Effect of Fermented Rice Bran used as Feed Ingredient on Apparent Digestibility Coefficient of Tilapia (Oreochromis niloticus) Feed

A N Putra12*, D Rohayati1, M B Syamsunarno1, Mustahal1

1Department Fisheries Science, Faculty of Agriculture, Universitas Sultan Ageng Tirtayasa, Jalan Raya Palka KM.03 Sindangsari, Serang, Indonesia 42163.
2Center of Excellence Local Food Innovation, Universitas Sultan Ageng Tirtayasa, Jalan Raya Palka KM.03 Sindangsari, Serang, Indonesia 42163.

*putra.achmadnp@untirta.ac.id

Abstract. This study aimed to investigate the effect of fermented rice bran used as a feed ingredient on the apparent digestibility coefficient (ADC) of tilapia feed. The fermentation process was conducted under aerobic conditions using 2% Aspergillus niger for 24 hours. Moreover, three different types of feed were prepared with 3 replicates and these include treatment A (reference feed), B (unfermented rice bran feed), and C (fermented rice bran feed). These treatments were provided for the tilapia for 30 days. Meanwhile, the tilapia used had an average weight of 5 g/fish and reared using a recirculation system with a density of 16 fish/aquarium. The results showed that the value of ADCenergy (78.11%) in treatment C was significantly highest (P<0.05) compared to other treatments, while the lowest was in treatment B with 72.24%. The values of ADCdry matter (67.56%) and feed efficiency (64.20%) in treatment C were also higher (P<0.05) compared to treatment B with 57.02% and 54.74% respectively. However, there was no significantly difference (p>0.05) in the value of ADCprotein, ADClipid, and SGR among the treatments. These results indicate the rice bran fermented using A. niger is potential to be used as a raw material ingredient for tilapia feed.

1. Introduction
Tilapia is an aquaculture commodity with development potential due to its ability to grow effectively with low protein and high carbohydrate feed [1]. [2] showed tilapia to be an economically important freshwater fish which is relatively easy to be cultivated, has a fast growth rate, a high tolerance for the environment, and resistant to disease. Meanwhile, one of the factors limiting the development of the aquaculture industry is the high price of feed which occupies the largest percentage of production costs [3]. This means an increase in feed raw materials, especially fish meal, affects the income to be obtained by fish farmers [4]. The price of fish meals, however, continues to increase with the quality reported to be uncertain while the supply is sometimes limited [5,6]. One of the alternative solutions to this problem is by incorporating local raw materials in the feed-making process and an example of these materials with sufficient supply is rice bran.

Bran is a by-product of the rice milling process which is produced while stripping the husks of grain and processing rice [7]. According to [8], the milling produces 65% rice, 25% husks, and 12% bran while [9] showed the rice bran is an energy source often found in feed ingredients with high fiber
content. Its nutritional contents include 11.66% protein, 11.95% fat, 10.77% water, 9.51% ash, and 10.57% crude fiber, and [10] showed the way to increase its nutritional and digestibility values as well as safe use is through biological means using fermentation techniques. Meanwhile, one of the microbes usually applied in the fermentation process is *Aspergillus niger*.

*A. niger* is a species of mold with the ability to secrete cellulase, chitinase, α-amylase, glucoamylase, catalase, pectinase, lipase, lactase, invertase, and acid protease enzymes [11, 12]. According to [13], the fermentation of bran using the mold *Aspergillus* spp. can produce phytase enzymes, thereby, reducing the phytate content in the bran. In addition to phytase, the lipase enzymes produced in the bran fermentation process also have the ability to reduce the rancidity of the bran. Therefore, this research was conducted to evaluate the effect of fermented rice bran as a raw material ingredient for fish feed on the digestibility value of tilapia (*Oreochromis niloticus*).

