Identification and Prevalence of Fungi on African Catfish (Clarias gariepinus) Fed with Pellets from Smoked Fish Waste

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Abstract. Smoked fish processing waste has a high nutritional content that can be used as a raw material for fish feed (pellets). One standard of fish pellets based on BSN is there must be no aflatoxin content produced from Aspergilus flavus. Qualitative analysis of aflatoxin content can be seen from the presence or absence of A. flavus fungi. In this study the identification of fungi on pellets, fish, and water for maintenance is discussed. Pellets made from smoked fish processing waste are grouped into: K.0 (control), K.1 (100% raw material + 0% fish feed), K.2 (70% raw material + 30% fish feed), K.3 (40% raw material + 60% fish feed), and K.4 (10% raw material + 90% fish feed). Pellets were given to African catfish for 30 days of maintenance. The identification of fungi was carried out on pellets, African catfish, and pond water. The identification results in three fungi namely Rhizopus oryzae, Aspergillus niger, and Fusarium oxysporum.

Keywords: Clarias gariepinus, fungi, pellets, smoked fish waste.

1. Introduction
The fishermen community living in the Bulak District of Surabaya commonly processes their farmed fish locally. The community cleanses fishes and shells as a part of the process. The processed products generate waste mainly from the abdominal part of the fish. The waste was often dumped into the environment without prior treatment. This could generate a strong and unpleasant smell and eventually trigger the presence of disease vectors. On the other hand, fish waste can be used for fish pellets as it contains high calcium and nutrient contents.

Fish feed is one of the factors that play an important role in the growth process of fishes. Fish growth reaches the optimum level once the amount, quality, and nutrient contents (e.g. protein, fat, carbohydrate, vitamin, and mineral) of the feed are balanced. The fish feed consists of organic and artificial feeds. An organic feed is commonly used in the natural state and rather difficult to be further developed. An artificial feed is a feed originating from the mix of several ingredients to fulfill the fish nutrition [1-3].

One of the most common fish feed is pellets. Pellets belong to the artificial feeds made from several ingredients and formed into bar or round shapes [1,4]. Pellets can be made from natural ingredients such as lymph, liver, and hearth of fishes. Pellet grouped according to its form includes
moist and dry pellets. The prominent feature of moist pellets is that it is easy to digest by fry and fingerling, although it has the potential to contaminate water [5]. Fish farmers often use commercial fish pellets despite the price reaches 60-70% of the overall farming price [6]. An effort that is promising to be considered is to self-produce the fish feeds using cheaper raw materials [1,7]. Raw materials that can be used for fish feeds include Patin bones, biogas sludge, Jaloh leaves (Salix tetrasperna), and Tongkol waste flour [13,8].

Smoked fish processing waste is one of the materials that can be used as fish feeds. The waste contains significant nutrition levels that can be used as fish feed including 29.70% of protein, 18.83% of fat, 1.94% of carbohydrate, 8.97% of water content, and 1.07% of crude fiber [7]. Unmanaged fish waste could generate organic pollution, unpleasant smells, and decreasing environmental cleanliness [2,7]. Before using the waste for fish feed, the waste has to be processed properly to avoid the presence of pathogens inside the fish bodies [2,9].

Commercial fish feeds quality standards aiming to be an appropriate indicator for fish growth. One of the microbiological indicators present in the quality standards of African catfish pellets according to BSN is the absence of *Salmonella* and aflatoxin [10]. Aflatoxin is produced mainly by *Aspergillus flavus* and *A. parasiticus* [11]. Aflatoxin contamination within the food materials and animal feed is present more often in areas with the tropical and sub-tropical climate as the temperature and humidity in these areas are suitable for the growth of fungi.

2. Methods
This study was conducted from April – September 2018. The research was conducted in the Laboratory of Zoology and Engineered Animals as well as Laboratory of Mycology, Department of Biology, Faculty of Natural Science, Institut Teknologi Sepuluh Nopember Surabaya.

