crude fiber	Crude protein	Crude fat	NFE
F0 (0%)	7.65±0.71^{a}	10.52±0.54^{a}	12.99±0.32^{c}	21.17±0.04^{a}	5.76±0.29^{a}	41.92±1.23^{b}
F1 (2%)	8.29±0.66^{a}	15.18±0.33^{b}	11.79±0.90^{c}	25.56±0.49^{c}	7.42±0.18^{b}	31.78±0.58^{a}
F2 (4%)	8.52±0.41^{ab}	16.26±1.54^{ab}	9.36±0.21^{b}	23.51±1.31^{bc}	7.11±0.36^{b}	35.25±2.60^{a}
F3 (6%)	10.02±0.33^{b}	18.74±0.49^{c}	6.93±0.17^{a}	22.96±0.54^{ab}	6.68±0.69^{ab}	34.68±0.03^{a}

The values within the column with different superscripts differ significantly ($P<0.05$)

The CF content of MOLM fermented with 6% *A. niger* (F3) was significantly lower comparing to other treatments ($P<0.05$) as shown in Table 1. The ability of the mold to degrade lignocellulose aerobically resulted in the decrease in the content of CF of the MOLM fermentation. It was in agreement with the findings of Winarno and Fardiaz [18], who declared that the presence of enzymes in *A. niger* cause a reduction in CF contents of treatments after fermentation. During fermentation process, these enzymes have the ability to convert cellulose to simple glucose. Cellulase is a complex enzyme that works slowly to degrade cellulose into glucose, which is then used as a source of carbon and energy.

The treatment with 6% *A. niger* (F3) resulted in the highest reducing in CF content of fermented MOLM. This could be caused by the fact the dosage of *A. niger* used in the treatment F3 was greater comparing to others, resulting in a decreased the content of CF. It is consistent with Suharman et al. [19] who carried-out the experiment on fermented water hyacinth leaf meal. The reduction in the content of CF is likely due to the increased *A. niger* concentrations, which resulted in the higher capability to break down fiber. The present of *A. niger*, which produces cellulose enzymes, are able to degrade cellulose to simpler forms; thus, decrease in the content of CF of fermented MOLM was attained [20]. These findings were similar to the results of Gloria et al. [21], who mentioned that fermenting apu-apu meal (*Pistia stratiotes* L.) with starter of *A. niger* could decrease the content of CF from 17.33% to 14.83% and the rice bran fermented using *A. niger* for 72 hours reduced the CF content [22].

It was a significant increased ($P<0.05$) in CP content of the MOLM fermented with *A. niger* comparing to the control group (F0). The increasing of the content of CP in fermented MOLM was due to the destruction of the protein in MOLM as the presence of protease enzyme produced by *A. niger* that convert CP into amino acids. These amino acids are used by fungi to help them grow. On the other hand, the increased CP content could be due to increased sporulation caused by increased *A. niger* biomass. All of these processes were easily detected by the decreased NFE of fermented MOLM, while the increase in CP was due to a decrease in carbohydrate used for fungi growth.

Since the body of fungi body is made up of nitrogen-containing elements, the increased in CP content also corresponds to the growth of the fungi [23] that were added during the fermentation process. Furthermore, fungi also contain proteases that can break down proteins into simpler forms [24]. The increase in CP content in fermented MOLM could be caused by higher in mold colony populations as evidenced by a rise in biomass of *A. niger* during process of fermentation [25]. Suharman et al. [19] found that the fermentation of water hyacinth leaf meal using *A. niger* increased the content of CP from 5.56 to 18.15%. The higher content of CP could be attributed to increasing the *A. niger* biomass where majority of the cells contains high amount of proteins (single cell protein/SCP) [26].

4. Conclusion

The current study demonstrated that fermentation with *A. niger* as a starter can improve nutrition quality of *Moringa oleifera* leaves. The best dosage of *A. niger* to reduce crude fiber in M. *oleifera* leaves is 6%.

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