2. Method

2.1 Rice Bran Fermentation and Feed Making

The rice bran fermentation process was conducted by steaming 600 g of bran for 45 minutes from the time the steam water boils after which it was cooled. The *A. niger* was added at a dose of 2% into 200 g of rice bran in each treatment, placed in polyethylene plastic, and incubated at room temperature for 24 hours. The production of the test feed was in line with [14] which consisted of 70% reference feed and 30% test material. Moreover, 5% chromium oxide was used as a digestibility indicator while tapioca flour was used as a binder in the test feed. This study used a completely randomized design (CRD) consisting of 3 feed treatments with 3 replications which include treatment A which was used as reference feed containing 100% commercial feed, treatment B with 70% reference feed and 30% rice bran without fermentation, and treatment C with 70% reference feed and 30% fermented rice bran. The test feed formulation used is presented in Table 1.

| Feed composition      | Treatment (g)a |
|-----------------------|----------------|
|                       | A     | B     | C     |
| Commercial feed       | 965   | 665   | 665   |
| Rice bran             |       | 300   |       |
| Fermented rice bran   |       |       | 300   |
| Tapioca flour         | 30    | 30    | 30    |
| Cr₂O₃                 |       | 5     | 5     |
| Total                 | 1000  | 1000  | 1000  |

*a A (Reference feed: 100% commercial feed, B (Unfermented rice bran feed: 70% reference feed and 30% rice bran without fermentation), C (fermented rice bran feed: 70% reference feed and 30% fermented rice bran).

2.2 Fish Rearing

The tilapias used were male monosex with an average weight of 5 g per fish and reared at a density of 14-16 fish/aquarium in nine aquariums designed to be 60 x 30 x 30 cm and arranged randomly. The rearing process was conducted for 30 days using a recirculation and aeration system in each container after which the fish were first acclimatized to the environment, fed for 7 days, and fasted for 24 hours with the aim of eliminating the feed residue in the body. They were fed three times a day at 07:00 am, 12:00 pm, and 05:00 pm at satiation. Moreover, the water quality in the treatment containers was maintained by siphoning the aquarium every day, morning and evening, to remove feed residue and dirt after feeding and replaced by ±30% of the total aquarium volume.
2.3 Study Parameters

The fish feces were collected to calculate the apparent digestibility coefficient (ADC) of the feed which consisted of ADC\textsubscript{dry matter} (1), ADC\textsubscript{nutrients} (2) included ADC\textsubscript{protein}, ADC\textsubscript{lipid}, ADC\textsubscript{dry matter}, and ADC\textsubscript{energy} and ADC\textsubscript{ingredient} (3). The collection was made by siphoning after 2 hours of feeding and the 7th day after the fish rearing was initiated and the feces were stored at -20°C before the chemical analysis. The ADC value was calculated based on the equation proposed by [14] as follows.

\[
\text{ADC}_{\text{dry matter}} = 100 - (100 \times \frac{\text{dietary chromium in feed}}{\text{dietary chromium in feces}})
\]

(1)

\[
\text{ADC}_{\text{nutrients}} = 100 - (100 \times \frac{\text{dietary chromium in feed}}{\text{dietary chromium in feces}} \times \frac{\text{dietary nutrients in feed}}{\text{dietary nutrients in feces}})
\]

(2)

\[
\text{ADC}_{\text{ingredient}} = \frac{\text{ADC}_{\text{dry matter}} - 0.7 \text{ADC}_{\text{rev}}}{0.3}
\]

(3)

The initial and final fish weights were weighed to calculate the specific growth rate (SGR) value (4) while the number of fish at the beginning and end of rearing was also measured to determine the effect of treatment on their survival rate value (5). Moreover, the amount of feed given each day was weighed to determine the feed efficiency value (6). These calculations were made using the formula proposed by [15] as follows.

\[
\text{SGR (\%/day)} = 100 \times \left( \frac{\ln \text{final biomass} - \ln \text{initial biomass}}{30 \text{ days}} \right)
\]

(4)

\[
\text{Survival Rate (\%)} = 100 \times \left( \frac{\text{final number of tilapia}}{\text{initial number of tilapia}} \right)
\]

(5)

\[
\text{Feed efficiency (\%)} = 100 \times \left( \frac{\text{change in fish biomass}}{\text{feed intake}} \right)
\]

(6)

2.4 Chemical Analysis

Proximate chemical analysis was conducted after the end of the fish rearing period with a focus on the protein, carbohydrate, fat, and crude fiber content of experimental feed and fish feces. This analysis was conducted at the Fish Nutrition Laboratory, Department of Aquaculture, Faculty of Fisheries and Marine Sciences, Bogor Agricultural University (IPB).

