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Assessment of the nutritional profile of fermented noni (*Morinda citrifolia*) leaf meal using *Rhizopus* sp. as a potential fish feed ingredient

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Abstract. This study aimed to assess the nutritional quality of noni (*Morinda citrifolia*) leaf meal (NLM) fermented with varying concentrations of *Rhizopus* sp. as a potential fish feed ingredient. A completely randomized design (CRD) with five treatments and three replicates was employed. The levels of *Rhizopus* sp. applied to NLM were 0%, 5%, 10%, 15%, and 20%. The results revealed that fermentation of NLM with *Rhizopus* sp. significantly ($P < 0.05$) decreased crude fiber content while increasing crude protein levels. These findings suggest that fermenting NLM with 20% *Rhizopus* sp. effectively enhances its nutritional value, particularly by improving protein content and reducing fiber.

1. Introduction

Morinda citrifolia (commonly known as noni) leaves are a locally available raw material with promising potential as a source of plant-based protein in aquaculture feed. These leaves are nutritionally rich, containing a high crude protein content (18.48%) [1], as well as essential minerals such as calcium, phosphorus, magnesium, and iron [2]. *M. citrifolia* leaves have been utilized in livestock diets [3-6], including for various fish species [7-14].

The use of noni leaf meal in aquafeeds is currently limited by several factors such as high crude fibre content and nutritional values that can vary [8,15], which may impair digestibility. Fermentation is widely recognized as an effective bioprocess to enhance the nutritional quality and digestibility of plant-based feed ingredients. Among the variables that significantly impact fermentation outcomes, the concentration of the starter culture plays a pivotal role in determining enzyme production, substrate breakdown, and overall nutrient bioavailability. *Rhizopus* sp., a fungus commonly used in tempeh production, produces various enzymes such as protease, amylase, and lipase, which help break down complex compounds in organic matter, making nutrients more available for absorption [16,17]. Besides that, fermentation with *Rhizopus* sp. altered the proximate composition of banana peel flour. The carbohydrate content significantly decreased, while the crude protein content significantly increased [18]. Additionally, cocoa pod husk meal fermented with *Rhizopus* sp. showed an increase in



crude protein and a reduction in crude fiber and anti-nutrient content, which could lead to improved palatability, better feed intake, and enhanced utilization [19].

Limited information is available regarding the optimal inoculum dose of *Rhizopus* sp. for fermenting noni (*M. citrifolia*) leaf meal, which is rich in crude fiber and presents challenges for direct inclusion in fish diets. Using different concentrations of *Rhizopus* sp. during fermentation is essential to determine the optimal conditions for enhancing the nutritional quality of agro-industrial by-products, particularly in terms of increasing crude protein content and reducing crude fiber content. Therefore, this study aims to systematically investigate the effects of various *Rhizopus* sp. inoculum concentrations on the proximate composition and nutritional enhancement of fermented noni leaf meal, with the goal of developing an efficient and cost-effective method to produce high-quality, plant-based fish feed ingredients.

2. Materials and Methods

2.1. Collection and preparation of noni leaf meal

Fresh *Morinda citrifolia* (noni) leaves were collected from the area surrounding Pekanbaru. The leaves were thoroughly washed under running water and soaked in clean water for 12 hours to reduce anti-nutritional compounds, as described by Alam et al. [20]. After soaking, the leaves were cut into small pieces and sun-dried until they became brown and brittle. The dried leaves were then ground using a blender and sieved through a fine mesh to obtain noni leaf meal (NLM).

2.2. Fermentation process of noni leaf meal

The finely ground NLM was subjected to fermentation following the method of [21], with slight modifications. Initially, water was added to the NLM at a 1:1 (w/v) ratio, and the mixture was steamed for 15 minutes to reduce any remaining anti-nutritional content. After cooling to room temperature, the steamed NLM was inoculated with *Rhizopus* sp. at concentrations of 0% (T0), 5% (T5), 10% (T10), 15% (T15), and 20% (T20) relative to the dry weight of the NLM. The inoculated samples were thoroughly mixed and transferred into perforated, heat-resistant plastic containers to create aerobic conditions. The fermentation process was conducted at ambient temperature (approximately 28–30°C) for 48 hours. During this period, *Rhizopus* sp. efficiently produced microbial biomass, contributing to an increase in crude protein content. Simultaneously, it released enzymes that degraded complex lignocellulosic fibers, resulting in a reduction in crude fiber content [22]. Successful fermentation was indicated by visible brown mycelial growth, a characteristic fermented aroma, and a moist texture [8]. To terminate microbial activity, the fermented NLM was steamed for an additional 15 minutes. The material was then sun-dried until completely dry and stored in airtight containers at room temperature for further analysis.