2.1. Tools and Materials
Tools used in this study were weighing scale, washbasin, blender, stove, boiler, meat grinder, mixer, baking sheet, five fish container in the form of a dark-colored bucket with a volume of 20 liters, stationery, logbook, analytical balance, sewing machine, petri dish, Erlenmeyer, micropipette, measuring pipette, mortar, autoclave, and light microscopy.

Materials used in this study included smoked fish waste, paddy snails, bran, vitamin and mineral premix, tapioca flour, and *tempeh* yeast, 25 *C. gariepinus*, piped water, PDA medium, *lactophenol blue*, and distilled water.

2.2. Procedure
This research consisted of several steps including pellets development from the smoked fish waste, preservation of *C. gariepinus*, measurement of the growths, identification of fungi on pellets, African catfish, and preservation water.

2.3. Development of pellets from the smoked fish waste, preservation of *C. gariepinus*
The smoked fish waste was cleansed, chopped, and boiled for approximately two hours. After that, it was rinsed and dried. Dried smoked fished waste was crushed into flour. Paddy snails were boiled in salty water, its flesh was taken out of the shells, washed, and later dried before crushing it into flour [7].

Flour from the smoked fish waste was mixed with the raw materials consisted of 200 g of paddy snails flour, 270 g of bran, 10 g of tapioca, as well as vitamin and 20 g of concentrated mineral until well-mixed (Table 1).
Table 1. Pellet materials composition.

| Combination | Smoked Fish Waste | Raw Materials |
|-------------|-------------------|---------------|
| K.0         | -                 | -             |
| K.1         | -                 | 100           |
| K.2         | 30                | 70            |
| K.3         | 60                | 40            |
| K.4         | 90                | 10            |

Annotation: K.0 = Control (Commercial Pellet), K.1 = Combination 1 (BB 100% + 0% fish flour), K.2 = Combination 2 (BB 70% + 30% fish flour), K.3 = Combination 3 (BB 40% + 60% fish flour), and K.4 = Combination 4 (BB 10% + 90% fish flour), BB = Raw materials for making fish feed including concentrated premix, rice bran, tempeh yeast, tapioca flour, and paddy snails.

The dough was mixed with 1.25 g of tempeh yeast, then allowed to rest for ± 12 hours. After the fermentation process, the dough was molded with a pellet and then dried. Pellets were stored in a clean and dry place.

2.3.1. Preservation of C. gariepinus. C. gariepinus was grouped into K.0, K.1, K.2, K.3, and K.4 with five fish bones and preserved for 30 days. C. gariepinus had an initial weight of ± 8 g, a length of ± 9 cm, and the age of five weeks. During fish preservation, the feed was given twice a day in the morning and evening. The tested feed was fish pellets originating from smoked fish waste and commercial pellets. The fish was fed with pellets as much as 3% of the bodyweight of the fish. The fish preservation period was carried out for 30 days.

2.3.2. Identification of fungi on pellets, African catfish, and preservation water. Identification of fungi on pellets, fishes, and water preservation was carried out on PDA (Potato Dextrose Agar) medium. The incubation process was carried at 25°C for 2—7 days. The identification process was carried out according to the procedure of Identification of Health Fungi [14]. Fungi’s prevalence was calculated using the following formula:

\[
\text{Fungi’s prevalence} = \frac{\text{the number of fishes affected by fungi}}{\text{the number of fishes observed}} \times 100\% \hspace{1cm} (1)
\]

3. Result

3.1. Identification of fungi on pellets, African catfish, and preservation water

Through the macroscopic and microscopic identification, three types of isolate were observed, namely Aspergillus niger, Rizhopus oryzae, and Fusarium oxysporum (Table 2).