2.5 Data Analysis

The data obtained were analyzed using an analysis of variance with a 95% confidence level after which further test was conducted with Duncan's Multiple Range test using the Statistical Product and Service Solution (SPSS) software version 20.0. to determine the difference.

3. Results and Discussion

3.1 Apparent Digestibility Coefficient

The apparent digestibility coefficient (ADC) values of tilapia after feeding for 30 days are presented in Table 2. The digestibility of a feed describes the percentage of nutrients the digestive tract of the fish body has the ability to absorb such that a greater value indicates the presence of more feed nutrients to be used by the fish [16]. The results showed the highest ADC\textsubscript{dry matter} value was found in treatment C.
with 67.56% followed by treatment A with 66.59% while the lowest was in treatment B with 57.02% at \( P < 0.05 \) level of significance. Similar results have also been reported by [17] which showed that \( A.\ niger \) fermentation on soybean meal and rapeseed meal produced a higher ADC\text{dry matter} value compared to the treatment without fermentation on vannamei shrimp rearing at \( P < 0.05 \) level of significance.

| Test Parameters | Test Feed\textsuperscript{a} | A     | B       | C      |
|-----------------|-----------------------------|-------|---------|--------|
| ADC\text{dry matter} (%) | 66.59 ± 1.45\textsuperscript{b} | 57.02 ± 2.89\textsuperscript{a} | 67.56 ± 2.35\textsuperscript{b} |
| ADC\text{protein} (%) | 58.58 ± 3.71 | 58.36 ± 13.09 | 64.40 ± 14.88 |
| ADC\text{lipid} (%) | 84.84 ± 6.33 | 78.48 ± 0.66 | 78.35 ± 0.69 |
| ADC\text{energy} (%) | 75.13 ± 2.80\textsuperscript{b} | 72.24 ± 0.83\textsuperscript{a} | 78.11 ± 2.90\textsuperscript{b} |
| ADC\text{ingredient} (%) | - | 88.90 ± 0.5 | 92.16 ± 0.8 |

\textsuperscript{a}Different superscript letters on the same line show significantly different results \( (P<0.05) \)

\textsuperscript{b} A (Reference feed), B (Unfermented rice bran feed), C (fermented rice bran feed).

The same trend was observed for the ADC\text{energy} with the highest value recorded in treatment C with 78.11% followed by treatment A with 75.13% while the smallest was found in treatment B with 72.24%. This is possible due to the quite high nutrient content in rice bran especially fat and carbohydrate which are non-protein energy sources used by the fish. [18] also showed that herbivorous fish which tend to be omnivorous such as tilapia have a better ability to absorb energy which does not come from protein. The high energy digestibility value recorded, therefore, indicates the ability of tilapia to use energy from carbohydrates. This is in line with the findings of [19] that rice bran contains 17.50% protein, 13.10% fat, and 52.33% carbohydrates. Moreover, the same result was recorded by [20] that the fermentation of waste cocoa pods and lamtoro leaves using \( A.\ niger \) was able to increase the ADC\text{dry matter} and ADC\text{energy} values in the tilapia rearing compared to the treatment without fermentation. However, there was no difference in ADC\text{protein} and ADC\text{lipid} in this study. The value of ADC\text{protein} ranged from 58.58 to 64.64% and ADC\text{lipid} of 78.35-84.84%. This might be due to the lack of production of protease and lipase enzyme by \( A.\ niger \). Similar result was obtained by [21], the fermentation of \( A.\ niger \) in the substitution of fish meal by plant protein mix showed no influence on ADC\text{protein} between control and 50-70% substitutions level on \textit{Penaeus vannamei} Boone, 1931.