2.3. Statistical analysis

Data are expressed as mean $\pm$ standard deviation (SD). When significant differences were detected ($P < 0.05$), a one-way analysis of variance (ANOVA) was conducted, followed by Duncan's multiple range test to compare group means. Statistical analyses were performed using SPSS version 21.0 (SPSS Inc., Chicago, IL, USA).

2.4. Ethical statement

Ethical approval was not required for this study, as the dataset did not include any animal samples.

3. Results and Discussion

The proximate composition of noni leaf meal (NLM) fermented with varying concentrations of *Rhizopus* sp. is summarized in Table 1. An increase in *Rhizopus* sp. supplementation corresponded with a rise in crude protein (CP) content, whereas crude fiber (CF) levels showed a declining trend.

Table 1. Proximate composition of NLM fermented with varying doses of *Rhizopus* sp. supplementation¹

Dose of <i>Rhizopus</i> sp	Proximate composition (%)					
	Moisture	Crude protein	Crude fat	Crude fiber	Ash	NFE
T0 (0%)/control	8.11±0.01 ^d	26.13±0.47 ^a	7.84±0.01 ^e	13.79±0.04 ^e	4.98±0.040 ^d	47.27±0.41 ^b
T5 (5%)	7.96±0.08 ^{cd}	30.73±0.04 ^b	7.53±0.04 ^d	10.67±0.03 ^d	4.80±0.11 ^c	46.28±0.16 ^a
T10 (10%)	7.88±0.01 ^c	31.19±0.57 ^{bc}	7.29±0.02 ^c	9.69±0.06 ^c	4.71±0.01 ^{bc}	47.13±0.03 ^b
T15 (15%)	7.51±0.06 ^b	31.38±0.05 ^c	7.12±0.05 ^b	9.34±0.08 ^b	4.57±0.05 ^{ab}	47.61±0.13 ^{bc}
T20 (20%)	7.28±0.11 ^a	31.59±0.09 ^c	6.99±0.04 ^a	9.08±0.09 ^a	4.49±0.02 ^a	47.23±0.59 ^c
Changes (%)		+20.90		-34.16		

¹Values are means ± SD. Based on Duncan test, values within the column with different superscripts are significantly different ($P<0.05$)

Based on the results presented in Table 1, fermentation of NLM with *Rhizopus* sp. increased the crude protein (CP) content by 20.90%, from 26.13% to 31.59%, while reducing the crude fiber (CF) content by 34.16%, from 13.79% to 9.08%. The CP content in the T20 treatment was significantly higher than that in the T0 (control) group ($P<0.05$), but did not differ significantly from the T10 and T15 treatments.

The increase in CP content following fermentation with *Rhizopus* sp. is primarily attributed to microbial degradation of complex macromolecules into simpler, more digestible compounds. This increase may also be associated with the synthesis of enzymes by the microbes, as enzymes themselves are proteins. In addition, during fermentation, microbial growth contributes single-cell protein and enzymatic proteins, both of which raise the overall CP content. The accumulation of microbial biomass further amplifies this effect. These results are in agreement with the findings of Sigiuro et al. [23] and Laining et al. [24], who reported that fermentation using *Rhizopus* sp. improved the protein quality of coconut meal and copra cake meal by increasing their CP levels.

The data in Table 1 demonstrate that fermentation with *Rhizopus* sp. reduced the crude fiber (CF) content in NLM by 34.16%. A notable decrease in CF was observed starting at a 20% *Rhizopus* sp. inoculum combined with a fermentation period of 48 hours. Rizal et al. [25] reported that *Rhizopus* sp. produces various enzymes, including α -amylase, protease, and lipase. The reduction in CF is primarily attributed to α -amylase, which catalyzes the breakdown of starch into simpler sugars [26]. Supporting these findings, Paramadini et al. [27] reported a reduction of CF in commercial feed from 28.76% to 21.16% after fermentation with *Rhizopus* sp. In this study, the most effective treatment for CF reduction in NLM was a 20% *Rhizopus* sp. dosage with a 48-hour fermentation period, resulting in a CF content of 9.08%, which was significantly different ($P<0.05$) from other treatments.

4. Conclusions

This study demonstrated that fermenting noni leaves with *Rhizopus* sp. improves their nutritional quality. A 20% supplementation of *Rhizopus* sp. in noni leaves was determined to be the optimal level for increasing protein content and decreasing crude fiber. These findings collectively suggest that *Rhizopus* sp. is an effective microorganism for fermenting NLM, thereby enhancing its nutritional quality for use as a dietary feed ingredient.

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