Table 2. Identification of fungi on pellets, African catfish, and preservation water.

| Group   | A. niger | R. oryzae | F. oxysporum |
|---------|----------|-----------|--------------|
| Pellets | K.0      | +         | -            | -            |
|         | K.1      | -         | +            | -            |
|         | K.2      | -         | +            | -            |
|         | K.3      | -         | +            | -            |
|         | K.4      | -         | +            | -            |
| Fishes  | K.0      | +         | -            | +            |
|         | K.1      | +         | +            | +            |
|         | K.2      | +         | +            | +            |
In this study, *A. niger* was found in fish and water samples (K.0, K.1, K.2, K.3, and K.4) (Table 3). This cosmopolitan fungus could exist in air and water. It grows fast and pH-tolerant [21]. In this study, *A. niger* spores were suspected to spread through the air and water from the outside environment [16], but they could also be derived from pellet raw materials such as rice bran. *A. niger* can be found in agricultural products such as rice, coffee, beans, and sunflower [22]. *A. niger* requires cellulose nutrition that will later be degraded into glucose [23].

| Group               | *A. niger* | *R. oryzae* | *F. oxysporum* |
|---------------------|------------|-------------|---------------|
| Preservation water  | +          | +           | +             |
| K.0                 | +          | -           | +             |
| K.1                 | +          | +           | +             |
| K.2                 | +          | +           | +             |
| K.3                 | +          | +           | +             |
| K.4                 | +          | +           | +             |

**Table 3.** Classification of fungi found in pellets, fishes, and preservation water.

| Division         | Ascomycota | Zygomycota | Eumycota |
|------------------|------------|------------|----------|
| Class            | Eurotiomycetes | Zygomycetes | Deuteromycetes |
| Ordo             | Eurotiales | Mucorales | Moniliales |
| Family           | Trichocomaceae | Mucoraceae | Tuberculariaceae |
| Genus            | Aspergillus | Rhizopus | Fusarium |
| Species          | *A. niger* | *R. oryzae* | *F. oxysporum* |

*R. oryzae* was found in pellets, fish, and preservation water (K.1, K.2, K.3, and K.4) (Table 3.1). The fungus *R. oryzae* was suspected to have originated from the raw material for making pellets as *R. oryzae* is one of the raw material components for making pellets. *R. oryzae* acts as a probiotic since it could break down complex fats into triglycerides and amino acids, and it could produce protease enzymes. *R. oryzae* could also function to inhibit the growth of *E. coli* and *A. flavus* [23] [24].

*A. flavus* was not found in water, fish, or pellet samples. We argue that this is because *A. flavus* can live as saprophytes in the soil by utilizing nutrition from plant residues [17]. In pellets made from smoked fish waste (K.1, K.2, K.3, and K.4), *A. flavus* was not found since the drying process with high temperatures (> 60 °C) could inhibit its growth. The optimum temperature for *A. flavus* to grow is 37°C to 48°C [18], hence there was no *A. flavus* on pellets. This indicates that the K.0 to K.4 pellets contained no aflatoxins, according to the quality requirements of African catfish feed that aflatoxins should be below 50 ppb [10]. Aflatoxin is a mycotoxin produced by *A. flavus* [19]. Aflatoxins in fish feed could cause diseases [20].

*F. oxysporum* was found in all fish and water samples. We argue that it has originated from plants near the pond. The fallen leaves carried spores and later proliferated in all fish and water samples. This fungus utilizes hemicellulose in plants as its energy source [26] [27].

3.2. Microscopic and macroscopic identification of fungi on pellets, African catfish, and preservation water

Macroscopic morphology of *A. niger* colony is blackish gray in color with a downy texture like cotton [14]. Microscopically, it has round-shaped vesicles, transparent conidiophores, and conidia that are
brownish black in color (Figure 1). Conidiophores are smooth and black and have round-shaped vesicles. Meanwhile, conidia are brown to black, rough, and have round-shaped [14] [29].