The digestibility was measured as an attempt to determine the quantity of feed substances absorbed in the digestive tract. The digestibility of the test material was, therefore, calculated to determine the feed feasibility with the value reported to be needed in preparing and assessing the quality of feed [22]. Moreover, the ADC\text{ingredient} indicates the percentage of materials usable by the fish [18] and the results showed the values for treatment C and B were 92.16% and 88.90%, respectively. This means treatment C is better than treatment B and is considered suitable to be used as a substitute for fish feed. This is associated with the ability of the bran fermented by \( A.\ niger \) to change the structural components of the material such as crude fiber to make it digestible.

### 3.2 Growth of Tilapia

The growth parameter values of tilapia after feeding for 30 days are presented in Table 3 and the results showed the treatments A, B, and C provided the feed intake values of 261.33 g, 166.33 g, and 187.00 g respectively. The differences in these amounts can be influenced by the content and physical characteristics of the feed such as size, shape, color, texture, taste, and smell [23]. Moreover, the low feed intake in treatments B and C was possible due to the smell of bran which was less favored by the fish and the absence of attractants which could have triggered their appetite. According to [24], attractants are compounds added to feed ingredients to improve the overall palatability of the feed.
Table 3. Growth parameter of tilapia

| Test Parameters       | Test Feed a,b |
|-----------------------|---------------|
| Feed intake (g)       | A 261.33 ± 7.37 b 166.33 ± 16.01 a 187.00 ± 6.08 a |
|                       | B 5.20 ± 0.07 5.26 ± 0.21 5.35 ± 0.26 |
| Initial weight (g)    | A 16.53 ± 1.22 14.80 ± 0.82 15.82 ± 1.28 |
| Final weight (g)      | A 3.85 ± 0.29 3.44 ± 0.20 3.61 ± 0.11 |
| SGR (%/day)           | A 62.39 ± 0.99 b 54.74 ± 0.52 a 64.20 ± 1.98 b |
| Survival Rate (%)     | A 79.97 ± 13.35 78.73 ± 12.93 78.03 ± 4.72 |

a Different superscript letters on the same line show significantly different results (P<0.05)
b A (Reference feed), B (Unfermented rice bran feed), C (fermented rice bran feed).

There was no difference in the values of final weight and SGR for all the treatments. The final weight ranged from 14.80 to 16.53 g and SGR of 3.44-3.85 %/day. Similar results were reported by [25] that the fermentation of *A. niger* in soybean meal and sunflower oil cake to replace fish meal the produced a daily growth coefficient which was not different from the treatment without fermentation on *Penaeus vannamei* Boone, 1931. This was associated with the lack of enzyme activity in the digestive tract of the test fish. Meanwhile, [26] stated that the chemical digestibility of the feed is related to the enzymatic activity found in the digestive tract of fish and that the ability of fish to digest feed is highly dependent on the completeness of digestive organs and the availability of digestive enzymes. [22] also showed that the protein digestion process requires protease enzymes such as trypsin, chymotrypsin, pepsin, and others. The value of feed efficiency was significantly (P<0.05) higher in treatment A (62.39%) and C (64.20%) than that of value in B (54.74%). This might be due to the high value of ADC dry matter and ADC energy in treatment A and C. No significant difference (P>0.05) in survival rate among the treatment (79.97, 54.73, 78.03%, repectively. This result indicated that the using fermented rice bran by *A. niger* as feed ingredient in the diet have not negative impact to tilapia. The same result was found in other species when feed ingredient was fermented by *A. niger*, such as: in *Labeo rohita* [27] and in *Penaeus vannamei* [17, 21, 25].

4. Conclusions
The fermented rice bran feed could increase ADC dry matter, ADC energy and feed efficiency of tilapia than unfermented rice bran feed. The rice bran fermention by *A. niger* is potential to be used as an ingredient in the tilapia feed.

Acknowledgments
The authors are grateful to the Faculty of Agriculture, Sultan Ageng Tirtayasa University for providing financial support for this research through the Internal Research Scheme, Intermediate Research Scheme in 2021.