The macroscopic morphology of the *R. oryzae* colony is gray with a downy texture like cotton, while the microscopic morphology of the globus sporangium is black and has a sporangiophore (Figure 2). *R. oryzae* colony’s white color gradually turned gray [14]. The sporangia on *R. oryzae* are globus- or sub-globus-shaped, sporangiophores that grow from Stolon towards the air, either individually or in groups (up to 5 sporangiophores) with rhizoids [14].

From the macroscopic observations, it was found that the morphology of *F. oxysporum* which were found in all water and fish samples (K.0, K.1, K.2, K.3, and K.4) is white colored in the middle and there was a combination of white and orange with cotton-like colonies on the sides. *F. oxysporum* colony is white colored in the middle and orange on the edge of the colony with a shape like cotton. From the microscopic observations, it appeared that the morphology of *F. oxysporum*’s macroconidia was curved, long, short at the tip, and microconidia which are 1-or-2-celled conidia smaller than macroconidia (3-5 cells) (Figure 3 and Figure 4) [14].
Figure 4. Morphology of (1) *A. niger*; (2) *Rhizopus oryzae*; (3) *Fusarium oxysporum* found in commercial pellets, treated fish, and pond water. The staining process used *Lactophenol blue*. 400x magnification.

During the development of pellets, *R. oryzae* was also added. It functions as a binding agent for the components of fish feed as well as a floating agent as it has mycelium. Mold mycelium fills the cavities between the substrate grains, trapping the air in the gaps between the substrates [25].

3.3. **Fungi prevalence on African catfish**
The prevalence of fungi in African catfish in this study is related to the combination of feeds given, namely K.0, K.1, K.2, K.3, and K.4. The prevalence results can be seen in Table 4. The highest prevalence value was found in sample K.1 with an average prevalence of fish, pellets, and water reaching 100%. While the lowest prevalence value was found in the samples K.3, K.4 for fish (77.7%) and treatment water (77.7%) and K.0 for fish reaching 66.6%.

| No | Combination | Types of fungi | Prevalence Level (%) | Averaged Prevalence (%) |
|----|-------------|----------------|----------------------|-------------------------|
| 1  | K.0         | *A. niger*     | 66.6                 | 66.6                    |
|    |             | *F. oxysporum* |                      |                         |
| 2  | K.1         | *A. niger*     | 100                  | 100                     |
|    |             | *R. oryzae*    | 100                  |                         |
|    |             | *F. oxysporum* | 100                  |                         |
| 3  | K.2         | *A. niger*     | 100                  | 88.8                    |
|    |             | *R. oryzae*    | 100                  |                         |
|    |             | *F. oxysporum* | 66.6                 |                         |
| 4  | K.3         | *A. niger*     | 100                  | 77.7                    |
|    |             | *R. oryzae*    | 100                  |                         |
|    |             | *F. oxysporum* | 33.3                 |                         |
| 5  | K.4         | *A. niger*     | 100                  | 77.7                    |
|    |             | *R. oryzae*    | 100                  |                         |
|    |             | *F. oxysporum* | 33.3                 |                         |

The lowest prevalence number in fish samples K.3 and K.4 was presumed to have been influenced by the high protein content from the feed making. *C. gariepinus* has a good immune system. The low number of leaf debris falling into the pond was presumed to have made the prevalence of *F. oxysporum* was only around 33.3%. African catfish has a good immune system since it has high leukocytes of 64.75 x 10^3 cells / mm^3 compared to rainbow trout of 7.8 - 29 x 10^3 cells / mm^3 [9]. Another factor affecting the low prevalence number of fish in K.0 was due to the commercial feeds that are suspected to contain *A. niger*. The high prevalence number of fungi can cause toxicity and mycosis in fishes [26] [27].
4. Conclusion
The conclusion that can be derived from this study is that A. niger, R. oryzae, and F. oxysporum were identified in samples. Meanwhile, the highest average prevalence number for fish and water is K.3 and K.4 where they reach 77.7%. As for fish is K.0 that reaches 66.6%.

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