References
[1] Mohamad SN, Noordin WNM, Ismail NF, Hamzah A. 2021. Asian Fisheries Science 34: 73-81.
[2] Irawati D, Rachmawati D, Pinandoyo. 2015. Journal of Aquaculture Management and Technology. 4(1): 1-9.
[3] Hakim AR, Kurniawan K, Siregar ZA. Jurnal Riset Akuakultur, 14:77-85.
[4] Li W, Zheng L, Wang YY, Zhang J, Yu Z, Zhang Y, Li Q. 2015 Bioresource Technology, 194: 276-282.
[5] Lee KW, Kim HS, Choi DG, Jang BI, Kim HJ, Yun A, Cho SH, Min B-H, Kim K-D, Han H-S. 2017. Turkish Journal of Fisheries and Aquatic Sciences 17: 519-526.
[6] Zhang D, Zhang Y, Liul B, Jiang Y, Zhou Q, Wang J, Wang H, Xie J, Kuang Q. 2017. Journal of Fisheries and Aquatic Sciences 17: 1039-1048.

[7] Akbarillah T, Hidayat, Khoiriyah T. 2007. Jurnal Sain Peternakan Indonesia. 2 (1): 36-40.

[8] Wizna & Muis H. 2012. Jurnal Peternakan Indonesia, 14(2): 398-403.

[9] Sidiq F & Anggreini REA. 2015. Trouw Add Science. 1(6): 1-4.

[10] Mulijianti SL, Tedy S, Nurhayeti. 2014. Jurnal Peternakan Indonesia, 16(3): 179-187.

[11] Purkanc P, Baktir A dan Sayyidah AR. 2016. Jurnal Kimia Riset. 1(1): 34-41.

[12] Djunaidi IH, Nuningtyas YF, Muharlien. 2020. Jurnal Peternakan Indonesia, 30(2): 167-172.

[13] Soares D, Djunaidi IH, Natsir MH. 2018. Jurnal Ilmu-Ilmu Peternakan 28(1): 90-95.

[14] Takeuchi T. 1988. Tokyo: Kanagawa Internasional Fisheries Training. Japan Internasional Cooperation Agency (JICA). 256 pp.

[15] Huisman EA. 1987Netherland: Departemen of Fish Culture and Fisheries, Wageningen Agricultural University. 170 p.

[16] NRC, National Research Council. 1993. Washington DC: National Academic Press. 128 pp.

[17] Jannathulla R, Dayal JS, Ambasankar K, Eugene AC, Muralidhar M. Aquaculture Research 49: 2891-2902.

[18] Mulyasari, Kurniawati F, Setiawati S. 2013. Jurnal Akuakultur Indonesia, 12(2): 178-185

[19] Bhosale S & vijayalakshmi D. 2015. Current Research in Nutrition and Food Science, 3(1): 74-80.

[20] Indariyanti N & Rakhamawati. 2013. Jurnal Penelitian Pertanian Terapan 13(2): 108-115.

[21] Dayal JS, Jannathulla, Ambasankar K, Muralidhar M. 2020. Aquaculture Nutrition, 26(3): 853-865.

[22] NRC, National Research Council. 2011. National Academy Press, Washington DC, USA. 376 pp.

[23] Abidin Z, Junaidi M, Paryono, Cokrowati N, Yuniarti S. 2015. Depik, 4(1): 33-39.

[24] Thomas H. 2009. Columbia: The Faculty of Graduate Studies (Food Science), The University of British Columbia. 124 pp.

[25] Jannathulla R, Dayal JS, Ambasankar K, Muralidhar M. Aquaculture 486: 1-8.

[26] Putra AN, Pradana AC, Novriansyah D, Mustahal. 2019. Jurnal Rekayasa dan Teknologi Budidaya Perairan 8: 951-964.

[27] Shamma N, Sardar P, Sahu NP, Pal AKm Jain KK, Phulia V. Aquaculture Nutrition, 21(1): 33-42.